Chapter 6

SUMMARY AND CONCLUSIONS

Bacterial infection in the mammary gland parenchyma induces local inflammation that can lead to a complex disease called mastitis. Globally *S. aureus* is the single largest mastitis pathogen and the infection can ultimately result in either subclinical or chronic and sometimes lifelong infection. The critical host pathogen interactions during mastitis can either result in the elimination of invading pathogens and restoration of immune homeostasis or lead to uncontrolled inflammatory response that can disrupt normal mammary gland function. Any such deregulation in the complex and well-coordinated response to inflammation can lead to unresolved inflammation.

In this study, we have identified *S. aureus* is the largest pathogenic species present in the milk from the subclinical cases of mastitis. Molecular characterization of the *S. aureus* isolates highlighted the predominance of *spa* t267, t359 and t6877 and emergence of new clones associated with mastitis from the geographical province. Further, the *spa*-based phylogenetic analysis suggested t267 as ancestral clone, endemic in the region causing subclinical bovine mastitis. The heterogenous virulence profile of predominant *S. aureus* isolates indicated the complex nature of *S. aureus* pathogenicity. In addition, the predominance of *S. aureus agr* group I suggests their involvement in persistent IMI in subclinical mastitis. The present study significantly expands our current knowledge on genetic background and specific traits of the lineages of *S. aureus* associated with bovine subclinical mastitis in the zone.

This molecular characterization allowed selection of representative strains of *S. aureus* for subsequent studies in mice model. Intramammary infection studies in established mice model with the selected strains of *S. aureus* allowed understanding of the host microbe interaction at cellular and molecular levels. The study addressed the differential inflammatory
response in the mice mammary tissue during intramammary infection and the altered epigenetic context induced by two closely related strains of *S. aureus*. Immunohistochemical and immunoblot analysis showed strain specific hyperacetylation at histone H3K9 and H3K14 residues. Real-time PCR and transcriptome profiling showed expression of a set of proinflammatory genes and cytokines in a temporal manner. Remarkably, over expression of the genes significantly correlated with the promoter specific acetylation in these residues. Furthermore, genome wide miRNA expression analyses have revealed vital role for these small regulatory molecules in mastitis pathobiology. We have identified several differentially expressed known miRNAs and 3 novel miRNAs in the *S. aureus* infected mice mammary tissue by small RNA sequencing. Of these, a bunch of miRNA regulating NFkB has been determined which had differential expression status in both the IMI. By employing these gene expression data, an attempt has been made to delineate the gene regulatory networks in the strain specific inflammatory response. The integrative data analysis apparently showed, one of the isolates of *S. aureus* activated the NFkB signaling leading to drastic inflammatory response and induction of immune surveillance, which could lead to rapid clearance of the pathogen. However, this drastic inflammatory response can also lead to detrimental effect if fails to return to homeostasis. On the other hand, the other strain repressed most of the inflammatory response, which might help in its sustainance in the host tissue. This differential mechanisms adopted by the pathogen, could partially elucidate why *S. aureus* vaccine has not been successful so far. The current study strengthens and adds a novel layer of information to our current understanding regarding the strain specific complex reaction of the host immune response in *S. aureus* induced mastitis.

This study brings together the field of pathogenesis with the field of epigenetics. Taken together, this study shed substantial lights to comprehend the mechanisms of strain specific differential inflammatory response to *S. aureus* infection during mastitis. The study reveals that histone H3 Acetylation and microRNA(s) are key players in regulating host
immune response and thus decides the outcome of the infection. These observations give additional insights into the panoply of changes that occur in host cells infected with *S. aureus*. This elucidation and finding offers novel opportunities to develop therapeutic regimes for *S. aureus* mastitis. Study suggests manipulation of immune responsiveness targeting these epigenetic modulators might provide new avenue for treatment and prevention strategies in mastitis or other staphylococcal infections in future.

Continued efforts in this burgeoning field to translate our understanding of the mastitis epigenome will surely lead to successful interventional strategies in the fields in future.