CONTENTS

Chapter 1 Introduction 1-15
1.1 Mastitis 1
1.2 Impact of Bovine Mastitis 2
1.3 Multiple etiological agents in mastitis 3
1.4 Host pathogen interaction 5
   1.4.1 Pathogenesis 5
   1.4.2 Host immune response patterns 8
   1.4.3 Epigenetic reprogramming of host genes in bacterial pathogenesis 9
1.5 Management of mastitis including treatment and vaccination 14
1.6 Objectives 16

Chapter 2 Review of Literature 17-43
2.1 Impact of S. aureus Bovine mastitis 17
2.2 General Characteristics of S. aureus 18
   2.2.1 Genomic content 18
   2.2.2 Virulence factors 19
   2.2.3 Cell wall components and role in the inflammatory response 21
2.3 Population structure of S. aureus 23
2.4 Clinical Significance 27
2.5 Clinical manifestation and outcome 27
2.6 Pathogenesis of bovine S. aureus intramammary infection 28
   2.6.1 The anatomical physical barrier of teat canal 28
   2.6.2 Innate immune response of mammary gland 30
2.7 Epigenetic Regulation of Host Response 36
   2.7.1 Histone modifications during bacterial infection 36
   2.7.2 miRNA mediated regulation 40

Chapter 3 Materials and Methods 44-80
3.1 Sampling 44
   3.1.1 Clinical examination of the cows 44
   3.1.2 Raw milk sampling 44
3.2 Bacterial Isolation 46
3.3 DNA extraction 46
3.4 Molecular Identification of S. aureus species 46
   3.4.1 Genus specific PCR 46
   3.4.2 Species specific mPCR 47
   3.4.3 Partial 16S rRNA sequencing 49
3.5 Characterization of S. aureus isolates 50
   3.5.1 spa typing 50
   3.5.2 PFGE 51
   3.5.3 agr typing 54
   3.5.4 PCR detection of staphylococcal toxin genes 55
   3.5.5 Antimicrobial Susceptibility testing 58
   3.5.6 MLST analysis 58
3.6 S. aureus intramammary infection in established mastitis mice model 59
   3.6.1 Establishment of mastitis in Swiss albino mice 59
      3.6.1.1 Tissue Collection from S. aureus infected mice mammary gland 60

Page No
3.6.2 Histopathological analysis of mice mammary tissue 61

3.7 Immune gene expression in Time Course manner 61
3.7.1 RNA extraction 61
3.7.2 cDNA preparation 63
3.7.3 Quantitative real-time PCR 64

3.8 Global Gene expression and transcriptome profiling 66
3.8.1 Microarray hybridization 67
3.8.2 RNA sequencing (NGS) 67
3.8.3 Microarray Data Analysis 68
3.8.4 Bioinformatics analysis of RNA-seq data 69
3.8.5 Gene ontology and pathway analysis 69

3.9 Small RNA sequencing 70

3.10 Real Time Validation for mRNA and miRNA 71

3.11 Integration of microarray, RNA sequencing and small RNA sequencing data 74

3.12 Epigenetic modification in mice mammary tissue 74
3.12.1 Immunohistochemical analysis of mice mammary tissue 74
3.12.2 Tissue lysate preparation and Western blot analysis 76
3.12.3 Chromatin immunoprecipitation (ChIP) assay 79

Chapter 4 Results 81-125

4.1 Isolation, Identification and Characterization of S. aureus isolates 81
4.1.1 Isolation 82
4.1.2 Identification of S. aureus 82
4.1.3 Characterization of S. aureus isolates 84
4.1.3.1 Toxin gene profiles of S. aureus isolates 84
4.1.3.2 agr typing 85
4.1.3.3 spa typing 85
4.1.3.4 PFGE analysis 91
4.1.3.5 MLST analysis 91
4.1.3.6 Antimicrobial Susceptibility testing 93

4.2 S. aureus induced strain specific molecular events in experimental IMI 95
4.2.1 Histopathology analysis of mice mammary tissue 95
4.2.2 Temporal expression of immune genes 95
4.2.3 S. aureus infection induces specific histone H3 hyperacetylation in alveolar epithelial cells.
4.2.3.1 Immunohistochemical (IHC) analysis 98
4.2.3.2 Western blot analysis 103
4.2.4 S. aureus infection induces differential gene expression in mice mammary tissue 105
4.2.5 Transcriptome profiling of S. aureus induced IMI 107
4.2.6 S. aureus infection induces differential expression of a set of miRNAs
4.2.6.1 Deep sequencing of Small RNA in mammary gland tissue samples from mice infected with S. aureus 116
4.2.6.2 Multiple miRNAs are differentially expressed in response to S. aureus infection 117
4.2.7 Histone H3K14 acetylation is selectively enriched at the over expressed gene promoters
4.2.7.1 ChIP assay 120