Molecular characterization of *Staphylococcus aureus* from bovine mastitis and unraveling molecular events in *S. aureus* intramammary infection in mice model

**ABSTRACT**

Bacterial infection in the mammary gland parenchyma induces local inflammation that can lead to a complex disease called mastitis. Globally *Staphylococcus aureus* is the leading mastitis pathogen and the infection can ultimately result in either subclinical or chronic and sometimes lifelong infection.

The impact of bovine mastitis to dairy sector is huge. India is the largest milk producer but per capita production is less than half of the world average. Estimated annual economic losses due to bovine mastitis has increased 135 folds in about almost 5 decades from 1962 (INR 529 million/annum) to 2009 (INR 71655.1million/annum).

*S. aureus* is a multifaceted pathogen which has the potential to express a myriad of virulence factors and is fully capable of evading immune surveillance and treatment compounds. These complexities are illustrated by the lack of efficacy of currently available vaccines and antimicrobial treatments. Being the chief pathogen causing mastitis, understanding of *S. aureus* pathogenesis is of paramount importance.

In the present study we have evaluated the virulence determinants and genetic diversity of *S. aureus* from bovine subclinical mastitis milk. PCR detection of virulence genes was performed for 173 *S. aureus* from bovine subclinical mastitis milk. Further, genetic diversity was analysed by *agr* and *spa* typing followed by pulsed field gel electrophoresis (PFGE) of selected isolates. Screening of virulence genes (n = 19) showed the adherence genes viz. *fnbA*, *clfA*, *fnbB* and *cna* in 98.8, 97.1, 68.8 and 28.3 percentage of isolates, respectively, and 80 strains (46.24%) positive for enterotoxin genes were distributed as 23 toxinotypes, of which, 5 genotypes contained a single gene and the rest comprised of multiple toxin genes. Out of *agr* type-1 (87.3%), 74.2 per cent belonged to the three predominant *spa* types. Of 27 *spa* types, 11 were identified for the first time. The predominant spa types were t267 (N =44), t359 (N = 42) and t6877 (N =29), which together accounts to 66.5 per cent of isolates. PFGE analysis of isolates (N = 45) covering all the *spa* types revealed mostly similar or closely related pulsotypes. Local emergence of *spa* type t6877 in herd-dependant manner was observed. *spa* sequence-based phylogenetic analysis suggested t267 as the ancestral clone of t359, t6877 and other *spa* types except two. Heterogeneous virulence profile of the isolates had no significant association with the genotype. High prevalence of *agr* group I reaffirms their association with persistent subclinical mastitis. The *spa* type t267 appears to be the ancestral clone endemic in the region causing subclinical mastitis. In addition, few new *spa* types have emerged in the geographic region. This study gives an insight into the genetic and evolutionary behaviour of *S. aureus* associated with bovine subclinical mastitis in India.

Subsequently, we have used mice model, to address 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* selected based on molecular characterization profile. Immunohistochemical and immunoblotting analysis showed strain specific hyperacetylation at histone H3K9 and H3K14 residues. Global transcriptional response analysis in the *S. aureus* infected mice mammary tissue revealed a
selective set of up regulated genes that significantly correlated with the promoter specific H3K14 acetylation. Furthermore, we have identified several differentially expressed known miRNAs and 3 novel miRNAs in the *S. aureus* infected mice mammary tissue by small RNA sequencing. By employing these gene expression data, an attempt has been made to delineate the gene regulatory networks in the strain specific inflammatory response. Apparently, 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. The other strain repressed most of the inflammatory response, which might help in its sustenance in the host tissue. Taken together, our studies shed substantial lights to understand the mechanisms of strain specific differential inflammatory response to *S. aureus* infection during mastitis. All these data indicates that *S. aureus* infection creates an environment favourable for sustenance of pathogen in the host tissue in a strain specific manner. Taken together we have tried to explore the mechanisms of regulation of gene expression through chromatin remodelling during bacterial infection. 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.