CHAPTER IV: CONCLUSION

Over the past two decades, MRSA has rapidly spread throughout the hospital and community, despite control efforts and policies. In the late 1960s and 1970s, the prevalence of MRSA infection was below 5% in most hospitals worldwide. Currently, the prevalence of MRSA infection has increased up to 40% in several hospitals. Treatment of infections caused by MRSA is costly due to the requirement for prolonged hospitalizations and increased laboratory use for extensive surveillance or screening. Therefore, in order to reduce the cost of treatment and to prevent morbidity and mortality associated with MRSA infections an effective vaccine is urgently required.

Several reviews on *S. aureus* vaccines suggested that a mixture of proteins would enhance the efficacy of protection against the pathogen. Cocktail vaccine formulated with AM, LytN, LytR and LytM was capable of protecting mice against lethal challenge of *S. aureus* Newman. The combinatorial cocktail vaccine provided 100% protection post 7 days of lethal challenge. Vaccine induced the production of high levels of specific IgGs that could efficiently induce opsonophagocytosis of *S. aureus*. The cocktail vaccine also helped in enhanced clearance of *S. aureus* that was observed from bacterial enumeration of internal organs.

Although a cocktail produces significantly better protection, the role of individual proteins were also tested to verify whether the protection observed was a cumulative protection or whether a single protein mediated the vaccine efficacy. Hence AM, LytR, LytN and LytM were individually tested for protective potential. Up on lethal challenge with *S. aureus*, AM alone generated 87.5% survival in vaccinated mice followed by 50% survival in the case of LytM and LytR group. LytN was the least efficient providing only 37.5% survival through the 7-day post lethal challenge. There was dissemination of bacteria into internal organs: heart, kidney, and liver, in the case of mock immunized mice and mice that received LytR, LytN or LytM alone. Interestingly, mice injected with AM alone showed lesser bacterial dissemination and very little bacterial load in the internal organs, implying that vaccination with AM alone induced clearance of the bacteria from the system. AM-vaccinated mice sera showed high specificity in recognizing *S. aureus* in *in vitro* analysis as it induced the production of opsonophagocytic antibodies. AM immunized mice sera produced more IgG2b, IgG2a, and IgG1 subtypes. Vaccination with AM induced a mixed Th1 and Th2 immune response. It was able to induce Th1, Th2, and Th17 cells, as observed by flow cytometric analysis of intracellular cytokine
staining of lymphocytes. Splenocyte proliferation assay result had shown the higher immunogenic potential of AM. It was observed through in vivo studies that vaccination with AM alone is sufficient to provide significant protection against lethal challenge by clinical drug resistant strains of S. aureus as well as S. epidermidis.

This study provides a strong evidence for an efficacious vaccine against S. aureus using non-covalently surface associated cell wall hydrolase AM. Even though a combinatorial vaccine was hypothesized, the results confirmed that a highly potent single antigen is sufficient to provide significant protection. This study proved that AM vaccinated mice was even protected from infection with drug resistant clinical strains of S. aureus. However, several additional experiments need to be done for further confirmation of the vaccine efficacy of AM. The durability of the vaccine efficacy needs to be investigated. Additionally, all animal models involving inbred mice, including BALB/c mice was used for the study, have some limitations in terms of extrapolation of experimental findings to humans. Therefore, the results obtained in mice experiment may not necessarily be reflected in humans. The selected protein, AM, has to be tested for efficacy using human approved adjuvants like alum or new and improved adjuvants that can be used in humans etc (Salgado-Pabon & Schlievert, 2014). Along with finding an effective vaccine, it is also important to explore mode of vaccine delivery, extended release strategies. One such interesting study is that of an O-carboxymethyl chitosan nanoparticle encapsulated Amidase protein vaccine delivery system that showed hemo- and cyto-compatibility and sustained release of the vaccine protein (AM) which would prolong immunogenicity (Smitha et al., 2014). One limitation of this study is that vaccine efficacy of AM has been validated only using hospital isolates of S. aureus and not using any of its community isolates, which are known to be more virulent. Nevertheless, this study provides strong evidence that AM is a potent vaccine candidate, and should be considered for future vaccine design against S. aureus and S. epidermidis. The scope of amidase vaccine can be extended to other opportunistic strains of staphylococci like the S. lugdunensis, S. saprophyticus, S. schleiferi, S. caprae etc. and can be tested for its protective potential against other staphylococcus strains as well.