Summary
Diarrheal diseases have remained the major cause of juvenile mortality, prevalent in underdeveloped world. Among other diarrhea causing pathogens, *Shigella* is responsible for high morbidity and mortality. The continuous use of antibiotics as a remedial therapy against *Shigella* had led to the emergence of multiple drug resistant strains which limits the available treatment. To prevent the infection and disease establishment, development of protective prophylactic could serve as an effective alternative. Since decades, research is going on for development of a vaccine against shigellosis through a variety of strategies, however, none of them has yet resulted in successful vaccine candidate.

Previously developed candidates conferred protection against only a few species of *Shigella*. Most of the vaccine candidates based on use of live attenuated strains such as CVD 1208, CVD 1208S developed by University of Maryland, WRSS1, WRSs3, WRSf3 developed by WRAIR, Silver Spring, Maryland USA provided strain specific protection against *S. sonnei*. In addition, the developed candidate vaccines were not equally effective in different geographic regions. A series of clinical trials with the immunization of $10^4$ C.F.U. of live attenuated *S. flexneri 2a* SC602 strain showed the remarkable results in terms of tolerance, immunogenicity, protection and replication in North American volunteers, whereas, the same study revealed very low immunogenicity in children and adults residing in Bangladesh (Levine et al., 2007). Due to the geographic disparity and diversity of the afflicted populations the candidates would not exhibit similar results worldwide. Therefore, there is a need for a better vaccine candidate(s) that can be effective against most if not all pathogenic species and which can be effective in most if not all endemic regions.

The conserved antigenic regions (epitopes) which are immunodominant in nature could be used for designing such kind of vaccine candidate. The ever evolving use of multiple prediction tools of the immunoinformatics could assist in identifying such regions (epitopes). The vaccine candidate developed by the incorporation of epitopes would encompass the varied properties with the optimum immunogenicity. Moreover, the epitope based vaccines are safe to use as they do not have the risk of virulence reversion, which is often encountered with the whole cell inactivated vaccines or attenuated strains. Hence, in the present study we designed and developed the epitope based vaccine candidate against shigellosis.
To achieve the objectives of the study, we started with the selection and characterisation of potent antigens. OMPs of *Shigella* were selected and isolated from *S. flexneri*, *S. sonnei*, *S. boydii* and *S. dysenteriae*. The isolated OMPs from all four species of *Shigella* were molecularly characterised by proteomic analysis. In addition, the functional conservancy and immunogenic properties of OMPs were also assessed by immunoblotting, bactericidal and opsonophagocytic activity of antisera raised against OMPs. The molecular and immunogenic characterisation of isolated OMPs revealed the conserved nature and immunogenic potential of OMPs, which was described in Chapter-3.

Among the isolated OMPs, OmpA and OmpC were selected as potent antigens which were found to be highly conserved among all four *Shigella spp*. The selected antigens OmpA and OmpC were cloned into pET28a expression vector, expressed using conventional expression system BL21 and purified by affinity chromatography. Moreover, the OmpA and OmpC purification method of affinity chromatography was also optimized. The cloning, expression, purification of OmpA and OmpC was described in Chapter-4.

After purifying the recombinant proteins OmpA and OmpC, the purified antigens were used to immunize Balb/c mice to evaluate the immunogenic potential of selected antigens. Elicitation of OmpA and OmpC specific humoral and cellular immune response was analysed. Moreover, the protective efficacy of OmpA and OmpC was ascertained using the in-house developed human like murine shigellosis model. The immunized mice were interaperitoneally challenged with *S. flexneri* and observed for the alleviation of disease parameters as described in Chapter-5.

After confirming the immunogenic and protective potential of selected antigens (OmpA and OmpC), the immunodominant regions (epitopes) were derived from OMPs of *Shigella* by the sequential deployment of immunoinformatics tools in a rational way, moreover, the workflow for the prediction and selection of epitopes was also developed. The epitopes were screened through multiple screening criteria such as surface exposure, binding to maximum number of MHC alleles and population coverage. The selection of epitopes through stringent screening criteria resulted in the pool of 18 potent epitopes against shigellosis. The prediction, screening and selection of epitopes by rational workflow were described in Chapter-6.

Moreover, the developed workflow was validated by implementing it for the prediction of potent epitopes against salmonellosis. B cell and T cell epitopes were predicted using similar immunoinformatic tools and stringent selection criteria. In addition, population coverage of
predicted epitopes was analysed among the world population and 23 countries lying on the equatorial region as salmonellosis is majorly a tropical disease. The reverse vaccinology approach implemented for the identification of potent epitopes against *Salmonella* infection was described in Chapter-7.

In continuation with the epitope selection against shigellosis, the high scoring epitopes derived from OmpA and OmpC were selected, chemically synthesized and conjugated with Ovalbumin (the carrier protein). The potential of conjugated epitopes to elicit humoral, mucosal and cellular responses was evaluated. Further, the immunologically characterised epitopes were used to develop EpiMix- the epitope based vaccine candidate against shigellosis. The protective efficacy of developed EpiMix was evaluated using shigellosis murine model, along with the parental proteins and carrier proteins as a control. The intramuscular immunization with EpiMix led to the elicitation of desired immune response required to confer protection against the intraperitoneal challenge of *Shigella* which has been described in Chapter 8.

Comprehensively, the strategy developed to rationally design the vaccine resulted in the potential epitope based candidate. However, the future detailed study would substantiate the prospective use of EpiMix as a prophylactic candidate.