Conclusions

The following conclusions can be drawn from the present study:

1) The OMPs were isolated from *S. flexneri*, *S. boydii*, *S. dysenteriae* and *S. sonnei*. The antisera raised against *S. flexneri* showed binding with OMPs of other *Shigella* indicating the conservancy of proteins. The antisera raised against OMPs were able to kill and opsonise *Shigella* confirming the immunogenic potential of OMPs against the *Shigella*. Among the OMPs, OmpA and OmpC were found to be highly conserved and were selected for further characterisation.

2) In order to individually characterise the selected potent immunogens, OmpA and OmpC were successfully cloned and expressed into the conventional *E. coli* expression system. The purification of OmpA and OmpC was achieved successfully using Ni-affinity chromatography. Additionally, we have also developed the new method; hybrid affinity purification to obtain the better yield of purified OMPs (~1.4 mg of OmpA from 100ml of bacterial culture and ~1.8 mg of OmpC from 100 ml of bacterial culture).

3) The purified OmpA and OmpC were immunologically characterised, the intramuscular immunization of Balb/c mice with OmpA and OmpC resulted in elicitation of high OmpA and OmpC specific IgG titres, and induction of IFN-γ, IL-4, IL-10 & IL-2 cytokines. The elicited immune response successfully protected Balb/c mice from intraperitoneal challenge with *Shigella flexneri* ATCC 1202, demonstrating the protective efficacy of selected immunogens OmpA and OmpC.

4) After the confirming the immunogenic and protective potential of selected OMPs, the immunodominant regions of OmpA, OmpC, OmpE, OmpF, OmpW and OmpX were successfully identified using the designed workflow of immunoinformatic tools. The pool of B and T-cell epitopes were derived from OMPs of *Shigella*. The selected epitopes were found to be topologically exposed, binding to a maximum number of MHC alleles and predicted to be efficacious in shigellosis afflicted Southeast Asian population. Further, the high scoring epitopes derived from OmpA and OmpC showed binding with IgG raised against OmpA and OmpC and also successfully elicited the set of cytokines (IFN-γ, IL-4, IL-10 & IL-2).

5) Moreover, B and T-cell epitopes against salmonellosis were also derived by implementing the similar immunoinformatic workflow. The B-cell epitopes were found to be surface exposed. The selected T-cell epitopes were predicted to be...
efficacious in the entire world population. More than 75% of world population was predicted to be responding against the set of selected epitopes. In addition, ~88% of Indian population was also predicted to be responsive against the selected epitope set against salmonellosis. Therefore, the implementation of developed workflow resulted in the set of epitopes that are predicted to be effective in the different populations residing at diverse geographic locations.

6) In the continuation with the identification of *Shigella* epitopes, the immune characterisation of chemically synthesized high scoring five epitopes derived from the OmpA and OmpC revealed the ability to elicit humoral, mucosal and cellular immune responses. The immunization with EpiMix (developed from immune-characterised five epitopes) resulted in elicitation of EpiMix specific IgG, IgA titres, and induction of high levels of IFN-γ, IL-4, IL-10 & IL-2 cytokines. Additionally, the EpiMix immunized mice were protected from the intraperitoneal challenge of *Shigella flexneri* ATCC 12022. EpiMix immunization also resulted in IFN-γ surge indicating the possible dominance of Th1 response required for removal of an intracellular pathogen. Thus, the developed, epitope based vaccine candidate proved to be efficacious against murine shigellosis.