Chapter-6

SUMMARY

There has been a worldwide trend of increasing incidence of salmonellosis in both man and animals. Amongst the well recognized bacterial agents of diarrhoeal diseases, non-typhoidal *Salmonella* has been identified as the major causative agent in many countries. Due to the ubiquitous distribution of *Salmonella* in the natural environment, prevalence in global food chain and virulence nature, there is a need for continued vigilance and stringent control measures at all levels. The virulence of *Salmonella* depends on virulence factors encoded by specific genes clustered within *Salmonella* pathogenicity islands. The present study describes the development of a simple and rapid multiplex PCR (m-PCR) assay for simultaneous detection of seven major virulence genes of *Salmonella* (*inv*A, *inv*H, *stn*, *sop*B, *sop*E, *sef*C and *pef*A). Also, a number of control strategies have been aimed at reducing infection of host by these organisms, of which vaccination best fulfils the requirement for practical anti-*Salmonella* intervention. Due to inherent shortcomings of various vaccines, researchers in recent past have extensively studied complex antigens of *Salmonella* for its utility as a vaccine candidate. In this regard attention is now being focused towards recombinant vaccines including possible use of outer membrane proteins of *Salmonella*. The product of *invH* gene of *Salmonella*, an important component of the needle complex in Type 3 Secretion System (T3SS) plays an important role in bacterial adherence and entry into the epithelial cells. The present study also envisaged in expressing the 15 kDa recombinant InvH surface protein of *Salmonella* Typhimurium in *E. coli* host and to evaluate its potential as a probable vaccine candidate by testing its immunogenicity in mice.

A total of 495 samples collected from different sources were examined for isolation of *Salmonella,* of which 76 (15.35%) were found to be positive for *Salmonella*. The isolation rate was highest in poultry (21.36%) followed by pig (15.9%), wild bird (14.85%), human (11.76%) and cattle (10%). The isolated *Salmonella* strains belonged to ten different serotypes namely *S*. Typhi, *S*. Typhimurium, *S*.Newport, *S*. Dublin, *S*. Enteritidis, *S*. Gallinarum, *S*. Litchfield, *S*. Weltevreden, *S*. Choleraesuis and *S*. Kentucky. In the present study, *S*. Weltevreden was found to be the most predominant serotype, irrespective of the host species.

Serotyping of the 76 (15.35%) isolates recovered in the present study from different sources revealed that 23 (30.26%) isolates belonged to *Salmonella enterica* serovar Weltevreden, 15 (19.74%) isolates belonged to *S.* Enteritidis, 10 (13.2%) to serovar Typhimurium, 7(9.21%) to serovar *S*.Newport, 5(6.6%) to serovar Dublin, 4 (5.26%) each to serovars Typhi, Gallinarum and Choleraesius and 2(2.63%) each to serovars Kentucky and Litchfield.

A multiplex PCR assay was developed for detection of seven major virulence genes of *Salmonella* i.e. *invA, invH, stn, sopB, sopE, pefA and sefC.* All the 76 isolates and 17 standard strains were screened for the presence of these genes encoding important virulence proteins that play significant role in the pathogenesis of *Salmonella.* Isolates belonging to different serovars showed variable results in respect of possession of different virulence genes. *invA, invH, stn* and *sopB* were present in all the isolates including the standard strains irrespective of their serovars while the rest three virulence genes *sefC, sopE* and *pefA* genes were present only in 23 (30.26%), 39 (51.32%) and 20 (26.32 %) of the samples in varying percentages among the 76 isolates of the *Salmonella* serovars

Enteritidis, Weltevreden, Typhi, Newport, Litchfield, Kentucky, Typhimurium, Choleraesuis, Dublin and Gallinarum.

Genomic DNA was isolated from *S*. Typhimurium and the *invH* gene was amplified by self designed PCR primers. The *invH* fragment of size 463 bp was gel eluted. The eluted PCR product was ligated into pGEMT vector and transformed in *E.coli DH5*α cells. Recombinant clones thus obtained produced white colonies on LB agar plate, which was confirmed by colony PCR. Plasmids extracted from the recombinant clones were digested with enzymes XbaI and XhoI to release the *invH* fragment from pGEM-T vector. The digested fragment was then ligated into the linearised pET303CT/His vector and transformed. Positive clones were confirmed by colony PCR using T7 forward and reverse primers.

Further, plasmids extracted from the positive clones were transformed into BL21competant cells. BL21 cells harbouring recombinant plasmids were induced with 1mM IPTG. The expressed protein was confirmed by SDS-PAGE and western blot which revealed the presence of 15kDa expressed protein. The recombinant protein was purified through affinity chromatography using Ni-NTA column under denaturing condition. After purification, the eluted protein was refolded by dialysis with sequential changes of buffer that contained 4, 2, 1 and 0 M urea, to provide gradual refolding of InvH. The refolded protein was them concentrated with PEG-8000 and quantified by Lowry's method which was found to be 7mg/ml. Immunoreactivity of the expressed protein was confirmed by strong reactivity of the recombinant InvH serum, raised in rabbit against the protein.

Two groups of mice were chosen for analyzing immune competence of r-InvH with FCA and bacterin vaccine with FCA.Booster dose was administered on the 14th day

post primary vaccination. Both the immunized groups (r-InvH with FCA and bacterin with FCA) have shown the maximum mean antibody titre on 21st day post primary vaccination with high level of significant difference between them. The mean anti-r (InvH) IgG response in serum of mice was significantly higher on 21st day post-immunization (16.69 ± 0.16), which gradually declined till 42nd day (3.99 ± 0.01). In the group injected with bacterin vaccine, there was a significant decline in IgG response ($P<0.05$) after 21st day (9.77± 0.14) till 42nd day (3.53 ± 0.01). The difference in antibody titre between the two groups was statistically significant ($P<0.01$).