ABSTRACT

Salmonellae the gram-negative bacilli of the family *Enterobacteriaceae* are widely distributed in nature and can cause diseases ranging from gastroenteritis to typhoid fever. 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). Presence of these genes was studied using 17 standard cultures and 76 field isolates from different sources. Seventeen non-*Salmonella* strains were also tested for specificity through optimization of multiplex PCR conditions. A spiked control experiment was done to detect the sensitivity of m-PCR assay. The primer pairs were found to be specific for *Salmonella* only. All the field isolates and standard strains of *Salmonella* were found to harbour *inv*A, *inv*H, *stn* and *sop*B genes, while variability was observed with respect to *sopE*, *sef* C and *pef*A genes. This multiplex PCR assay has been tested as a simple and rapid method for detection of major virulence factors in important clinical serovars of *Salmonella*.

In addition, this study was also undertaken to express the 15 kDa recombinant InvH surface protein of *Salmonella* Typhimurium in *E. coli* host and to evaluate its potential as a vaccine candidate by testing its immunogenicity in mice. The *invH* gene was cloned and expressed in *E. coli* and the recombinant protein was purified under denaturing condition and identified by SDS-PAGE and western blot analysis. The purified recombinant InvH protein provoked a significant rise of IgG in the inoculated mice. 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). The immunized mice were completely (100%) protected against challenge with $10^{7.5}$ LD_{50} of homologous *Salmonella* serovar, while protection against challenge with the same dose of heterologous serovars appeared to be 95 per cent. To conclude, the recombinant invH protein could induce effective protective immunity in tested mice. The present findings suggest that the potential use of the recombinant InvH protein of *S.* Typhimurium can be used as an effective vaccine candidate against *Salmonella* infections.