Salmonella, a genus of the Gram-negative family Enterobacteriaceae comprises a large, closely related group of important bacteria, whose nomenclature is complex (Brenner et al., 2000; Tindall et al., 2005). The American Society for Microbiology (ASM) and the Centres for Diseases Control Prevention (CDC) use a system based on recommendations from the World Health Organisation Collaborating Centre that recognizes two Salmonella species, Salmonella enterica and Salmonella bongori (Board 1999; Brenner et al., 2000). S. enterica, which is medically the more significant of the two species, is classified into six subspecies (enterica, salamae, arizonae, diarizonae, houtenae and indica) which are further divided into serovars based on the antigenicity of the LPS (O-antigen) and flagella (H-antigen). In total >2,500 serovars of S. enterica have been described. Host range and virulence varies greatly between the different serovars (Coburn et al., 2007; Martins et al., 2013).

Salmonellosis, a foodborne illness caused by Salmonella is an important public health problem in both man and animals, all over the world (Shahane et al., 2007). Salmonella serovars (S. Typhimurium, S. Enteritidis) cause infections in domestic animals which can be transmitted to humans and represent a serious concern for the food industry (Mastroeni, 2002). Salmonella enterica serovar Typhi (S. Typhi) is a facultative intracellular pathogen that causes typhoid fever in humans (the only known natural hosts and reservoir of infection) (Mweu and English, 2008). S. Typhi is noncapsulated, nonsporulating, Gram-negative anaerobic bacilli, which have characteristic flagellar, somatic and outer coat antigens (Shahane et al., 2007). S. Typhi harbors the virulence capsular polysaccharide (Vi; an important virulence factor during infection) thereby making it different from that of other serovars of Salmonella (Hornick et al., 1970; Kaur and Jain, 2012b).

Typhoid remains a major health problem, especially in developing world where there is substandard water supply and lack of sanitation (Crump and Mintz, 2010; Dougan et al., 2011; Kaur and Jain, 2012a). S. Typhi is transmitted through contaminated food and water, and much less commonly by direct finger-to-mouth contact with faeces, urine or other secretions. High epidemiology of typhoid has been frequently reported in the African (Sub-Saharan Africa) (Muyembe-Tamfum et al., 2009), Asian regions (India, Indonesia, China, Pakistan, and Vietnam) (Ochiai et al., 2008, Crump et al., 2010), Central and South America, the Middle East and a few Southern and Eastern European countries (Paterson and muskell, 2010). The worldwide incidence of typhoid fever is estimated to be around 22 million with at least 200,000 deaths annually (Crump et al., 2004, Paterson and muskell, 2010). People residing in slump
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areas, under unhygienic conditions and travellers to regions where the disease is endemic are more prone to infection (Majumder et al., 2009; Farmakiotis et al., 2013).

It’s a disease with range of symptoms like chills, perspiration, diarrhoea or constipation, headache, anorexia, cough, weakness, sore throat, dizziness, and muscle pains are frequently present before the onset of fever in typhoid. During active invasion, bacilli disseminate from the intestine via blood to liver, spleen, bone marrow, gall bladder and Peyer’s patches in the terminal ileum, where they proliferate (Charles et al., 2010). Infection of Peyer’s patches leads to lymphoid hyperplasia, which can resolve without scarring or can progress to capillary thrombosis, with necrosis and ulceration. The most frequent and severe complication is intestinal perforation with peritonitis (Inian et al., 2006; Meltzer et al., 2006). Complications occur in 10% to 15% of patients, usually in the third and fourth week of infection such as gastrointestinal bleeding, intestinal perforation, and typhoid encephalopathy (Parry, 2002).

The first antibiotic (1948-1970) introduced for the treatment of typhoid fever was Chloramphenicol (Woodward et al., 1948). However, with this drug the relapse rate was not reduced and were are unable to cure convalescent excretors and chronic carriers. Moreover, strains of S. Typhi became resistant to chloramphenicol. Towards the end of the 1980s and 1990s, S. Typhi developed resistance simultaneously to all the first line drugs (chloramphenicol, trimethoprim, sulphamethoxazole and ampicillin) (Rowe et al., 1990). The appearance of Multi drug resistant (MDR) S. Typhi in Asia led to the widespread use of fluoroquinolones and extended spectrum cefephalosporins for treatment. Isolates with low-level resistance to fluoroquinolones appeared within a few years of this change and have become common in Asia (Brown et al., 1996; Dutta et al., 2001). Now, S. Typhi has acquired increased virulence, communicability and survivability. The MDR organisms are thus known for their notorious role in causing complications leading to increased morbidity and mortality.

Typhoid fever is becoming increasingly difficult to diagnose and treat due to this rapid and widespread emergence of S. Typhi serotypes with resistance to multiple antibiotics and changing modes of bacterial presentation (Yang et al., 2010; Allam et al., 2011). The emergence of MDR strains of S. Typhi has added a sense of urgency to develop more effective typhoid vaccines (Levine et al., 2004). Though a number of vaccines for typhoid are currently available, none of these is ideal. The first line of parenteral whole cell vaccines has been associated with fever and systemic reactions. Although it was licensed, it is considered unsuitable for mass immunization and no longer in use (Garmony et al., 2002). Currently two licensed vaccines against Salmonella
are in use globally, Vi polysaccharide vaccine and Ty21a live attenuated vaccine (Cheminay and Hensel, 2008). Vivotif Berna® is an attenuated live S. Typhi Ty21a strain, generated by chemical mutagenesis of parental virulent strain of S. Typhi Ty21 using nitrosoguanidine (Germanier and Fuer, 1975). The other licensed typhoid vaccine is Typhim-Vi® which is the purified Vi antigen from S. Typhi (Keitel et al., 1994; Allam et al., 2011). Both these vaccines are well tolerated but are only moderately protective. However, due to the shortfalls of these vaccines such as certain unacceptable adverse reactions and less than desired efficacy, efforts are being made to use bivalent Salmonella strains to deliver DNA vaccines, a number of new genetically defined attenuated strains of S. Typhi have been constructed as candidate live oral vaccines (Garmony et al., 2002). The most promising strains include CVD 908-htrA, CVD 906, CVD 909, Ty800, and M01ZH09 (Hohmann, et al., 1996; Tacket, et al., 2000; Levine et al., 2001; Levine et al., 2004; Jain, 2009). These attenuated strains of S. Typhi could also be candidates to serve as carrier of foreign antigens from other pathogens. Therefore, the emergence of MDR strains of S. Typhi and certain drawbacks in presently available vaccines against typhoid has necessitated exploring new immunogens to develop more effective typhoid vaccine.

Over the past years, several Outer membrane proteins (OMPs) have been investigated as potential vaccine candidates. Although significant advances have been made regarding the structure and function of OMPs, the number of OMPs that have been characterized represents only a small portion of the total OMPs revealed by bacterial genome sequences (Lin et al., 2002). The OMPs apart from virulence attributes and transport functions are also known to have an important role in evoking immune response. Studies of bacterial systems have focused attention to the OMPs of Salmonella as a possible candidate for vaccine development suggesting the existence of protective immunogenic components in S. Typhi. Although a few reports are available describing the role of OMPs in immunity to salmonellosis (Kuusi et al., 1979; Bhatnagar et al., 1982), a complete study of their involvement in humoral and cellular aspects of the immune response is lacking. Recent Studies have shown that porins (Aslam et al., 2012; Saxena et al., 2012) and also novel non-porin OMP from the outer membranes of S. Typhi evokes humoral and cell-mediated immune responses, confers protection and provides a promising target for the development of candidate vaccine against typhoid (Hamid and Jain, 2008; 2010).

The development of a number of important vaccines and immunotherapies has been stymied by the inability to induce appropriate immune responses in the recipient. To circumvent such adversities, various approaches of immunological manipulations, either alone or in combination
with chemotherapy and vaccination has been explored. Now, adjuvants are being used as essential components of new subunit and killed vaccines that boost the immune responses to an antigen creating protective immunity in the vaccinated individual, to control a range of medical problems including infectious disease, cancer, neurological and addictive disorders. Recent advances in vaccine development and in particular, the increasing use of recombinant subunit and synthetic vaccines makes the need for adjuvants all the more acute. Use of well-defined recombinant protein in combination with appropriate adjuvant is more likely to overcome many limitations and provide species or strain specificity. Better technological possibilities combined with increased knowledge in related fields such as immunology and molecular biology allows for new vaccination strategies (Nasser et al., 2002; Charles et al., 2010).

Hence, in the background of above facts the main objectives undertaken for my PhD. work were:

1. Cloning and Expression of the gene for 49-kDa protein from outer membranes of Salmonella enterica serovar Typhi
2. Immunological Evaluation of recombinant protein vis-à-vis natural protein.
3. Conjugation of the protein with adjuvants to further potentiates its immunogenecity.
4. Formulation of a conjugate vaccine against typhoid.