Immunoaffinity-based mass spectrometric characterization of immunoreactive proteins of *Salmonella* Typhi

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**Abstract**

*Salmonella* Typhi, a human-restricted Gram negative enterobacteriaceae, is the causative agent of typhoid fever in human being. The available serodiagnostic tools for the diagnosis of typhoid fever lack sensitivity and/or specificity. This study aimed to identify the immunoreactive proteins of *S*. Typhi that could help to develop improved diagnostic tools. Here, we performed immunoaffinity-based proteomic approach that uses charged columns to retrieve IgG and IgM antibodies from the plasma of typhoid patients followed by capture of *S*. Typhi proteins. These proteins were then characterized by mass spectrometry and bioinformatics tools. Using this approach, we identified 28 immunoreactive proteins of *S*. Typhi, in which 14 proteins were captured by IgG charged column and 4 proteins were captured by IgM column. We also identified 10 proteins (hlyE, rfbH, dapD, argl, glyA, pflB, trxB, groEL, tufA and pepD) captured by both columns. The prediction of antigenicity and immunogenicity resulted that 22 proteins were antigenic while 6 were non-antigenic on the scale of 0.4 threshold value of VaxiJen. These proteins successfully simulated the immune system in silico and in response higher amount of antibodies’ titers were recorded in C-IMMSIM, confirming the immunogenic nature of these proteins. The identified proteins are of diverse nature and functions including those involved in virulence and pathogenesis, energy metabolism, cell development, biosynthesis of amino acids, regulatory functions and biosynthesis of cofactors. The findings of this study would be helpful in the development of improved vaccines and diagnostic tools for typhoid fever.

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1. Introduction

*Salmonella enterica* serovar Typhi (*S*. Typhi) belongs to the family enterobacteriaceae, is a Gram negative human-restricted pathogen that causes typhoid (enteric) fever in human beings, transmitted by fecal-oral route through contaminated water and food. Typhoid is a generalized acute infection of reticulo-endothelial system, gall-bladder and intestinal lymphoid tissues and if left untreated, it may develop severe complications like meningitis and others (Gosh et al., 2011). Each year, about 21.7 million individuals are infected with *S*. Typhi with 600,000 death toll around the globe in which most of the cases, approximately 80 %, are reported from Asia, particularly from Indian subcontinent (Crump and Mintz, 2010). International Vaccine Institute, in 2010, has reported 11.9 million cases and 129,000 deaths due to typhoid in low and middle-income countries (Mogassale et al., 2014). Pakistan is among the typhoid endemic countries with incidence rate of 451.7 cases per 100,000 populations per year (Crump et al., 2010).

Currently, the available commercial vaccines for typhoid fever provide 50 % to 75 % affective for 2 to 5 years (Levine et al., 1999). The main dilemma, in the management of typhoid fever in the country, is the available poor sero-diagnostic tools with poor sensitivity and/or specificity (Olsen et al., 2004). Identification of immunogenic/immunoreactive proteins of *Salmonella* Typhi expressed during typhoid infection would help in development of...
better sero-diagnostic tools with improved sensitivity and/or specificity.

Many studies have demonstrated that S. Typhi infection can stimulate both cellular and humoral immune responses (Lundgren et al., 2009; Parry et al., 2002; Jones and Falkow, 1996). Being a facultative intracellular pathogen, both antibody-mediated and cellular immune responses can play role in restricting and removing infection of S. Typhi. Previous studies, conducted on antibody responses during typhoid infection, largely focused on few number of antigens, like Vi antigen, H antigen and O antigen (Jones and Falkow, 1996; Kirkpatrick et al., 2005). Despite this, a limited data is available on the immune responses against other specific antigens during the infection of wild-type S. Typhi in humans.

Previously, immunoaffinity-based approaches have been used for the identification of immuno reactive proteins and screening protein libraries of the pathogens (Harris et al., 2006; Rollins et al., 2005; Kudva et al., 2006). Other techniques, like in vivo-induced antigen technology (IVIAT) investigating antigens of S. Typhi during typhoid infection in humans (Rollins et al., 2005) and proteomic-based expression library screening (PELS) identifying antigens produced by Escherichia coli using antibody-charged columns and mass spectrometric analysis (Kudva et al., 2006) were used for the said purpose.

With a modification in the existing immunoaffinity approaches, here we directly screen the total cell proteins of the pathogen of interest by antibody-charged columns. Firstly, we recovered the antibody IgG and IgM fractions from plasma of typhoid-confirmed patients and fixed them on the columns and then total cell proteins of S. Typhi were run on the charged columns to capture the immunogenic proteins. The captured proteins were eluted, processed for mass spectrometry and characterized through bioinformatics tools to get further understandings of the immunogenic antigens of S. Typhi expressed during typhoid infection in humans.

2. Materials and methods

2.1. Bacterial strain and protein extraction

The study was approved from the university’s ethical committee (Ref. No. KUST/Ethical Committee/18/13). We used the ten molecularly confirmed S. Typhi strains obtained from blood samples of ten typhoid positive patients with current active infection (diagnosed as IgM/IgG positive by Typhidot) in our previous study (unpublished data). To get the maximum protein expression profile before applying bacterial lysate to columns charged with antibody, S. Typhi was grown separately to mid-log and then to stationary phase on Luria broth (LB) medium and on minimal media containing low-magnesium (10 µM MgCl2) at 37 °C. The low-magnesium condition stimulates Phop regulon that help in intracompartmental survival of bacteria (Charles et al., 2009); this condition was selected for maximum antigens’ expressions, mimicking the in vivo environment during typhoid infection in human. Total cell proteins were extracted by centrifugation followed by 6X Leamlin Buffer method containing 1.5 ml of 1 M Tris HCl (pH 6.8), 0.6g SDS, 3 ml glycerol, 0.468g DTT, 30 mg bromophenol blue and ddH2O for SDS PAGE. The concentration of proteins was measured by NanoDrop (DeNovix) using Protein A280 software of DS-11 Spectrophotometer with known BSA protein standard (Thermo Scientific™ Catalog number: 23209). While distilled water was used as blank sample at the start and in the middle of measurement to maintain the performance and accuracy. The overall immunoaffinity-based proteomics approach, we followed in this study, is depicted in the workflow chart in Fig. 1.

2.2. Proteins confirmation on 1D SDS PAGE

Prior to immunoaffinity and mass spectrometry, we confirm the presence and purity of proteins in cell lysates by loading them on one dimensional Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (1D SDS PAGE) of Mini-PROTEAN System (Bio-RAD Catalog# 165–8000) as per render instructions. Briefly in the procedure, 15 µl from each samples, containing equal amount of proteins mixed with loading buffer (Bio Rad catalog #161–0738) in ratio 1:1, were loaded on 12 % Mini-PROTEAN® TGX™ Prestac Protein Gel (Bio Rad catalog #4561043) and run for 45 min at 200 V having 1X SDS-PAGE running buffer (Bio Rad catalog #161–0732). After the run, gel was washed with milliQ water and stained overnight in Coomassie Brilliant Blue R-250 staining solution (Bio Rad catalog #161–0436). Then gel was destained in Coomassie Brilliant Blue R-250 (Bio Rad catalog #161–0438) destaining solution until clear bands were appeared on the gel.

2.3. Plasma preparation and enrichment of IgG and IgM fractions

Plasma, from ten typhoid positive patients with current active infection (diagnosed as IgM/IgG positive by Typhidot), was pooled together making a total of 5 ml volume plasma sample. The pooled plasma sample was diluted by 1:1 with freshly prepared sterile PBS of pH 7.4. The IgG and IgM fractions from plasma were separately recovered by using HiTrap™ protein G high-performance (HP) column (Merck® Germany, GE17–0404–01) and HiTrap® IgM Purification HP column (Merck® Germany, GE17–5110–01) respectively, as per manufacturers’ instructions with slight modifications. In the procedure, pooled plasma were diluted by ratio 1:4 in binding buffer containing 0.02 M Sodium phosphate buffer of pH 7.0 and loaded on protein G and IgM columns separately through syringe that were equilibrated with binding buffer of ten volumes. IgG and IgM antibodies present in the plasma bind with protein G and protein M respectively but no other immunoglobulin isotypes can bind. After washing with binding buffer, the bounded IgG and IgM polyclonal antibodies (pAbs) were separately eluted by 4 ml elution buffer composed of 0.1 M Glycine of pH 2.7 into tubes containing 200 µl of 1 M Tris–HCl of pH 9.0. The affinity-purified IgG and IgM pAbs were quantified through a nomograph followed by preparation for coupling.

2.4. Coupling of IgG and IgM pAbs to HiTrap NHS-activated HP columns

Polyclonal antibodies (pAbs) were affinity-purified from pooled cultured-confirmed humans’ sera as described above. The affinity-purified pAb fractions dialyzed at 4 °C against PBS of pH 7.4 overnight followed 4 h’ dialysis through dialysis membrane against coupling buffer containing 0.2 M NaHCO3, 0.5 M NaCl of pH 8.3 with molecular weight cut-off of 3500 Da. The affinity-purified IgG and IgM pAbs were coupled by amine groups separately as per render protocol with few modifications. Briefly, after removing isopropanol, the HiTrap N-Hydroxysuccinimide (NHS)-activated HP columns (Merck® Germany GE17–0716–01) were standardized with coupling buffer of 10 column volumes. Affinity-purified IgG and IgM pAbs were gently loaded separately on columns by syringe followed by attachment of another syringe to the outlets of columns for recirculation through the columns at room temperature for 30 min. The active groups, not coupled to columns, were quenched by 10 ml of 1 M tris (hydroxymethyl) aminomethane of pH 9.0. The nonspecifically bounded pAbs were eluted by 10 ml of 1 M acetic acid. We rinsed the immobilized IgG and IgM pAbs columns (charged columns) with 10 ml deionized water for removing quencher and eluant.
2.5. Capture of S. Typhi proteins

The “Charged” columns were calibrated with 0.2 % nonionic detergent n-octyl β-D-glucopyranoside (NOG) and 10 column volumes binding buffer containing PBS of pH 7.4. S. Typhi total cell proteins were diluted in 20 ml of PBS, 0.2 % NOG consisting two times concentration of Complete protease inhibitor mixture (Roche Diagnostics) followed by separately loading via syringe onto charged columns with a dictated gravity flow rate of approximately 0.75 to 1 ml/minute. Then columns were rinsed by 20 volumes of loading buffer for the removal of loosely bounded proteins followed by elution of specifically bounded proteins into 15-ml Falcon tubes having 0.5 ml ammonium hydroxide through 10 ml of 1 M acetic acid. To get the maximum number of captured S. Typhi proteins, the process was repeated three times. To assess nonspecific binding, cell lysates were passed through “uncharged” columns that had been blocked but not being coupled with antibodies having quenched active groups. Each of the eluted proteins, captured by IgG or IgM pAbs, were pooled separately, concentrated with a spin filter and stored at −70 °C until used.

2.6. Sample preparation for mass spectrometry

The eluted proteins from both groups were measured by Protein A280 of Nano-Drop DS-11 Spectrophotometer (DeNovix). Eluted protein samples (200 µg) from each group were separately prepared and digested (out gel digestion). Briefly, protein samples of 200 µg were reduced by 10 mM Dithiothreitol for 45 min at 56 °C followed by 30 min’ dark alkylation with 55 mM iodoacetamide at room temperature, then 4 times diluted with 50 mM ammonium bicarbonate followed by addition of 2 µg trypsin (Sigma-Aldrich, Steinheim, Germany) with a ratio of 1:100 (w/w). The digestions were incubated at 37 °C for 16 h. All the samples were filtered via centrifugal filters with 10 kDa cut-off (VWR International, West Chester, PA, USA) for 10 min at 10,000 × g.

2.7. Mass spectrometry

Digested protein samples in trypsin were processed to make the concentration equal (1 µg/µL) for mass spectrometry. For supporting retention time alignment; synthetic indexed retention time (iRT) peptides (Biognosys, Schlieren, Switzerland), within each data files, were spiked onto all digestions at 1:25 (v/v) in which each digest contained 1 µg/µL protein. The 5 µl samples were processed using a TripleTOF 6600 mass spectrometer (AB/Sciex, MA, USA) which was coupled with Eksigent NanoLC 415 system and DuoSpray Ion Source controlled by Analyst TF 1.7.1 software. For MS parameters: the ion spray was set to 5000 V, curtain gas to 30psi, ion source gas was set to 1 of 25psi, and positive scheduled MRM mode was set to 1 s targeted scan time on the basis of observing actual retention times for each peptide followed by monitoring window of 4 min around the observed RTs.

2.8. Peptide identification and statistical analysis

The data were exported for further statistical analyses for peptide identification in the samples through different bioinformatics tools. The raw scan files of Analyst software were searched via ProteinPilot 5.0.1 search engine by interrogating UniProtKB Salmonella typhi reference proteome database (https://www.uniprot.org/), downloaded on September 23th 2021 consisting 17,000 protein entries to which we added the iRT peptide sequences. The ProteinPilot search parameters were set as: in fixed modification, iodoacetamide carried out Cys alkylation; trypsin in digestion; TripleTOF 6600 mass spectrometer was the instrument; search effort by thorough ID; greater than0.05 was confidence score equals 1 % FDR at peptide level. The group files generated were exported to Skyline.
version 3.7 and used in SWATH data processing as ion reference library.

In Skyline, for SWATH data analysis, the appropriate transition and peptide settings were set to following: MS/MS filtering was set to DIA; product mass analyzer was TOF; isolation scheme was set to SWATH (VW 100) and 30,000 was the resolving power. Then from the imported iRT peptides query parameters in Skyline, over the measured retention times (RTs), a linear regression was made to check the compatibility of iRTs. The files of SWATH data were investigated against spectral library in which the fragment signals and peptide were checked manually and refined by peak selection. Eventually, a custom-defined report consisting protein SwissProt ID, gene locus, protein name, protein description and protein(s) bounded by IgG, IgM or both was generated.

2.9. Functional analysis by bioinformatics tools

The accession numbers of all immunoreactive proteins identified by immunoaffinity-based mass spectrometry were given to the STRING database (https://string-db.org/cgi/) (January 2022) to determine the protein–protein interaction (PPI) and their functional partners. Moreover, these proteins were subjected to different databases and bioinformatics tools such as GeneCodis (https://genecodis.genyo.es/), Cytoscape (https://www.cytoscape.org/) and KEGG (https://www.genome.jp/kegg/kegg4.html) and were categorized on the basis of their biological and molecular functional analysis, protein–protein interactions, cellular component localization and metabolic pathways.

These proteins were also further subjected to Vaxijen server V 2.0 (https://www.ddg-pharmfac.net/vaxijen/Vaxijen/Vaxijen.html) to predict the computational based antigenicity of the identified immunoreactive proteins on the scale of threshold value 0.4 in the Vaxijen server. We also subjected our selected eight immunoreactive proteins as antigens to the antigen-based simulator, the Computational-Immune Simulator (C-IMMSIM) online server (https://kraken.iac.rm.cnr.it/C-IMMSIM) to predict the immunogenicity of the identified proteins.

3. Results

3.1. Proteins on 1D SDS PAGE

Total cell proteins extracted from Salmonella Typhi culture were run parallel with a protein marker, Precision Plus Protein Dual Color Standard (Bio Rad catalog #1610374), gave a broad range (in terms of molecular weight) of bands containing proteins of low as well as high molecular weight (10–250 kDa) on SDS PAGE gel as shown in the Fig. 2. This also confirmed the purity and presence of proteins in the lysate samples prepared for immunoaffinity and mass spectrometry for further characterization.

3.2. Proteins identified by Immunoaffinity-based mass spectrometry

By using this immunoaffinity-based proteomic approach and mass spectrometry, we identified a large number of proteins captured by either IgG, IgM or both as shown in Fig. 3. However, after refinement and applying afore mentioned statistical parameters,
### Table 1
Mass spectrometric characterization of *S.* Typhi proteins captured by serum antibodies.

| Protein group                  | SwissProt ID | Protein name | Gene locus | Protein description                                      | Bound by Immunoglobulin |
|--------------------------------|--------------|--------------|------------|---------------------------------------------------------|-------------------------|
|                                |              |              |            | G            | M            |                  |
| Virulence and pathogenesis     | Q8Z727       | hlyE         | STY1498    | Hemolysin E                                           | X                       |
|                                | Q8Z6B2       | pagC         | STY1878    | Outer membrane invasion protein                       | X                       |
|                                | Q8Z6A7       | cdtB         | STY1886    | Cytolethal distending toxin subunit B homolog          | X                       |
|                                | Q8Z6A4       | pltA         | STY1890    | Putative pertussis-like toxin subunit                  | X                       |
| Cell envelope                  | Q8XH17       | ompX         | STY0872    | Outer membrane protein X                               |                         |
|                                | Q8Z750       | ompA         | STY1091    | Outer membrane protein A                               | X                       |
|                                | Q8Z5I6       | rfbH         | STY2300    | Putative dehydratase RfbH                              | X X                     |
|                                | P67913       | hldD         | STY4085    | ADP-c-glycero-a-manno-heptose-6-epimerase              | X                       |
| Amino acid biosynthesis        | Q8Z0A8       | dapB         | STY0236    | 2,3,4,5-Tetrahydropyridine-2,6-dicarboxylate           | X X                     |
|                                | Q8Z381       | ilvC         | STY3648    | Ketol acid reductoisomerase                            | X                       |
|                                | Q8Z123       | argI         | STY4807    | Ornithine carbamoyltransferase                         | X                       |
|                                | P66039       | ribH         | STY0456    | 6,7-Dimethyl-8-ribityllumazine synthase                | X                       |
|                                | P0A2E2       | glyA         | STY2802    | Serine hydroxymethyltransferase                       | X X                     |
|                                | Q8Z0E9       | aceF         | STY0176    | Dihydrolipoamide acetyltransferase component          | X                       |
|                                | Q8XCV7       | pflB         | STY0973    | Formate acetyltransferase 1                           | X                       |
|                                | P0A1F1       | gapA         | STY1825    | Glyceraldehyde 3-phosphate dehydrogenase              | X                       |
|                                | Q8Z5J3       | gnd          | STY2290    | 6-Phosphogluconate dehydrogenase, decarboxylating      | X                       |
|                                | P64077       | eno          | STY3081    | Enolase                                               | X                       |
|                                | P65703       | fba          | STY3226    | Fructose 1,6-bisphosphate aldolase                    | X                       |
|                                | Q8Z0U3       | degC         | STY4918    | Deoxysubphosphate aldolase                            | X                       |
|                                | Q8XF12       | trxB         | STY0956    | Thioredoxin reductase                                  | X X                     |
|                                | P0A1D4       | groEL        | STY4690    | 60-kDa chaperonin                                      | X X                     |
|                                | P0A1H6       | tufA         | STY4353    | Elongation factor Tu                                  | X X                     |
| Energy metabolism              | Q8Z3E9       | sspA         | STY3523    | Stringent starvation protein A                        | X                       |
|                                | Q8Z9R1       | dnaK         | STY0012    | Chaperone protein DnaK                                | X                       |
|                                | Q8Z934       | pepD         | STY0361    | Aminoacyl-histidine dipeptidase                        | X X                     |
|                                | Q8Z1Y0       | bfr          | STY4355    | Bacterioferritin                                      | X                       |
| Cofactors biosynthesis         |              |              |            | G: Bound by immunoglobulin IgG, M: Bound by immunoglobulin IgM, X: Presence. |                  |
| Regulatory function            |              |              |            |                                                         |                  |
| Transport and binding          |              |              |            |                                                         |                  |
we identified 28 proteins of Salmonella Typhi captured by IgG and IgM antibody fractions recovered from the plasma of S. Typhi bacteremia-confirmed patients. These captured proteins have reacted to the antibodies responses by binding with IgG, IgM or both serotypes as shown in Table 1. The captured-identified proteins were of diverse nature and functions and were categorized into groups based on the functions they perform. Among the 28 identified proteins, most of the proteins were involved in energy metabolism, few proteins including hlyE and pagC were involved in cellular processes, virulence and pathogenesis while proteins like ompX, ompA and rfbH that help in cell envelope synthesis were among the captured-identified proteins. We also identified heat shock proteins dnaK and groEL and other proteins like pltA and cdtB which were involved in virulence and pathogenesis.

3.3. Comparison of proteins bound by isotype

We identified a total of 28 proteins, captured on both IgG and IgM columns, through mass spectrometry. The captured proteins were of diverse nature, involved in different functions as shown in Fig. 4A. Among the total, we identified 14 proteins bound on IgG column and 4 proteins bound on IgM column. We also observed that among the total 38 captured proteins, there were 10 proteins which were bounded to both IgG and IgM columns as shown in Fig. 4B. These were hlyE, rfbH, dapD, argl, glyA, pfIB, trxB, groEL, elongation factor protein tufA and regulatory protein pepD as summarized in Fig. 5.

3.4. Functional analysis and interactions of immunoreactive proteins

The functional analysis of captured proteins in the STRING, GeneCodis and Cytoscape databases showed that these proteins had a higher-than-expected degree of interconnectivity, protein–protein interactions and were enriched in their characteristic biological and molecular functions such as aceF, gapA, eno, pagK and fba are involved in glycolytic process and dapD, glyA, argl and ilvC are involved in the amino acids biosynthetic process. The proteins dnaK and groEL give cellular response to the unfold proteins while hlyE is involved in hemolysis in other organisms as shown in Fig. 6A. Molecularly, most of the identified proteins such as ompX, aceF, sspA, pepD, ompA, tufa, eno, groEL, gapA and hldD are involved in protein binding. The proteins gnd, ilvC and hldD are involved in NADP binding while proteins dnaK and pgk are involved in ADP binding as shown in the Fig. 6B.

With respect to cellular-component localization, most of the proteins such as argl, tufa, aceF, dnaK, trxB, dapD, gapA, pfIB, glyA, hldD, pepD, gnd, ribH etc were found in cytoplasm and in the cytosol while some proteins like ompA, ompX and hlyE were in the
outer membrane as shown in the Fig. 7A. While the analysis of these proteins in the KEGG pathway resulted that dnaK, groEL and eno are involved in RNA degradation, gapA, aceF, pgk and fba are involved in glycolysis. The proteins agrI, ilvC and dapD help in the biosynthesis of amino acids while gndA, pgk and deoC are involved in pentose phosphate pathway as shown in Fig. 7B.

3.5. In silico prediction of antigenicity and immunogenicity of immunoreactive proteins

The antigenicity of the identified 28 immunoreactive proteins was predicted by Vaxijen v 2.0. The analysis resulted that among the total 28 immunoreactive proteins, 22 proteins were antigenic scoring more than 0.4 while 6 proteins rfbH, hldD, hlyE, ilvC, gnd, and bfr were found as non-antigenic as they did not cross the threshold value of 0.4 as shown in the Fig. 8.

The selected immunoreactive proteins were subjected to the C-IMMSIM server to check immune responses against them. The analysis resulted that all these proteins successfully simulated the immune system, contained B and T cell receptor epitopes as well as higher amount of antibodies titered were observed against these proteins. This means that if any of these proteins is exposed to immune system, the system will respond by producing antibodies from day 5 which will last for 35 days against these antigens. Among these immunogenic proteins, higher antibodies titer (10,000 to 16,000) were recorded against pflB, trxB, glyA and rfbH when these proteins were injected as antigens to C-IMMSIM as shown in the Fig. 9.

4. Discussion

Diagnosing typhoid fever in low-and meddle-income countries is a major health problem due to the available rapid
sero-diagnostic tools which lack sensitivity and/or specificity. Although these diagnostic kits are not recommended by National Institute of Health of Pakistan (Mehmood et al., 2015; NIH, 2018) and World Health Organization (WHO, 2003) but still widely used across the country as the standard techniques like blood culture, PCR and ELISA are time consuming, expensive and require skills.

![Graph: Vaxijen Threshold](image)

Fig. 8. Prediction of antigenic and non-antigenic immunoreactive proteins in Vaxijen.

![Graphs: Amount of proteins (antigens) vs antibodies' titers vs number of days](image)

Fig. 9. Amount of proteins (antigens) vs antibodies' titers vs number of days.
and sophisticated instruments. So, there is a definite need to work on the improvement and development of sero-diagnostic tools for typhoid fever with better sensitivity and specificity.

A number of immunoaffinity based screening approaches like IVIAT and PELS have been developed to investigate the interactions of host and pathogen during human infection (Harris et al., 2006; Kudva et al., 2006; Hang et al., 2003). Here, using one such advance immunoaffinity based proteomic approach, we identified 28 proteins of Salmonella Typhi captured by various isotypes of antibodies recovered from typhoid-confirmed patients in Pakistan. In comparison to other approaches used, our approach has the edge to use directly pathogen of interest to elucidate antibody responses and characterizing them by isotype class using S. Typhi as the protein pool for the immunoaffinity modification of PELS approach (Kudva et al., 2006).

In our study, to get the maximum number of S. Typhi proteins, we cultured the bacteria under low magnesium-growth condition that induced PhoP regulon to mimic the conditions that encountered during intracellular survival of pathogen in human infection (Charles et al., 2009). We also assessed the immunoreactive proteins of S. Typhi captured by columns charged with antibodies recovered from infected patients with the columns blocked but not charged with the antibodies as optimal negative control. Our identification of proteins along with the previously reported known immunoreactive proteins of Salmonella Typhi like HlyE, GroEL and PagC supported the validity of our followed approach (Faucher et al., 2009; Charles et al., 2010).

Hemolysin E (HlyE) and/or cytolysin A (ClyA), being a pore-forming toxin supporting the cytotoxicity and epithelial cells invasion that also influences the intracellular survival of bacteria during human infection (Faucher et al., 2009; Fuentes et al., 2008). PhoP regulon controls the expression of HlyE and shares about 90% amino acid similarity with ClyA in K-12 strain of Escherichia coli (Oscarsson et al., 2002). In both human and murine macrophages, ClyA is involved in the cytotoxic activity (Lai et al., 2000). Initially, the HlyE gene was thought to be unique in S. Typhi and Paratyphi A genome (Oscarsson et al., 2002; von Rhein et al., 2009) but a recent study suggested that it is also present in other Salmonella serovars that can cause humans’ systemic infection (Fuentes et al., 2008). PagC, which expresses under PhoP regulon control, is identified as immunoreactive in our study. In another study, PagC was identified in the IVIAT screening of immunogenic proteins in S. Typhi infection, demonstrated a specific antibody response in the sera of infected patients in Bangladesh (Harris et al., 2006). We also identified GroEL and DnaK, the heat shock proteins, as immunoreactive in our study.

Generally, the heat shock proteins are often considered as immunoreactive proteins and identified as immunoreactive against antibody responses in previous studies (Faucher et al., 2009; Charles et al., 2010). Proteins putative dehydratase (RfbH), thioredoxin reductase (TrxB) and elongation factor Tu (TuA) found immunoreactive against both isotypes responses were also identified as immunogenic in a previous study (Meyer et al., 2012).

Our study has limitations. The present study did not precisely confirm the immuno-reactivities of the identified proteins through other confirmatory immuno-assays like Enzyme Linked Immuno Sorbent Assay (ELISA), microarrays, western blotting etc. However, using advance approaches like immunoaffinity based-proteomic, mass spectrometric analysis and bioinformatics tools, the study successfully identified a large number of immunoreactive Salmonella Typhi proteins including some proteins that are known to be immunogenic in S. Typhi infection identified in previous studies.

In summary, immunoaffinity based-proteome profiling is a rapid and reliable approach for the identification of proteins in an infected host that can provoke serological immune responses. Using this approach, we identified 28 Salmonella Typhi immunoreactive proteins including some proteins of known immunogenicity that supports our findings and the validity of our approach. We also identified a subset of proteins under Phop regulon control that support the intracellular survival of bacteria, a subset that mainly present in human-specific S. Typhi and S. Paratyphi A genomes however largely absent in other Salmonella species. We identified 10 proteins that can elicit both IgG and IgM responses as these were captured by both IgG and IgM charged columns. The proteins, we identified as immunoreactive in our study, are of diverse nature and function including a large number supporting energy metabolism, protein biosynthesis, iron homeostasis, some are outer membrane components while a significant subset is involved in intracellular survival and virulence. Our findings may facilitate cellular immunological studies that target antigens of Salmonella Typhi. Additionally, our identification of proteins that reacted to both IgG and IgM responses, are of potential diagnostic significance demanding further work in the development improved of diagnostic tools with better sensitivity and specificity.

Taken together, all these analyses resulted that our identified proteins have highly antigenic as well as immunogenic properties.
and could be used as potential candidates in the development of vaccines as well as serodiagnostic tools for typhoid fever.

5. Data availability statement

All data generated or analyzed during this study are included in this article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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