




RESEARCH ARTICLE

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# Expression of Recombinant OmpA (rOmpA) and *In vitro* Validation of Antibody Mediated Cross Reactivity among the *Enterobacteriaceae* Pathogens

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## Abstract

*Enterobacteriaceae* pathogens such as *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Proteus* sp., and *Klebsiella pneumoniae* cause a wide range of gastrointestinal and other mucosal infections. These bacteria acquire antibiotic resistance very quickly and evolve into multi-drug resistant strains thereby making the treatment very difficult. The outer membrane proteins (OMPs) in *Enterobacteriaceae* are potential vaccine candidates owing for their high immunogenicity and amino acid conservation. The OmpA is one such protein which need to be investigated for the development of a potential subunit vaccine against multiple infections casued by the pathogens of *Enterobacteriaceae*. To investigate this, we expressed and purified the highly conserved OmpA of *S. typhimurium* and studied the antibody mediated cross reactivity with the other *Enterobacteriaceae* pathogens. This was validated through dot ELISA performed with the hyperimmune sera raised against rOmpA of *S. typhimurium*. We further analyzed the sequence of OmpA protein and clearly understood that the B-cell epitopes in the protein are highly conserved are responsible for cross reactivity among the *Enterobacteriaceae* pathogens. This work led to findings that provide strong evidence for the application of OmpA in broad-spectrum subunit vaccine against enteric infections.

**Keywords:** *Enterobacteriaceae*, OMPs, OmpA, Antibodies, B-cell Epitopes, Cross Reactivity

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## INTRODUCTION

*Enterobacteriaceae* pathogens namely *E. coli*, *K. pneumoniae*, and members of the genus *Salmonella*, *Shigella*, *Proteus* cause serious gastrointestinal infections, bacteremia, and other mucosal infections.<sup>1-5</sup> The *E. coli*, *Salmonella* and *Shigella* are also major foodborne pathogens transmitted by fecal-oral route. Primarily, these pathogens cause foodborne outbreaks globally by contaminating food and drinking water.<sup>1-5</sup> The *E. coli* is an opportunistic pathogen and has evolved into several serotypes namely enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC) and diffuse adherent *E. coli* (DAEC).<sup>6</sup> These pathogenic forms of *E. coli* cause serious gastrointestinal infections, sepsis, urinary tract infections (UTIs), meningitis in children and adults with poor immunity.<sup>7</sup> *Shigella* is phylogenetically a very close relative of *E. coli* in *Enterobacteriaceae* family, and it is associated with severe gastrointestinal infections even in low CFU count.<sup>8,9</sup> *Shigella* includes four species i.e., *S. boydii*, *S. dysenteriae*, *S. flexneri* and *S. sonnei*. These bacteria cause an acute intestinal infection called shigellosis.<sup>10</sup>

*Salmonella* is another prominent pathogenic member of *Enterobacteriaceae* that causes diarrhea, typhoid, paratyphoid, salmonellosis, and gastroenteritis etc.<sup>11</sup> Members of the genus *Proteus* like *P. mirabilis* and *P. vulgaris* cause UTIs and asymptomatic bacteriuria.<sup>12,13</sup> Apart from these pathogens, *K. pneumoniae* is another important pathogen of *Enterobacteriaceae* which is also an opportunistic pathogen that is responsible for nosocomial respiratory and urinary tract infections.<sup>14-16</sup> Also, *K. pneumoniae* is the second most frequent bacteria to cause UTIs and bacteremia.<sup>14-16</sup>

Treatment for these infections has remained a major concern because of their ability to evolve as multi-drug resistant strains over time.<sup>17</sup> In this scenario, vaccination and passive immune therapy are the promising alternatives to control widespread infections by these enteric pathogens.

For the development of any immunoprophylactic agent, selection of the right virulence factor as an antigen is very essential. Previous

reports from our lab and others suggest that outer membrane proteins (OMPs) of *Enterobacteriaceae* as strong immunogenic components in vaccine and passive immune therapies.<sup>18-21</sup> The OMPs comprise of  $\beta$ -barrel structures that are essential in maintaining membrane integrity and selective permeability in gram negative bacteria including *Enterobacteriaceae*.<sup>22-24</sup> These OMPs are highly immunogenic and are important virulent factors during infection. Furthermore, studies have shown that the OMPs in *Enterobacteriaceae* are highly conserved with greater amino acid sequence homology which will vouch for cross-protection against wide range of *Enterobacteriaceae* infections.<sup>18-21</sup> The OmpA is such a strong immunogen which elicited strong immune response against *Salmonella* and *E. coli* in mouse models.<sup>25,26</sup> However, there is lack of information on the nature of epitope conservation and antibody cross reactivity against this antigen among the pathogenic *Enterobacteriaceae* members. Therefore, our study will really help the current vaccinologists in developing a broad-spectrum epitope-based vaccine against *Enterobacteriaceae* pathogens simultaneously.

In the present study, we expressed the highly conserved OmpA protein of *S. typhimurium* and carried out several experiments to understand the cross reactivity by polyclonal antibodies against OmpA of the standard strains used in the study. The antibodies generated against the OmpA of *S. typhimurium* has shown cross reactivity against the other *Enterobacteriaceae* pathogens such as *E. coli*, *Salmonella*, *Shigella*, *Proteus* and *K. pneumoniae*. Sequence and immunoinformatics analysis of OmpA has revealed the presence of several conserved B-cell epitopes that are responsible for the cross reactivity by polyclonal antibodies.

## MATERIALS AND METHODS

### Bacterial strains, plasmids, and growth conditions

All the standard cultures used in this study were enlisted in Table 1. The bacteria were cultured overnight in Luria Bertani (LB) broth at 37°C. Dehydrated growth media and antibiotics were from HiMedia labs, Mumbai, India. Isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) was purchased from Thermo Scientific, India. The

*S. typhimurium* OmpA was cloned into pET22b vector between EcoRI and HindIII restriction sites using GeneRunner software and outsourced from Eurofins, Bengaluru, India. The DH5α and BL21 (DE3) pLys strains of *E. coli* were used as cloning and expression hosts to express rOmpA of *S. typhimurium*. These strains were purchased from Invitrogen, USA. Anti-6X histidine mouse monoclonal antibodies and the HRP-conjugated anti-mouse rabbit secondary antibodies were from Sigma Aldrich, India.

### Transformation, expression, and purification

The recombinant OmpA gene from *S. typhimurium* ATCC 14028 was cloned in pET22b vector and sequenced at Eurofins Analytical Services, Bengaluru, India. The recombinant plasmid was transformed into chemically competent *E. coli* DH5α host cells by heat shock at 42°C. The transformants were screened by colony PCR using T7 promoter and terminator primers with appropriate controls as reported elsewhere.<sup>27,28</sup> One of positive colonies was sub cultured and the plasmid DNA was isolated from the transformed cells for further transformation into *E. coli* BL21 (DE3) pLys expression host cells. The overnight culture was inoculated into 200 mL of LB broth (tryptone=2g; yeast extract=1g; NaCl=2g; pH=7.0) containing 50 µg/mL of ampicillin and 35 µg/mL of chloramphenicol and grown at 37°C overnight. After reaching desired O.D<sub>600</sub> value of ~0.6, the rOmpA gene was induced with 1mM of isopropyl β-D-galactopyranoside (IPTG) and cultured for another 5 hrs for the efficient protein expression. After this, the cells were harvested, subjected to osmotic shock and the periplasmic extracts were purified using imidazole and Ni-NTA metal affinity chromatography. The eluted protein extracts were pooled and subjected to buffer exchange in 1X sterile PBS (NaCl=137 mM; KCl=2.7 mM; Na<sub>2</sub>HPO<sub>4</sub>=4.3 mM; KH<sub>2</sub>PO<sub>4</sub>=1.47 mM; pH=7.4). The purified rOmpA protein was lyophilized, diluted with 1 X sterile PBS, and quantified by Lowry's assay with BSA standards.<sup>29</sup>

### Immunization

Five-week-old female BALB/c mice were caged in polypropylene cages with rice hulls as bedding material. The mice were provided with food pellets, mineral water from Kent water

purifier and maintained in 12-hr light and dark cycles. Fifty micrograms of purified rOmpA were emulsified with Freund's complete adjuvant and administered sub-cutaneous during the first immunization. Similarly, 50 µg of rOmpA antigen emulsified with Freund's incomplete adjuvant was administered as booster dose during the second and third immunizations on 14<sup>th</sup> and 28<sup>th</sup> day post first immunization. Forty-five days post first immunization, the mice were sacrificed, and blood was harvested by cardiac puncture for serum isolation. After this, Antibody cross reactivity was studied against the *Enterobacteriaceae* strains through dot ELISA.

### Dot ELISA

The standard *Enterobacteriaceae* cultures enlisted in table 1 were cultured overnight and immediately used for determining the polyclonal antibody cross reactivity using dot ELISA. Briefly, cells were harvested from the 500 µL of overnight culture and resuspended in carbonate-bicarbonate buffer (pH 9.2). Then 10 µL of the cell suspension was coated on to nitrocellulose strips and airdried followed by blocking in 5% milk solution for 1 hour at room temperature. The strips were washed with PBST solution 4-5 times and incubated in anti-OmpA polyclonal antibody serum. The unbound antibodies were washed, and strips were further incubated with anti-mouse HRP conjugate secondary antibody, washed, and developed with diaminobenzidine (DAB) tetrahydrochloride solution and 0.003% H<sub>2</sub>O<sub>2</sub>.

**Table 1.** List of Enterobacteriaceae strains used in the study.

Organism/strain	Strain
<i>Salmonella typhimurium</i>	ATCC 14028
<i>Salmonella paratyphi</i>	ATCC 9150
<i>Shigella flexneri</i>	ATCC 9199
<i>Shigella boydii</i>	ATCC 9207
<i>Klebsiella pneumoniae</i>	ATCC 10031
<i>Proteus vulgaris</i>	ATCC 13315
<i>Escherichia coli</i>	ATCC 10536
<i>Salmonella paratyphi</i>	MTCC 735
<i>Salmonella typhi</i>	Gwalior isolate
<i>Salmonella enteritidis</i>	Gwalior isolate

**Table 2.** Percentage similarity index showing the sequence homology of OmpA among the *Enterobacteriaceae* pathogens

	<i>P. mirabilis</i>	<i>P. vulgaris</i>	<i>K. pneumoniae</i>	<i>S. paratyphi B</i>	<i>S. paratyphi A</i>	<i>Styphimurium</i>	<i>S. enteritidis</i>	<i>S. typhi</i>	<i>S. paratyphi C</i>	<i>S. dysenteriae</i>	<i>S. boydii</i>	<i>E. coli</i>	<i>S. flexneri</i>	<i>S. sonnei</i>
<i>P. mirabilis</i>	100.00	92.60	70.86	70.25	70.25	70.25	70.25	70.25	70.25	70.25	70.25	70.86	71.47	71.17
<i>P. vulgaris</i>	92.60	100.00	70.86	70.25	70.25	70.25	70.25	70.25	70.25	70.25	70.25	70.86	71.47	71.17
<i>K. pneumoniae</i>	70.86	70.86	100.00	88.04	88.04	87.73	87.69	88.04	88.04	86.81	86.20	85.89	86.77	86.50
<i>S. paratyphi B</i>	70.25	70.25	88.04	100.00	99.09	99.09	99.09	99.39	99.39	94.85	95.15	93.64	94.53	95.15
<i>S. paratyphi A</i>	70.25	70.25	88.04	99.09	100.00	99.39	99.39	99.09	99.09	94.85	95.15	93.64	94.53	95.15
<i>Styphimurium</i>	70.25	70.25	87.73	99.09	99.39	100.00	100.00	99.70	99.70	95.15	95.15	93.64	94.53	95.15
<i>S. enteritidis</i>	70.25	70.25	87.69	99.09	99.39	100.00	100.00	99.70	99.70	95.14	95.14	93.62	94.53	95.14
<i>S. typhi</i>	70.25	70.25	88.04	99.39	99.09	99.70	99.70	100.00	100.00	95.15	95.15	93.64	94.53	95.15
<i>S. paratyphi C</i>	70.25	70.25	88.04	99.39	99.09	99.70	99.70	100.00	100.00	100.00	99.09	97.27	98.18	98.18
<i>S. dysenteriae</i>	70.25	70.25	86.81	94.85	94.85	95.15	95.14	95.15	95.15	100.00	100.00	97.58	98.48	98.48
<i>S. boydii</i>	70.25	70.25	86.20	95.15	95.15	95.15	95.14	95.15	95.15	99.09	100.00	100.00	99.09	98.48
<i>E. coli</i>	70.86	70.86	85.89	93.64	93.64	93.64	93.62	93.64	93.64	97.27	97.58	100.00	100.00	99.39
<i>S. flexneri</i>	71.47	71.47	86.77	94.53	94.53	94.53	94.53	94.53	94.53	98.18	98.48	99.09	100.00	99.39
<i>S. sonnei</i>	71.17	71.17	86.50	95.15	95.15	95.15	95.14	95.15	95.15	98.18	98.48	98.48	99.39	100.00

### Sequence retrieval and alignment

The complete amino acid sequence of OmpA protein of *S. typhimurium* LT12 strain bearing an accession ID of P02936 was retrieved from Uniprot resource (<https://www.uniprot.org/uniprotkb/P02936/entry>). Later this sequence was used to mine the complete amino acid sequences of OmpA from other *Enterobacteriaceae* such as *E. coli*, *Shigella* sp., *K. pneumoniae* and *Proteus* sp. (Table 2). Multiple sequence alignment (MSA) was performed for these sequences using CLUSTAL Omega tool (<https://www.ebi.ac.uk/Tools/msa/clustalo/>).

### B-cell epitopes

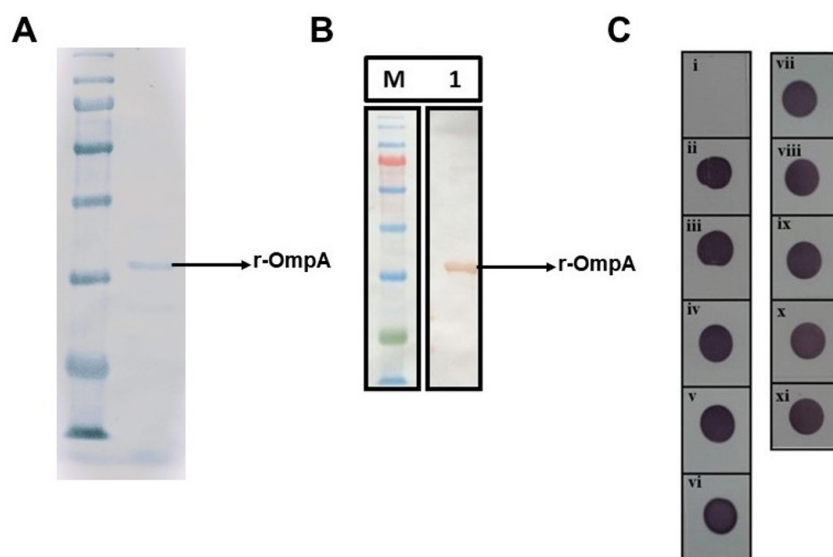
Initially B-cell epitopes were predicted in the core peptide region (21-350 aa) of OmpA of *S. typhimurium*. Then amino acid sequence conservation in the predicted B-cell epitopes of *S. typhimurium* OmpA was identified in sequence alignment file containing OmpA sequences of other *Enterobacteriaceae* pathogens. For the prediction

of B-cell epitopes in OmpA of *S. typhimurium*, we used BepiPred 2.0 (<https://services.healthtech.dtu.dk/services/BepiPred-2.0/>) tool<sup>30</sup> with default parameters.

## RESULTS

### Generation and purification of rOmpA

The IPTG induction of *E. coli* BL21 (DE3) pLys cells carrying rOmpA-pET22b plasmid resulted in expression of a ~ 38-kDa protein which was further purified from the periplasmic extracts through Ni-NTA chromatography (Figure 1a). Then after the pooled eluents were buffer exchanged and purified under native conditions to retain the protein structure for its efficient biological activity *in vivo*. The protein concentration was found to be ~ 1mg/mL which was determined by Lowry's method. The rOmpA protein of *S. typhimurium* also contains 6X His tag at the C-terminal end of the protein. Using the mouse monoclonal antibodies raised against the 6X-His tag, the rOmpA protein



**Figure 1.** Purification of r-OmpA protein and polyclonal antibody mediated cross reactivity towards the Enterobacteriaceae strains. A. SDS-PAGE image showing the purified r-OmpA. B. Western blot image showing the reactivity of anti-6X histidine antibodies to the purified r-OmpA at ~ 38-kDa. C. Cross reactivity of polyclonal antibodies in the hyperimmune sera was determined through dot ELISA. The polyclonal antibodies in the serum were not reactive to i. negative control, but reactive to ii. *Salmonella typhimurium* ATCC 14028, iii. *Salmonella paratyphi* ATCC 9150, iv. *Shigella flexneri* ATCC 9199, v. *Shigella boydii* ATCC 9207, vi. *Klebsiella pneumoniae* ATCC 10031, vii. *Proteus vulgaris* ATCC 13315, viii. *Escherichia coli* ATCC 10536, ix. *Salmonella paratyphi* MTCC 735, x. *Salmonella typhi* Gwalior isolate and xi. *Salmonella enteritidis* Gwalior isolate.

was detected through western blot (Figure 1b). Reactivity of the 6X-His antibodies was observed at the vicinity of 35-kDa band in western blot.

### Cross reactivity by polyclonal antibodies

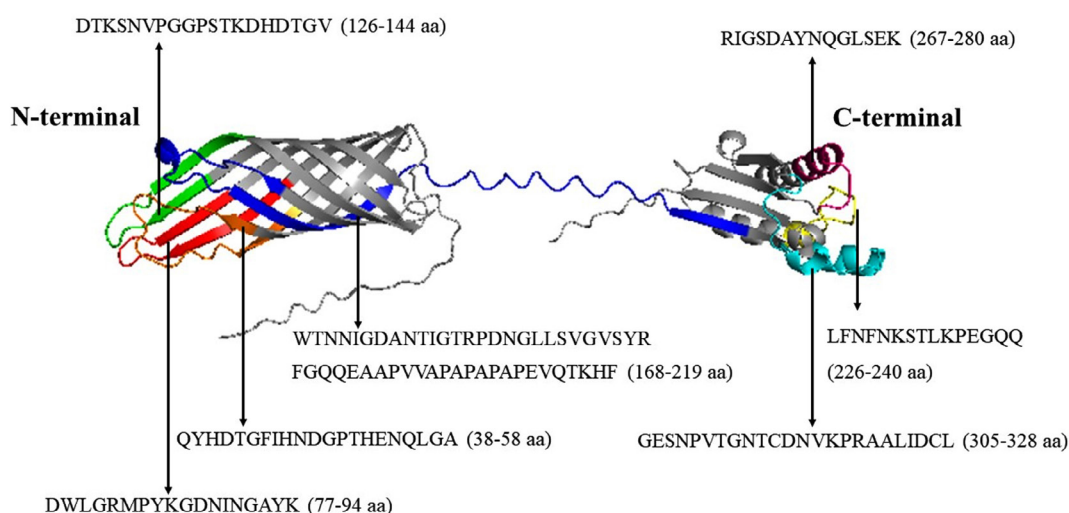
Upon confirming rOmpA through Western blot followed by the successful immunization of rOmpA protein, the mice were sacrificed once the antibody titer reached 1:64000 after the third booster dose. The high antibody titer indicates strong immunogenic potential of OmpA protein. The harvested serum was used for dot ELISA experiments to determine cross reactivity of anti-OmpA polyclonal antibodies against other *Enterobacteriaceae* pathogens. Dot ELISA results showed the cross reactivity of anti-OmpA antibodies against other *Enterobacteriaceae* strains such as *E. coli*, *Shigella* sp., *Proteus* sp., *K. pneumoniae* and other *Salmonella* serovars (Figure 1c). As we expected through our *in-silico* analysis, high degree of amino acid sequence conservation led to the presence of several conserved B-cell epitopes (Figure 2 and 3) which can mediate the cross reactivity by anti-*S. typhimurium* OmpA polyclonal antibodies towards overnight cultures of standard *Enterobacteriaceae* cultures we used in this study.

### Amino acid sequence conservation in OmpA

We performed MSA analysis for all the retrieved amino acid sequences of OmpA from the *Enterobacteriaceae* pathogens that are included in the study. The percent identity matrix of OmpA protein among different pathogens was represented in Table 2. The OmpA protein is highly conserved in the monophyletic group of *Enterobacteriaceae* such as *E. coli*, *Shigella*, *Salmonella* and *K. pneumoniae* sp. (Table 2). Moreover, considerable amount of amino acid sequence similarity is seen in *P. mirabilis* and *P. vulgaris* too which are somewhat distinct from *E. coli*, *Shigella*, *Salmonella* and *K. pneumoniae* (Table 2). Overall, almost ~66% of the amino acid residues in OmpA protein are highly conserved vowing to the existence of conserved B-cell epitopes to which the antibodies can cross react.

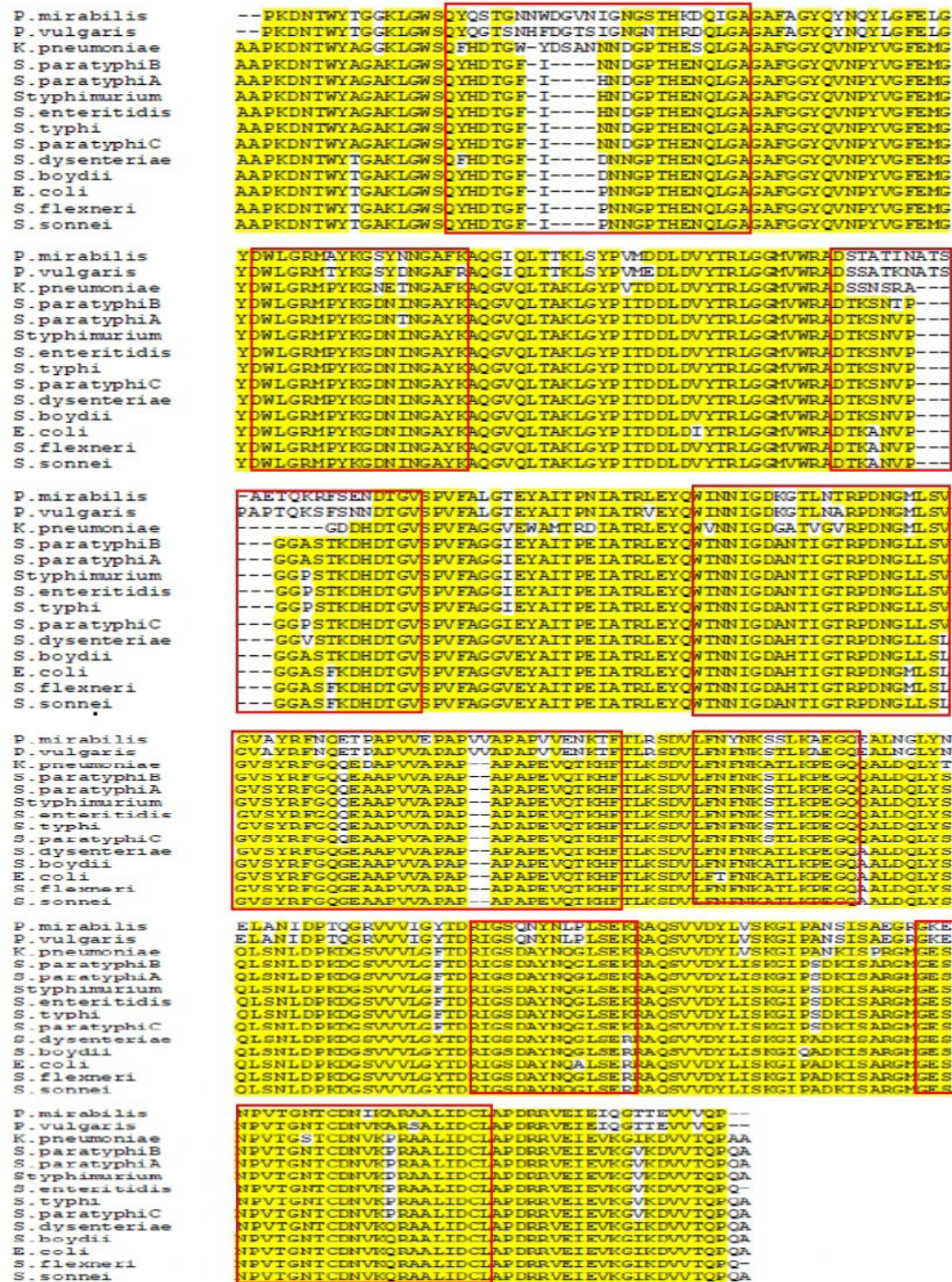
### B-cell epitope conservation in the OmpA

Using BepiPred 2.0 tool, we predicted a total of 7 B-cell epitopes in the OmpA of *S. typhimurium* (Figure 1). These are QYHDTGFIHNDGPTHEQLGA (38-58 aa), DWLGRMPYKGDNINGAYK (77-94 aa), DTKSNVPGGPSTKDHDGTGV (126-144 aa), WTNNIGDANTIGTRPDNGLLSVGVSYSYR FGQQEAAPVVAPAPAPAPEVQTKHF (168-219 aa), RIGSDAYNQGLSEK (267-280 aa), LFNFNKSTLKPEGQQ (226-240 aa), GESNPVTGNTCDNVKPRAALIDCL (305-328 aa)



**Figure 2.** The B-cell epitopes predicted in OmpA of *S. typhimurium*. The B-cell epitopes were highlighted in different colors (orange, red, green, blue, yellow, pink, and cyan, respectively) three-dimensional tertiary structure of OmpA of *S. typhimurium* LT2 strain.





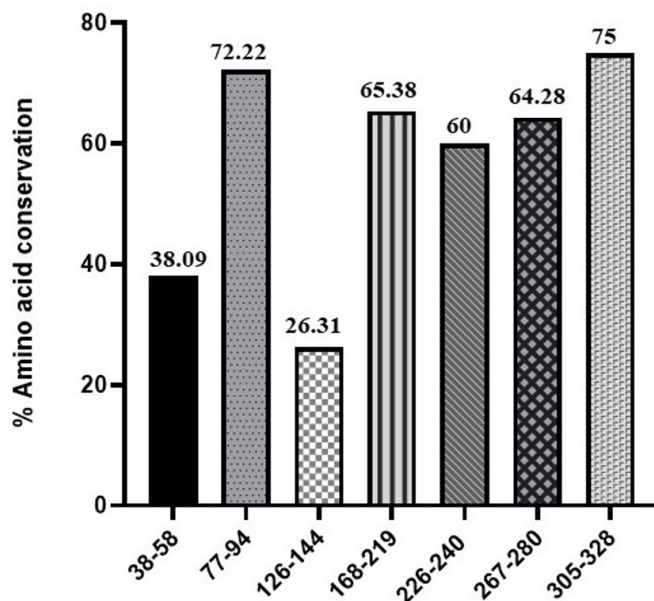
**Figure 3.** Multiple sequence alignment showing amino acid sequence conservation of B-cell epitopes in the OmpA among *Enterobacteriaceae* pathogens. The B-cell epitopes in the OmpA antigen of *S. typhimurium* are highly conserved in the other *Enterobacteriaceae* pathogens such as *E. coli*, *Shigella*, *K. pneumoniae* and *Proteus* sp. This indicates the likelihood of polyclonal antibody mediated cross reactivity among *Enterobacteriaceae* pathogens.

aa), RIGSDAYNQGLSEK (267-280 aa) and GESNPVTGNTCDNVKPRAALIDCL (305-328 aa) respectively (Figure 2). Later, the predicted B-cell epitopes were searched for their amino acid sequence conservation in the alignment of 14 sequences of *Enterobacteriaceae* pathogens we used for analysis (Figure 3). Among all the 7 predicted epitopes, DWLGRMPYKGDNINGAYK (77-94 aa) and GESNPVTGNTCDNVKPRAALIDCL (305-328 aa) are highly conserved with 72.22% and 75% of the amino acids covering all the 14 pathogens (Figure 3 and 4). Whereas three epitopes WTNNIGDANTIGTRPDNGLLSVGVSRYFG QQEAAPVVAPAPAPAPEVQTKHF (168-219 aa), LFNFNKSTLKPEGQQ (226-240 aa) and RIGSDAYNQGLSEK (267-280 aa) are the next highly conserved ones with at least 60% of amino acid residues covering all the 14 sequences (Figure 3 and 4). Only two epitopes QYHDTGFIHNDGPTHEQLGA (38-58 aa) and DTKSNVPGGPSTKDHDGTGV (126-144 aa) are with least amino acid conservation (38.09% and 26.31%) in the OmpA of all the 14 sequences (Figure 3 and 4).

## DISCUSSION

Antibody cross reactivity provides cross protective immune response against a broad range of closely related pathogens.<sup>31-33</sup> Shared epitopes because of amino acid sequence homology among species is the underlying factor for this. *Enterobacteriaceae* pathogens such as *E. coli*, *Salmonella*, *Shigella*, *Proteus* and *K. pneumoniae* are responsible for a wide range of gastrointestinal and other mucosal infections.<sup>1-5</sup> Moreover, the family members of *Enterobacteriaceae* share similarities in the antigenic properties. Significantly, the *Enterobacteriaceae* OMPs are strong immunogens and also highly conserved with greater amino acid identity.<sup>18-21,34</sup> The OMPs play a crucial role in the adherence of bacteria to the surface of host cells. Therefore, prevention or blocking of bacterial adhesion to cell surface will be a promising approach for immune therapy. The antibodies directed against these OMPs will prevent the establishment of infection.

Hence, application of these OMPs in vaccine development and passive immune



**Figure 4.** Amino acid sequence conservation in the B-cell epitopes of OmpA among the considered *Enterobacteriaceae* pathogens. The percent amino acid conservation in the B-cell epitopes was depicted in percent (%).



therapies might confer cross protection against multiple pathogens of *Enterobacteriaceae* by producing cross reactive antibodies in the host. Our recent study has explored the likelihood of conserved epitopes in the crude OMP of *Salmonella* and other *Enterobacteriaceae* pathogens which were revealed with a potent Sal-6 monoclonal antibody (mAb).<sup>18</sup> This mAb was raised using crude extracts of OMP proteins recovered from the *S. typhimurium* cells. Whereas the identity of the OMP to which Sal-6 reacts was not deduced.

In this study, we explored the antibody mediated cross reactivity against the *Enterobacteriaceae* pathogens as a primary attempt to uncover an important OMP, i.e., OmpA as broad-spectrum epitope vaccine for serious bacterial infections caused by *Enterobacteriaceae* pathogens. Our results show that the polyclonal antibodies that are raised against rOmpA of *S. typhimurium* are reactive towards *E. coli*, *Shigella*, *Salmonella*, *Proteus*, and *K. pneumoniae* (Figure 1c). The high degree of amino acid sequence conservation with ~ 66% of identity indicates the presence of conserved B-cell epitopes (Figure 3 and 4) Because of highly conserved B-cell epitopes in OmpA, the polyclonal antibodies are reactive against standard *Enterobacteriaceae* strains used in this study (Figure 1c). In support with the few other studies,<sup>18,21,34</sup> the initial finding in our study is critical to study the cross protective efficiency of OmpA as a subunit vaccine candidate against *Enterobacteriaceae* infections.

However, T-cell mediated immune response is also very crucial in generating immune response against the pathogen. We also presume the occurrence of several T-cell epitopes in OmpA with high sequence conservation among *Enterobacteriaceae*. But this presumption should be investigated with appropriate *in vitro* and *in vivo* experiments.

Finally, our work resulted in the identification of several conserved B-cell epitopes in the OmpA which are responsible for antibody mediated cross reactivity against the *Enterobacteriaceae* strains.

The rOmpA could be explored as a potential sub unit immunogen against multiple pathogens of *Enterobacteriaceae*. Additionally, the antibodies against OmpA could be used as an alternative for antibiotics to treat infections

caused by these prominent *Enterobacteriaceae* pathogens. However, *in vitro* experiments and pre-clinical evaluation in mice model are highly needed to accomplish the immunogenicity and safety of OmpA for immunoprophylaxis against broad range of *Enterobacteriaceae* infections.

## CONCLUSION

Finally, our work resulted in the identification of several conserved B-cell epitopes in the OmpA which are responsible for antibody mediated cross reactivity against the *Enterobacteriaceae* strains. The rOmpA could be explored as a potential subunit immunogen against multiple pathogens of *Enterobacteriaceae*. Additionally, the antibodies against OmpA could be used as an alternative for antibiotics to treat infections caused by these prominent *Enterobacteriaceae* pathogens. However, *in vitro* experiments and preclinical evaluation in mice model are highly needed to accomplish the immunogenicity and safety of OmpA for immunoprophylaxis against broad range of *Enterobacteriaceae* infections.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

PNR and SSM designed the study. HBK, SSL and DV performed experiments. HBK performed bioinformatics analysis. HBK, SSL and DV wrote the manuscript. PNR and SSM reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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#### DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

#### ETHICS STATEMENT

This study utilized BALB/c mice as animal model. The animals were bred and housed at animal house facility (Reg. No.: 2046/PO/ReBi/S/18/CPCSEA) of Vignan's Foundation for Science, Technology and Research (VFSTR). Necessary permission to use animals was provided by the Institutional Animal Ethical Committee (IAEC) of VFSTR. The protocols and procedures performed on the animals in the study abide by the guidelines laid by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Govt. of India.

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