Comparative Molecular Analysis and Antigenicity Prediction of an Outer Membrane Protein (ompC) of Non-typhoidal *Salmonella* Serovars Isolated from Different Food Animals in Lagos, Nigeria

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Morufat Yusuf[1], Abraham Ajayi[2], Utibeima Udo Essiet[2], Oyin Oduyebo[3], Adeyemi Isaac Adeleye[1] and Stella Ifeanyi Smith[2,4]

[1]Department of Microbiology, University of Lagos, Akoka, Nigeria. [2]Molecular Biology and Biotechnology Department, Nigerian Institute of Medical Research, Lagos, Nigeria. [3]Department of Medical Microbiology, College of Medicine, University of Lagos, Idi-Araba, Nigeria. [4]Department of Biological Sciences, Mountain Top University, Ibafo, Nigeria.

ABSTRACT: Non-typhoidal *Salmonella* (NTS) infections occur globally with high morbidity and mortality. The public health challenge caused is exacerbated by increasing rate of antibiotic resistance and absence of NTS vaccine. In this study, we characterized the outer membrane protein C (*OmpC*) serovars isolated from different food animals and predicted antigenicity. *ompC* of 27 NTS serovars were amplified by polymerase chain reaction (PCR) and sequenced. Sequence data were analysed and B-cell epitope prediction was done by BepiPred tool. T-cell epitope prediction was done by determining peptide-binding affinities of major histocompatibility complex (MHC) classes I and II using NetMHC pan 2.8 and NetMHC-II pan 3.2, respectively. *ompC* sequence analysis revealed conserved region among *ompC*s of *Salmonella* Serovars. A total of 66.7% of *ompC*s were stable with instability index value < 40 and molecular weight that ranged from 27 745.47 to 32 714.32 kDa. All *ompC*s were thermostable and hydrophilic with the exception of *S.* Pomona (14p) isolate that had *ompC* with GRAVY value of 0.028 making it hydrophobic. Linear B-cell epitope prediction revealed ability of *ompC* to elicit humoral immunity. Multiple B-cell epitopes that were exposed and buried were observed on several positions on the *ompC* sequences. T-cell epitope prediction revealed epitopes with strong binding affinity to MHC–I and -II. Strong binding to human leukocyte antigen (HLA-A) ligands, including HLA-A03:1, HLA-A24:02 and HLA-A26:01 in the case of MHC-I were observed. While binding affinity to H-2 IAs, H-2 IAq and H-2 IAu (H-2 mouse molecules) were strongest in the case of MHC-II. *ompC*s of NTS serovars isolated from different food animal sources indicated ability to elicit humoral and cell-mediated immunity. Hence, *ompC*s of NTS serovars are potential candidate for production of NTS vaccines.

KEYWORDS: Antigenicity, B-cell, outer membrane protein, *Salmonella*, T-cell

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CORRESPONDING AUTHOR: Stella Ifeanyi Smith, Molecular Biology and Biotechnology Department, Nigerian Institute of Medical Research, Lagos, Nigeria. Email: stellaismith@yahoo.com

Introduction

Salmonella enterica remains a pathogen of concern globally, since it continually causes infections that results in high morbidity and mortality.[1] Depending on clinical outcomes, serotypes of *Salmonella* are broadly grouped into typhoidal *Salmonella* (TS) and non-typhoidal *Salmonella* (NTS).[2] While TS serovars are human redistricted, NTS serovars cause infection in birds, mammals and humans.[3] Although NTS serovars cause self-limiting diarrhoea in individuals, infection in infants and immunocompromised persons could be severe or fatal.[4] It is estimated that NTS serovars cause 93 million infections with 155 000 associated deaths per year globally.[5] In 2022 alone, several outbreaks of NTS infections have been reported in Europe (*S.* Enteritidis and *S.* Typhimurium) and the United States (*S.* Enteritidis, *S.* Typhimurium, *S.* Hadar, *S.* Infantis, *S.* Indiana, 1 4,[5], 12:1 and *S.* Mbandaka) resulting in hospitalization and deaths.[6-9] Treatment of bacterial infections, including NTS infections are becoming difficult as a result of antimicrobial resistance (AMR). The World Health Organization (WHO) has categorized fluoroquinolone (FQ)-resistant *Salmonella* spp. as priority pathogen urgently in need of new antibiotics.[10] This underscores the need for NTS vaccines. There is currently no NTS vaccine in use, however, several vaccine candidates, including live-attenuated, glycoconjugate, outer membrane vesicle vaccines and many more are in various stages of development and clinical trials.[11] *Salmonella* live-attenuated vaccines (SLAVs) have the potential to elicit both humoral and cell-mediated responses, however, their safety in immunocompromised individuals at high risk of coming down with invasive non-typhoidal *Salmonella* (iNTS) has been brought to question.[5] Hence, the need to explore other cell components of NTS serovars for vaccine production is imperative. Returning to normal life during this COVID-19 pandemic could in part be attributed to the prompt production of vaccines especially the mRNA vaccine that initiates the production of viral spike proteins that in turn elicit immune response to severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) virus.[12] The outer membrane proteins (omps) of *Salmonella*, such as ompC, ompD and ompF, are known antigens that are highly immunogenic and elicit cellular immunity and efficient protective antibody.[13] OmpCs of *Salmonella* have been indicated as a potential broad spectrum *Salmonella* vaccine candidate as several highly conserved amino

acid sequences located in the transmembrane β-sheet have been identified in both typhoidal and non-typhoidal serovars.[13] Carreño et al[14] in a study demonstrated that OmpC of *S.* Typhi elicited specific B-cell responses and polyfunctional $CD4^+$ T-cell responses that evolved over a period of 2 months post-administration of live-attenuated vaccine. Similarly, OmpC of *S.* Typhimurium has been shown to elicit cell-mediated and humoral immune responses that conferred absolute protection against *S.* Typhimurium in rats.[15] Although omps of *Salmonella* are promising vaccine candidates, available data tend to centre majorly on *S.* Typhimurium that is just one among other numerous NTS serovars that have been implicated in outbreaks and invasive infections. Questions on spectrum of activity (broad spectrum) of NTS omps base vaccines have been raised. For instance, ompD has been shown to mediate immune response that limits clinical outcome of invasive *S.* Typhimurium infection.[11] However, Ashton et al[16] reported the absence of ompD locus in *S.* Typhimurium (ST313) strain U60 isolated in the United Kingdom. The need to study the omps of other NTS, including rear serovars for vaccine prospect is therefore imperative. This study aimed to molecularly characterize OmpC of NTS serovars isolated from different sources and predict their antigenicity.

Materials and Methods

Salmonella isolates

Thirty previously isolated *Salmonella* Serovars from animal sources (chicken, sheep and cattle) stored in the stock culture of Molecular Epidemiology Unit of the Molecular Biology and Biotechnology Department of Nigerian Institute of Medical Research (NIMR) Yaba Lagos were retrieved. To resuscitate the isolates, a loop full of the isolates in glycerol stock were inoculated into 1 mL of brain heart infusion broth (Oxoid, Basingstoke, UK) and incubated for 18 hour at 37°C after which they were streaked on nutrient agar plate and incubated for 24 hours at 37°C. Isolates were then stored in agar slants for further use.

DNA Extraction

Isolates were inoculated into 1 mL of brain heart infusion broth (Oxoid, Basingstoke, UK) and incubated for 18 hours at 37°C. Cells were harvested by centrifuging at 14 rpm for 3 minutes and supernatant was discarded retaining the cell pellet. Genomic DNA was extracted with bacterial genomic DNA isolation Kit (NorgenBiotek Corp., Canada) according to the manufacturer's instructions. Two hundred and fifty microliter of re-suspension solution A was added to cell pellet and vortexed to make a cell suspension to which 12 µL of lysozyme (400 mg/mL) was added and mixed. Then, 250 µL of lysis buffer P and 12 µL of proteinase K were added to the cell suspension vortexed and incubated at 37°C for 2 hours. Five hundred microliter was added to the lysate and mixed well with gentle vortexing after which 500 µL of 100% ethanol was added and mixed again by gentle vortexing. Spin columns were assembled into which lysate mixtures were dispensed and centrifuged at 14 000 rpm for 1 minute. After centrifugation, flow-through was discarded and spin column was reassembled. Five hundred microliter of wash solution A was added to spin column and centrifuged for 1 minute at 14 000 rpm. Flow-through was discarded and the washing process was repeated once more. After the second wash and discarding of the flow-through, the column was centrifuged for 2 minutes to ensure the column was thoroughly dried. The spin column was reassembled with a new 1.7 mL collection tube and 200 µL of elution buffer B was added to the centre of the column which was then centrifuged for 2 minute at 14 000 rpm and pure genomic DNA obtained was stored at −20°C for further use.

Detection and Sequencing of ompC

Salmonella ompC gene was amplified by PCR using primer set F-ATGAAAGTTAAAGTACTGTCCCTC and R-TTAGA ACTGGTAAACCAGACCC.[17] A 30 µL PCR mix was used. The reaction mix contained 16.2 µL nuclease-free water, 0.9 µL forward primer, 0.9 µL reverse primer, 6 µL DNA template and 6 µL of 5X PCR Master Mix (Solis BioDyne, Estonia). PCR programming parameter consisted of initial DNA denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 40 seconds and extension at 72°C for 2 minutes, followed by a final extension at 72°C for 10 minutes. Electrophoresis was done to visualize amplicons. Gel electrophoresis was ran at 100 V for 60 minutes and visualized under a trans-illuminator (Cleaver Scientific Ltd). A 100 bp DNA ladder (Solis BioDyne, Estonia) was used as a molecular weight marker. Fifteen microliter of PCR products, and 10 µL of forward and reverse primers were aliquoted into a microcentrifuge tube and sent to Inqaba Biotec, South Africa, for Sanger sequencing. Sequences obtained in this study have been deposited in the National Centre for Biotechnology Information (NCBI) database with accession numbers ON190078–ON190104.

Comparative Analysis of ompC Gene Sequences and Prediction of Antigenicity

Protein sequence analysis and phylogenetic tree construction

Nucleotide sequences of *ompC* were base called on Bioedit software[18] and exported as FASTA to note pad. The Basic Local Alignment Search Tool (BLAST) analysis was done on NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine sequence homology with reference sequences. Nucleotide sequences were translated to amino acid sequences using the sorted six-frame translation tool of

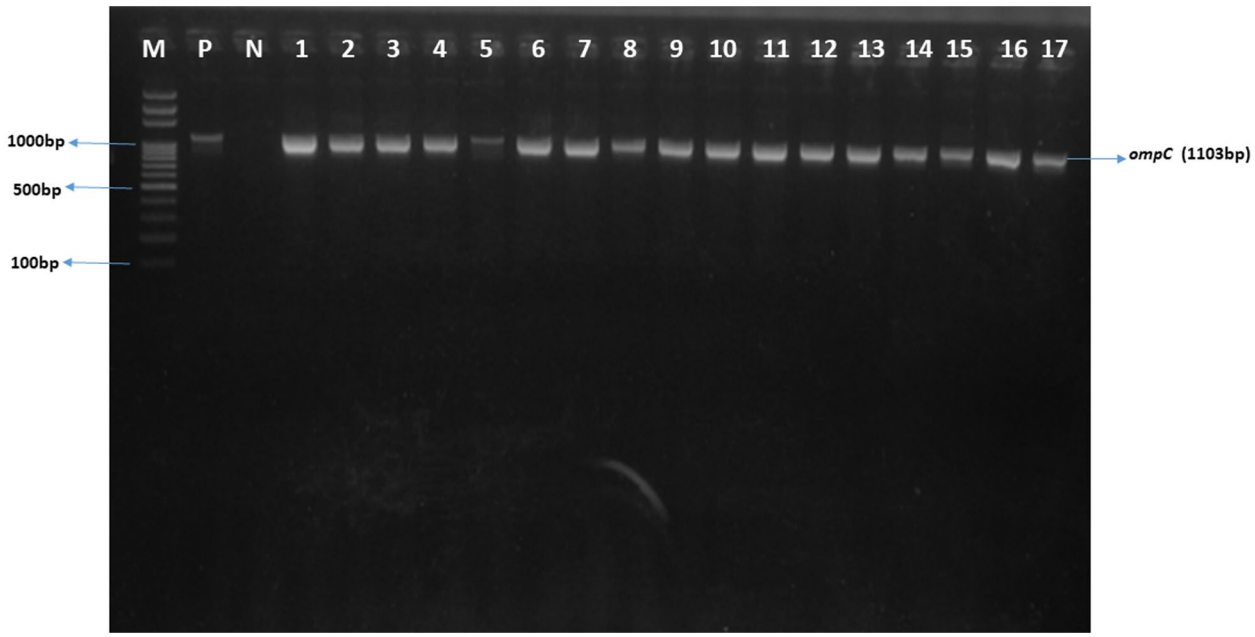

Figure 1. Gel image of amplified *ompC* gene in *Salmonella* isolates.
Lanes 1–17 positive *ompC* 1103 bp bands. Lane M indicated 100 bp molecular maker; lane N, negative control; lane P, positive control.

Bioedit software and BLAST analysis was done on UniProt database (https://www.uniprot.org/blast) to confirm protein identity. Multiple sequence alignment was done using CLUSTALW and phylogenetic tree was constructed using the maximum likelihood joining tree in MEGA 11.0 software[19] and variation were analysed. Protein 3D structure of *ompC* was determined from the PDBsum pictorial database in the protein data bank (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum).

Prediction of antigenicity

Properties of proteins were determined using ProtParam tool on the ExPASy platform and properties, such as number of amino acids, molecular weight, instability index, aliphatic index and theoretical isoelectric point (pI) was recorded. B-cell epitope prediction to determine B-cell epitope regions of *ompC* protein was done with BepiPred prediction tool on the cbs.dtu.dk server (http://www.cbs.dtu.dk/services/BepiPred/).[20] Scores of epitopes were recorded and peptides with 10 or more amino acids in length were considered as good B-cell epitopes. T-cell epitope prediction was done by determining peptide-binding affinities of MHC classes I and II by calculating epitope database analysis and ranking them according to their percentile using NetMHCpan 2.8 (https://services.healthtech.dtu.dk/service.php?NetMHCpan-2.8) and NetMHCIIpan 3.2 (https://services.healthtech.dtu.dk/service.php?NetMHCIIpan-3.2) servers for MHC-I and -II, respectively.[21] For MHC-I, affinity threshold for strong binding peptides was set at 5000 and affinity threshold for weak binding peptides was set at 500 000. While rank threshold for strong binding peptides was set at 0.500 and for weak binding peptides 2.00. On the other hand, rank threshold for strong and weak binding peptides for MHC-II was set at 2 and 10, respectively.

Results

Protein sequence analysis and properties of protein

ompC was detected in all (100%) the *Salmonella* isolates as the *ompC* gene of 1103 bp gene was amplified as shown in Figure 1. However, *ompC* of three of the *Salmonella* isolates failed sequencing quality control (QC) and could not be sequenced. *ompC* sequence analysis from multiple sequence alignment showed conserved regions among *ompC*s of *Salmonella* Serovars as shown in Figure 2. However, phylogenetic analysis of protein sequence revealed that three major clades (**A**, **B** and **C**) were formed by various serovars. Some *Salmonella* isolates that were the same serovar clustered with some other serovars; 18P_*S.* Gera (a dominant serovar in this study) clustered with *S.* Enteritidis in clade **B** that comprised *S.* Ealing, *S.* Muenster, *S.* Hadar, and *S.* Blockely as shown in Figure 3. Similarly, 31P_*S.* Gera, 33P_*S.* Gera, 23P_*S.* Gera and 27P_*S.* Gera clustered with other *Salmonella* Serovars in clade **C**.

Table 1 shows the physicochemical properties of *ompCs* of various *Salmonella* Serovars. Total number of negatively charged residues (Asp + Glu) in some of the *ompCs* of some of the serovars were low with concomitant high instability index resulting in unstable proteins. However, 66.7% of the proteins were stable with instability index values < 40. Molecular weight of *ompCs* ranged from 27 745.47 to

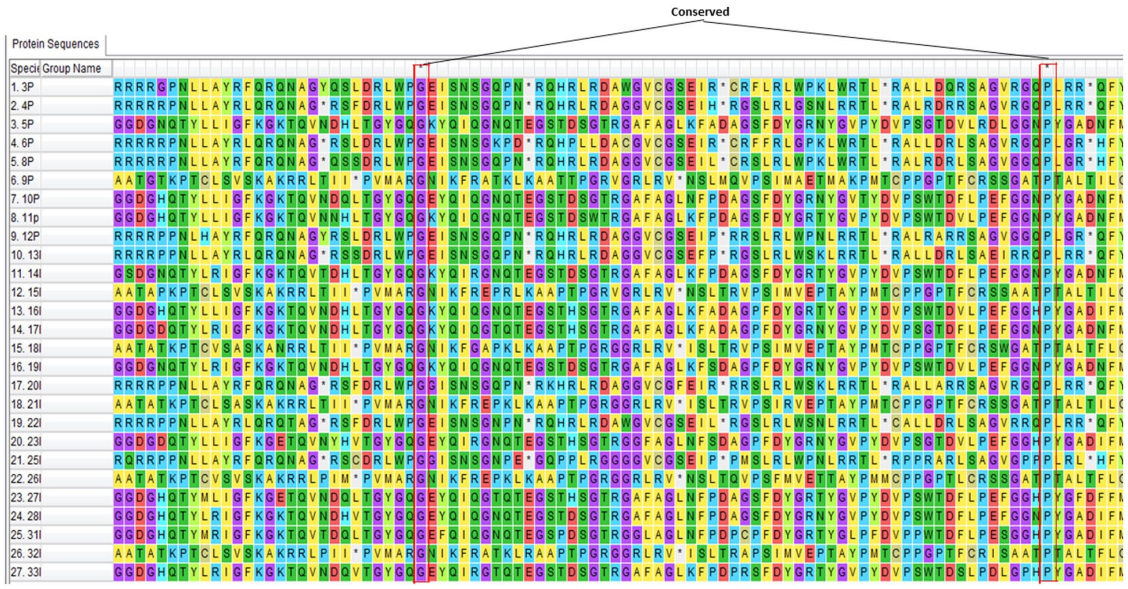

Figure 2. Multiple sequence alignment of *ompC* protein sequence showing conserved glycine (G) and proline (P) among *Salmonella* Serovars.

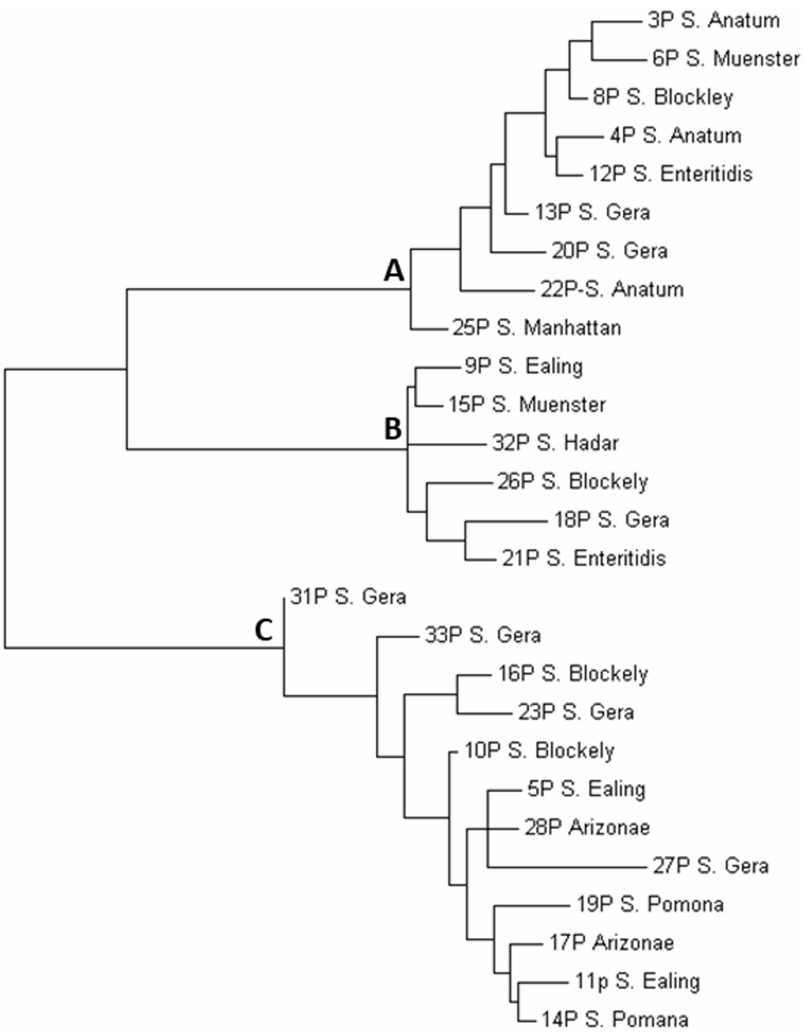

Figure 3. Phylogenetic analysis of *ompC* protein sequences of *Salmonella* Serovars. Three main clades **A**, **B** and **C** were formed.

Table 1. Various properties of *ompC*s of *Salmonella* Serovars, including number of amino acids, molecular weight, theoretical pI, instability index, aliphatic index and hydropathicity.

ID	NO. OF AA	MW	THEORETICAL PI	TOTAL NUMBER OF NEG. CHARGED RESIDUES	TOTAL NUMBER OF POS. CHARGED RESIDUE	INSTABILITY INDEX	ALIPHATIC INDEX	HYDROPATHICITY
3p	298	32714.32	9.27	22	31	29.70–stable	62.18	−0.605
4p	287	30907.14	9.10	22	28	19.55–stable	61.22	−0.530
5p	281	29990.89	8.90	21	25	13.28–stable	61.10	−0.555
6p	288	31225.62	6.57	25	24	15.48–stable	66.42	−0.528
8p	259	27745.47	9.16	21	26	17.22–stable	54.98	−0.699
9p	280	30521.66	9.14	23	29	21.88–stable	56.82	−0.695
10p	276	31678.89	11.81	11	54	73.60–unstable	81.34	−0.534
11p	286	29869.38	10.04	11	34	40.30–unstable	72.76	−0.107
12p	287	30375.33	8.84	22	26	20.61–stable	57.11	−0.607
13p	288	31484.89	9.24	21	29	29.46–stable	55.21	−0.645
14p	290	30744.08	10.45	10	36	44.63–unstable	80.48	0.028
15p	290	31311.76	8.70	20	24	23.52–stable	59.52	−0.524
16p	293	31176.08	9.90	14	36	42.26–unstable	77.41	−0.080
17p	291	31192.34	7.12	23	23	18.73–stable	66.08	−0.457
18p	287	30102.47	9.50	16	31	32.51–stable	73.90	−0.091
19p	290	31524.47	9.28	19	26	23.35–stable	74.34	−0.302
20p	293	31792.40	8.89	23	29	22.99–stable	64.91	−0.507
21p	290	31975.64	8.53	21	25	28.38–stable	65.62	−0.477
22p	287	32119.06	11.83	11	49	61.74–unstable	80.00	−0.485
23p	293	32256.94	8.76	24	29	23.43–stable	66.55	−0.481
25p	276	30675.35	9.74	19	33	43.23–unstable	60.76	−0.686
26p	283	31973.91	11.94	12	60	69.54–unstable	75.90	−0.705
27p	288	31388.26	8.76	22	26	23.90–stable	64.06	−0.370
28p	293	32016.52	8.99	23	29	17.04–stable	66.59	−0.539
31p	286	31239.30	10.48	12	44	40.36–unstable	67.27	−0.324
32p	282	32250.85	11.86	8	53	61.62–unstable	74.79	−0.513
33p	285	31457.03	9.58	21	34	33.15–stable	56.49	−0.715

Abbreviations: AA, amino acid; MW, molecular weight; pI, isoelectric point.

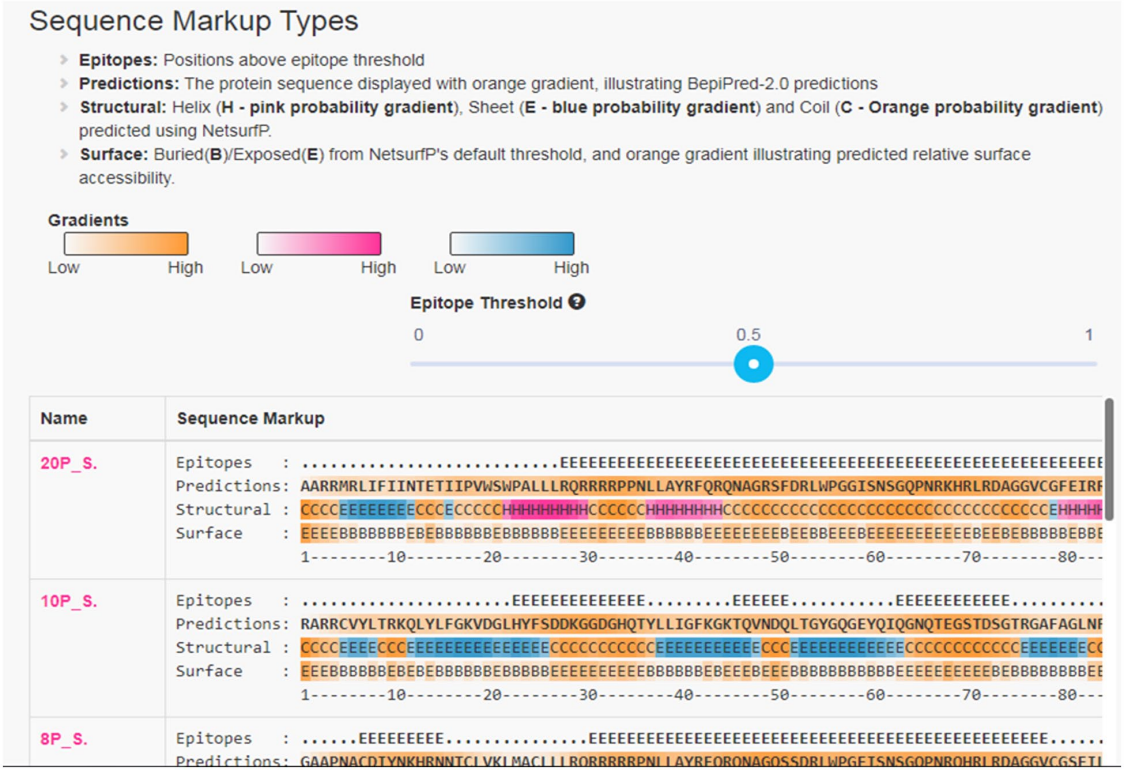

Figure 4. Result output of MHC 2.0 prediction tool showing positions of predicted epitopes that are buried or exposed form NetsurfP's default threshold, structure of proteins and protein sequences illustrating BepiPred-2.0 prediction.

32 714.32 kDa. While number of amino acids ranged from 259 to 298. *pI of ompC*s of *Salmonella* Serovars ranged from 7.12 to 11.94. Hydrophobicity or hydrophilicity measured as grand average of hydropathicity (GRAVY) values of *ompC*s indicated they were all hydrophilic with the exception of the *ompC* of isolate 14p (*S.* Pomona) that had a GRAVY value of 0.028 making it hydrophobic. Aliphatic index (thermostability of protein) indicated that *ompC*s of the *Salmonella* isolates were thermostable.

Prediction of B-cell epitopes

Linear B-cell epitope prediction was high in several positions of *ompC* sequence of *Salmonella* Serovars as depicted in Figure 4. Some of the predicted epitopes were buried. However, majority of the others were exposed indicating relative surface accessibility as predicted by NetSurfP's default threshold. 8P_*S.* Anatum had the highest number of predicted B-cell epitopes (218) of which 151 (69.27%) were exposed while 67 (30.73%) were buried; 18P gera had 134 predicted B-cell epitopes making it the least among epitopes possessed by other serovars as shown in Table 2. The number of predicted B-cell epitopes and their orientation (exposed or buried) among the same serovars varied. 12P_*S.* Enteritidis possessed 212 epitopes of which 142 (66.98%) were exposed while 21P_*S.* Enteritidis had 188 epitopes with 141 (75.00%) exposed. Although, 11P_*S.* Ealing possessed 204 predicted B-cell epitopes, only 81 (39.71) were exposed making the serovar with the least exposed epitopes.

Prediction of T-Cell Epitopes

MHC-I

Affinity and ranks of representative epitopes with the strongest binding affinity is shown in Table 3. Mapped set of 9mer peptides sequences of each isolate ranged from 246 to 290. Isolate 23P (*S.* Gera) had 19 the highest number of peptides that were strong binders followed by isolate 6P (*S.* Muenster) and 20P (*S.* Gera) that had 17 strong binding peptides each. Weak binding peptides were highest in isolate 3P with 51 weak binders followed by isolate 16P (*S.* Blockley). Not all peptides 9mers had binding affinity. More affinity to HLA-B ligands were recorded with rank ranging from 0.001 to 0.5 (Table 3). Although, strong binding affinity to HLA-A ligands, HLA-A03:1, HLA-A24:02 and HLA-A26:01 had more binders recorded compared to HLA-A01:01 and HLA-A02:01. No binding to any of the HLA alleles was recorded for isolate 21P (*S.* Enteritidis), however, 12P also an *S.* Enteritidis isolate had strong binders to only HLA-A03:1 and HLA-A24:02 of the HLA ligands.

Table 2. Number of predicted B-cell epitopes and surface orientation in *Salmonella* Serovars.

S/N	CODE	*SALMONELLA* SEROVARS	NO. OF EPITOPES	EXPOSED (%)	BURIED (%)
1	3P	*S.* Anatum	166	149 (89.76)	31 (18.67)
2	4P	*S.* Anatum	218	151 (69.27)	67 (30.73)
3	5P	*S.* Ealing	141	85 (60.28)	56 (39.72)
4	6P	*S.* Muenster	200	145 (72.50)	55 (27.50)
5	8P	*S.* Blockley	180	123 (68.33)	57 (31.66)
6	9P	*S.* Ealing	145	103 (71.03)	42 (28.97)
7	10P	*S.* Blockley	149	91 (61.07)	58 (38.93)
8	11P	*S.* Ealing	204	81 (39.71)	123 (60.29)
9	12P	*S.* Enteritidis	212	142 (66.98)	70 (33.02)
10	13P	*S.* Gera	189	137 (72.49)	52 (27.51)
11	14P	*S.* Pomona	122	77 (63.11)	45 (36.89)
12	15P	*S.* Muenster	205	136 (60.34)	69 (33.66)
13	16P	*S.* Blockley	168	110 (65.48)	58 (34.52)
14	17P	*S* 9:1, w:e,n,x	142	100 (70.42)	42 (29.58)
15	18P	*S.* Gera	134	88 (65.67)	46 (34.33)
16	19P	*S.* Pomona	141	91 (64.54)	50 (35.46)
17	20P	*S.* Gera	211	149 (70.62)	62 (29.38)
18	21P	*S.* Enteritidis	188	141 (75.00)	47 (25.00)
19	22P	*S.* Anatum	206	146 (70.87)	60 (29.13)
20	23P	*S.* Gera	151	102 (67.55)	49 (32.45)
21	25P	*S.* Muenster	181	119 (65.75)	62 (34.25)
22	26P	*S.* Blockley	199	114 (57.29)	85 (42.71)
23	27P	*S.* Gera	149	99 (66.44)	50 (33.56)
24	28P	9:1, w:e,n,x	140	95 (67.86)	45 (32.14)
25	31P	*S.* Gera	144	90 (62.50)	54 (37.50)
26	32P	*S.* Hadar	171	96 (56.14)	75 (43.86)
27	33P	*S.* Gera	157	99 (63.06)	58 (36.94)

MHC-II

Predicted MHC–peptide-binding affinity of the various H-2 mouse molecules revealed varied numbers of strong and weak binders. Molecules H-2 IAs had a total of 371 strong binders among *Salmonella* servers followed by molecule H-2 IAq with 348 strong binders and H-2 IAu with a total of 284 strong binders among the serovars (Figure 5). A total of 246 and 283 strong binders were detected in molecule H-2 IEd and H-2 IEk, respectively, among the serovars (Figure 6). The least number of binders among the serovars was found in the H-2 IAk molecule with 166 strong binders as shown in Figure 7.

Discussion

The genus *Salmonella* consist of six subspecies with over 2600 phenotypically, ecologically and genetically diverse serovars.[22] All NTS serovars in this study isolated from different food animal sources were positive for OmpC. This implies that OmpC is largely distributed among *Salmonella* Serovars. Jawad and Al-Charrakh[23] similarly reported the PCR amplification of OmpC in all *Salmonella* strains isolated from human and animal samples in Iraq. Serovars in this study showed genetic interrelatedness with similar serovars clustering with unrelated serovars inferred from phylogenetic analysis of OmpC sequences. Although variation existed in protein sequences of serovars there was a high level of sequence homology with

Table 3. Affinity and ranks of strongest binding representatives of peptides to various alleles of HLA-A and HLA-B ligands, including total numbers of 9mers peptides of various *Salmonella* isolates.

ID	TOTAL NUMBERS OF 9MERS PEPTIDES	VARIOUS ALLELES OF HLA-A AND HLA-B LIGANDS																TSB	TWB
		HLA-A01:01		HLA-A02:01		HLA-A03: 01		HLA-A24:02		HLA-A26:01		HLA-B07:02		HLA-B08:01		HLA-B27:05			
		1-LOG50K	RANK	1-LOG50K	RANK	1-LOG50K	RANK	1-LOG50K	RANK	1-LOG50K	RANK	1-LOG50K	RANK	1-LOG50K	RANK	1-LOG50K	RANK		
3P	290	–	–	–	–	0.6668	0.175	0.6296	0.3	–	–	0.8058	0.075	0.7664	0.025	0.6535	0.1	14	51
4P	277	–	–	–	–	0.6668	0.175	–	–	–	–	0.678	0.3	0.7319	0.05	0.7246	0.025	11	31
5P	274	0.7769	0.025	–	–	0.678	0.15	0.6296	0.3	0.7846	0.05	–	–			–	–	4	16
6P	273	–	–	0.794	0.4	–	–	0.7432	0.075	–	–	0.7886	0.1	0.7312	0.05	0.7308	0.025	17	21
8P	246	–	–	–	–	0.6369	0.25	–	–	–	–	–	–	0.5631	0.4	0.695	0.05	16	35
9P	268	–	–	0.8311	0.2	0.6949	0.125	–	–	–	–	0.8384	0.05	0.6422	0.15	0.7689	0.01	11	30
10P	278	0.605	0.125	0.8672	0.1	0.678	0.15	–	–	0.6293	0.125	–	–	0.5495	0.4	0.6154	0.175	8	22
11P	279	0.5035	0.25	0.794	0.4	0.697	0.1	0.7164	0.125	0.6281	0.1	0.0221	0.2	–	–	0.7033	0.05	11	23
12P	274	–	–	–	–	0.6256	0.3	–	–	0.7224	0.075	–	–	0.7319	0.05	0.6625	0.075	10	35
13P	278	0.507	0.25	–	–	0.6668	0.175	–	–	0.5779	0.2	0.8113	0.05	0.6225	0.2	0.695	0.05	14	42
14P	285	0.5035	0.25	0.9027	0.05	0.7177	0.075	0.6469	0.25	0.7839	0.05	0.6009	0.5	–	–	–	–	6	24
15P	281	0.5096	0.2	–	–	–	–	–	–	0.6116	0.15	0.852	0.025	0.6772	0.1	0.7689	0.01	11	25
16P	286	0.7559	0.025	–	–	0.678	0.15	0.5495	0.5	0.7486	0.05	–	–	0.7044	0.075	0.6767	0.075	7	35
17P	284	0.7559	0.025	–	–	0.7177	0.075	0.616	0.3	0.7486	0.05	0.1023	0.5	–	–	0.5212	0.5	7	25
18P	280	–	–	0.8672	0.1	0.6541	0.2	0.6176	0.3	0.4662	0.5	0.6591	0.4	–	–	0.7689	0.01	6	25
19P	282	0.8541	0.01	–	–	0.7177	0.075	0.616	0.3	0.7486	0.5	0.0463	0.05	0.6084	0.25	0.5228	0.5	10	29
20P	283	–	–	0.912	0.025	0.6668	0.175	0.6474	0.25	–	–	0.7886	0.1	0.7177	0.075	0.75	0.01	17	47
21P	279	–	–	–	–	–	–	–	–	–	–	0.0735	0.3	0.5448	0.4	0.7689	0.01	8	25
22P	280	–	–	–	–	0.6377	0.25	–	–	–	–	0.8672	0.025	0.7665	0.025	0.6645	0.075	16	33
23P	286	0.8541	0.01	0.8405	0.175	0.726	0.075	0.6724	0.175	0.6651	0.1	–	–	0.86	0.01	0.6725	0.075	19	16
25P	263	–	–	–	–	–	–	–	–	–	–	0.8727	0.025	0.5389	0.5	0.6	0.2	6	25
26P	276	–	–	–	–	0.7212	0.075	–	–	–	–	0.7431	0.15	0.6644	0.125	0.7074	0.05	12	26
27P	281	0.5632	0.15	–	–	0.7013	0.1	–	–	0.6293	0.125	0.7997	0.015	0.6542	0.125	–	–	5	27
28P	286	0.5196	0.2	–	–	0.7177	0.075	0.5584	0.5	0.713	0.075	–	–	–	–	0.5904	0.25	8	24
31P	278	–	–	–	–	0.7338	0.05	–	–	0.6077	0.175	0.6246	0.5	0.6419	0.15	0.6734	0.075	3	24
32P	281	–	–	0.794	0.4	–	–	0.5517	0.5	0.4662	0.5	0.8384	0.05	0.6422	0.15	0.7517	0.01	6	31
33P	278	0.5196	0.2	–	–	0.7177	0.075	0.801	0.05	0.5821	0.2	0.6928	0.25	0.7995	0.025	0.6436	0.1	11	24

Abbreviations: 1-log50k, prediction score of affinity; 9mers, protein fragments of length 9; Rank, % rank; TSB, total strong binders; TWB, total weak binders.

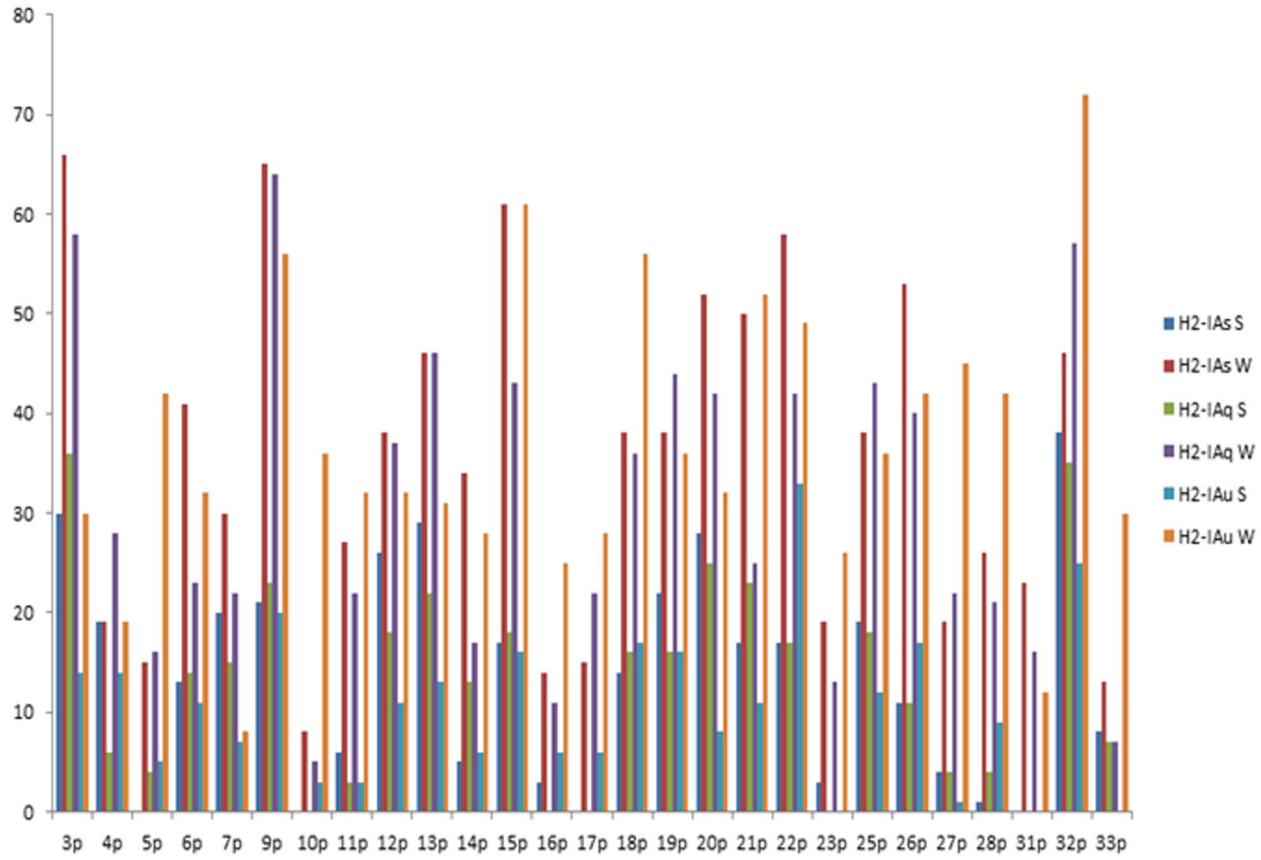

Figure 5. Predicted MHC-II–peptide-binding affinity of H2-IAs, H2-IAq and H2-IAu showing numbers of strong and weak binders.

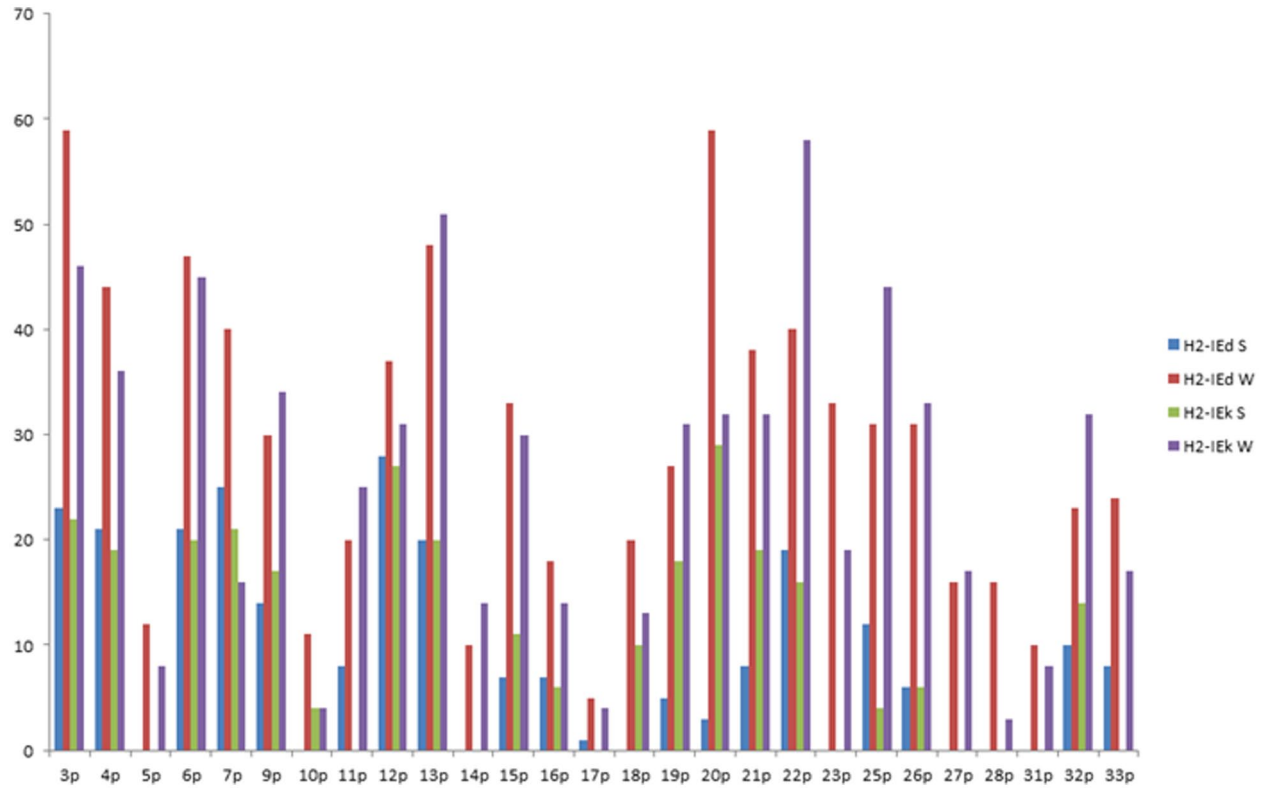

Figure 6. Predicted MHC-II–peptide-binding affinity of H2-IEd and H2-IEk showing numbers of strong and weak binders.

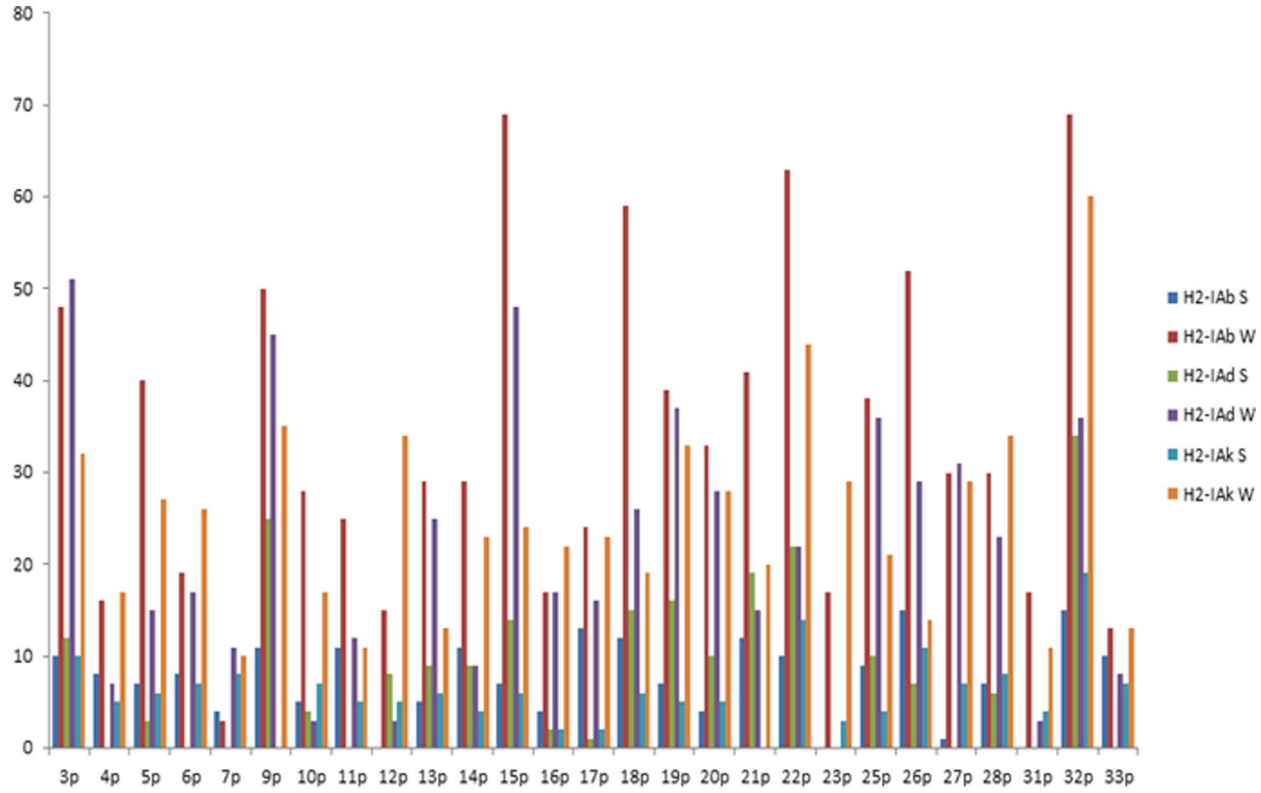

Figure 7. Predicted MHC-II–peptide-binding affinity of H2-IAb, H2-IAd and H2-IAk showing numbers of strong and weak binders.

identified conserved regions. Valero-Pacheco et al[13] in a previous study have reported highly conserved amino acid sequences of OmpC among both TS and NTS serovars. The need for prospecting for NTS vaccine is important considering the increasing number of iNTS infections coupled with the high burden of AMR.[24] Vaccine potential of the OmpC of NTS as earlier highlighted tends to be a resource that needs to be explored and exploited to solve the problem. Beyond being conserved among *Salmonella* Serovars,[25] OmpC of NTS possess physicochemical parameters that make them ideal antigens. Functional and physicochemical properties of vaccine antigens are core determinants of the level of antigen-adjuvant interaction and vaccine response.[26] In this study, a large percentage of the OmpCs of serovars, including *S.* Enteritidis were stable with instability index of < 40. They had aliphatic index that indicated they were thermostable and GRAVY values that showed they were hydrophilic. Rabbani et al[27] in a previous study have demonstrated the conformation and thermostability of *omp* of *S.* Typhi to different pH conditions. Similar physicochemical properties have been reported for ompC and ompF of *S.* Typhimurium by Jha et al[28] and Soman et al,[29] respectively.

Functional properties of OmpC as an ideal vaccine antigen have been demonstrated in previous studies. However, most studies have only evaluated and reported on the OmpCs of *S.* Typhimurium and *S.* Typhi.[13-15,28] Concerns have been raised on the limitation of the use of certain omps, such as ompD, as broad spectrum vaccine against NTS infections.[11] ompCs of *Salmonella* Serovars investigated in this study all possessed linear B-cell epitopes on several positions with majority exposed capable of evoking immune response. This is in line with the findings of Jha et al[28] who reported B-cell epitopes with antigenic index of 1.7 from the mapping of the OmpC of *S.* Typhimurium. Studies have highlighted the importance of the accessibility of epitopes to MHC-I and MHC-II to elicit optimal immune response.[30] Immunogenic potential of OmpC for MHC-I predicted in this study showed strong binders with more affinity to HLA-B ligand. On the other hand immunogenic potential of OmpC for MHC-II showed strong binding with high preference to H-2 IAs a variant of H-2 mouse molecule. Valero-Pacheco et al[13] in their study reported conserved OmpC amino acid sequences with immunogenic potential for MHC-II binding.

Conclusion

In conclusion, findings from our study revealed that OmpC of various *Salmonella* Serovars possessed B-cell and T-cell epitopes that have the potential of evoking immune response. Hence, OmpC of these serovars can be considered for design of broad spectrum vaccine against NTS.

Author Contributions

MY, AA and EUU contributed to data collection; MY, AA and EUU contributed to data analysis and preparation of draft;

AA, AIA and SIS contributed to conceptualization of study; and OO, AIA and SIS supervision and correction of draft. All authors read and approved final draft.

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