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Full Length Research

Prevalence and characterization of *Salmonella typhi* isolated from internally displaced persons in Jos, Plateau State, Nigeria

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ABSTRACT: *Salmonella typhi*, is estimated to cause 21.6 million illnesses and 216,000 deaths. The plight of displaced persons has in recent times become a formidable problem of global significance and implication. This work was aimed at isolating and characterizing *S. typhi* from two internally displaced camps in Jos, Plateau State, Nigeria. A total of 183 stool samples were collected randomly and cultured. Isolates were serotyped and identified by PCR targeting the invasive gene. An antimicrobial susceptibility test was carried out on isolates using the disc diffusion method. The study showed a prevalence of 1.1% for *S. typhi* and 1.6% for non-typhoid Salmonella. All the isolates were sensitive to ciprofloxacin, ofloxacin ceftriaxone, chloramphenicol and gentamicin except for nalidixic acid and cefuroxime. The *Salmonella typhi* isolates were sequenced using Fastp for quality control and the genotype was determined. Comparative genomics on the two *S. typhi* assembled showed *S. typhi* 1(SO1) contains 38 contigs and *S. typhi* 2 (SO2) had 35 contigs. The result shows that the genome is similar to the reference strain CT18. More care and adequate health facilities should be provided in other to further reduce the rate of infection in the IDP camps.

Keywords: Disease, isolates, Jos, Plateau State, prevalence, *Salmonella typhi*.

INTRODUCTION

Salmonella enterica subspecies enterica serovar typhi, the cause of typhoid fever, is transmitted primarily by ingestion of contaminated food and water. It was estimated to cause 21.6 million illnesses and 216,000 deaths in the year 2000 (Crump *et al*., 2004). Even though the incidence of typhoid fever has declined markedly in developed countries, it remains a major cause of morbidity in less-developed countries (Connor and Schwartz,2005). In recent decades, cases of typhoid fever in developed countries have been associated predominantly with travellers returning from areas where typhoid fever is endemic (Lynch et al., 2009). *Salmonella* organisms are gram-negative, flagellate, non-sporulating, facultative anaerobic bacilli that ferment glucose and reduce nitrate to nitrite. They are ingested in food and survive passage through the gastric acid barrier. They subsequently invade the mucosa of the small and large intestines and produce toxins. *Salmonella typhi* possesses virulence factors allowing it to be more rapidly invasive through the mucosa. Molecular methods are increasingly important in detecting and typing *Salmonella* PCR examination technique with the invA gene is very sensitive and specific in detecting *Salmonella.* The first step in the intracellular pathogenicity cycle of Salmonella is the invasion of intestinal epithelial cells, and this step is controlled by the invA gene. The invA gene encodes proteins in bacterial cell membranes that are needed for

invasion into host epithelial cells (Sharma and Das, 2016).

The organism spreads through contaminated water or food causing fever, headache, and other symptoms. If not treated, the disease can lead to complications like gastrointestinal perforation and kills up to 20% of patients. Estimates range from 10 million to 30 million cases a year (Kupferschmidt, 2005). Antibiotic resistance is a serious problem that has the potential to drag the world into a pre-antibiotic era (Tiwari and Kaur, 2010). The most important reason is the widespread use of antibiotics and the often choosing an inappropriate drug. The misuse of antibiotics stems primarily from an inherent inclination of clinicians towards prescribing potent antibiotics. There is a need for periodic analysis of the pattern and the sensitivity of organisms isolated and results need to be communicated to clinicians (Tiwari and Taur, 2010).

An internally displaced person (IDP) is someone who is forced to flee his or her home but who remains within his or her country's borders (Mooney, 2005). They are often referred to as refugees, although they do not fall within the legal definitions of a refugee. Most are victims as a result of the insurgency and do not have access to clean water and poor sanitation has remained the greatest challenge facing internally displaced persons (IDMC, 2018). The plight of the displaced person has in recent times become a formidable problem of global significance and implication (Lam *et al*., 2015). The United Nations Refugee Agency (UNHCR) estimates the number of forcibly displaced people increased from 22.7 million people in 1996 to 67.7 million people in 2016. *Desai et al.* (2020) identified that the prevalence of infectious diseases has increased in internally displaced persons. Owoaje (2016) found that children are the worst hit by disease outbreaks occurring in IDP camps. Therefore, the study was aimed at isolation, characterization and antimicrobial susceptibility testing of *Salmonella* enterica serovar *typhi* from persons in IDP Camps in the Jos metropolis.

MATERIALS AND METHODS

Study area

The study was carried out among children and adult in two camps housing Internally Displaced Persons within Jos metropolis. Jos is a city in North Central region of Nigeria located at 09° 55' 00" N 08° 53' 25" E. The internally displaced persons are victims of insurgency and do not have access to clean water and poor sanitation has remained their greatest challenge. A cross-sectional longitudinal study was carried out to determine the prevalence of typhoid fever among 183 persons in IDP camp.

Sampling method

Randomized sampling method was used to select 183 subject who consented to participate in IDP camp while individuals who declined consent were excluded.

Ethical approval

This was obtained from the State Emergency Management Agency (SEMA) Plateau state. Informed consent form and questionnaire were given to individuals in the study population.

Sample collection

Stool samples were collected in a sterile universal bottle and transferred to the central diagnostic laboratory, National Veterinary Research Institute, Vom, Plateau State, Nigeria for culture.

Isolation and characterization

The stool samples were inoculated in selenite F broth and incubated at 37°C for 24 hours. After 24 hours incubation it was sub cultured onto Salmonella Shigella agar (SSA) and Deoxycholate citrate agar (DCA). The isolates were characterized based on motility, and biochemical tests (Citrate utilization test, Urease test, indole test, oxidase test, sugar fermentation test on TSI) was carried out on the non-lactose fermenting isolate.

Serotyping of *Salmonella* isolates: A Vi-specific monoclonal antibody was used in a passive latex agglutination test for the serotyping. A method in which surface antigen are identified based on agglutination reaction with specific antibodies (Kauffmann-white Le minor scheme). This is based on phenotypic characterization (Wattiau *et al*., 2011).

Determination of O-phase

After confirmation of isolates on TSI slant, a drop of antisera O (Polyvalent) was placed on a slide and isolate was picked from muller Hinton agar and placed on it. It was mixed thoroughly and rocked in a circular motion for 30 seconds and observed for agglutination. As agglutination is observed with the polyvalent antisera serotyping continued using antisera O (Monovalent) O:4, O:9, O:12, and O:6 and observed for agglutination (Rabiu *et al*., 2018).

Determination of H-phase and VI antigen

After confirmation of isolates on TSI slant a drop of antisera H (Phase 1& 2) and VI was placed on a slide and isolate was picked from muller Hinton agar and placed on it. It was mixed thoroughly and rocked in a circular motion for 30 seconds and observed for agglutination. Agglutination is positive and no agglutination is negative.

DNA extraction

The isolates were extracted using chelex resin. The isolates cells were re-suspended in 200 ul of physiological buffer saline and vortex. It was centrifuged at 13000 rpm for 1 minute and supernatant was discarded. 200 ul of 10% chelex suspension was added and incubated at 57°C for 15 minutes. It was vortexed briefly and pulse spun for 10 seconds. It was incubated at 100°C for 8 minutes, vortex for 10 seconds and then centrifuge at 13000 rpm for 3 minutes. The supernatant was transferred into plain Eppendorf tube and immediately frozen at -80°C (Walsh *et al*., 2018). The InvA gene contains sequences specific to the genus Salmonella and is considered the international standard for its characterization. Salmonella specific primer s139 and s141 have respectively the following nucleotide sequence based on the Inv A gene of Salmonella 5'-GTG AAA TTA TCG CCA CGT TCG GGC AA- 3' and 5' TCA TCG CAC CGT CA A AGG AAC C- 3' (Rahn *et al*., 1992). Reaction mixture with these primers were carried out with amplification mixture consisting (1x master mix composition) 10Mm Tris HCl, 50Mm KCl, 1.5Mm Mgcl2, 0.2Mm dNTPs, 5% Glycerol, 0.08% IGEPAL CA-630, 0.05% Tween 20, 25units/ml Taq DNA Polymerase and pH 8.6 at 25˚C. The primer used was for all amplification of 284 bp (base pair) fragment of InvAgene. For PCR amplification 12.5ul volume reaction mixture was used, containing 6.25ul master mix (1x master mix Taq), 2.5ul nucleus free water, 0.625 each InvA (forward and reverse) and 2.5 template DNA (Salehi *et al*., 2005).

Polymerase chain reaction amplification and electrophoresis

The mixture was vortexed, centrifuged briefly and inserted into PCR machine on Taq quick load with positive (known Salmonella isolate) and negative control (nucleus free water) included. The PCR cycling for reaction mixture was: initial mixture 95°C for 2 minutes, denaturing 95°C for 30seconds, annealing 53°C for 30 seconds, extension 72°C for 1 minute and final extension 72°C for 7 minutes which was repeated 35 times at 100 voltage for 40 minutes. The final PCR product was run on 1.5% agarose gel with 1 kb plus DNA ladder (75, 200, 300, 400, 500). It was observed under UV light (Panezai *et al*., 2018).

Antimicrobial susceptibility testing

A disc diffusion method was used and a total of seven (7) antibiotics (Oxoid) were used based on Clinical and Laboratory Standard Institute (CLSI, 2013) guideline (Cefuroxime 30 ug, Ceftriaxone 30ug, Gentamicin 10 ug, Chloramphenicol 30 ug, Nalidixic acid 30 ug, Ciprofloxacin 5ug and Ofloxacin 5 ug). The standardized inoculum was poured on a well-dried muller Hinton agar plate and excess were poured into a disinfectant jar, it was allowed to stand for 10 minutes and the antibiotic disc was placed on it and allows to diffuse into the medium. It was incubated at 37°C for 24 hours. After incubation, the zone of inhibition was observed and measured. The result was interpreted using the Clinical Laboratory Standard Institute (2013) and result reported as susceptible, intermediate and resistance.

Genome sequencing, assembly and annotation

The *Salmonella typhi* isolates were sequenced using Fastp for quality control of the reads (Chen *et al*., 2018). The command line used (supplementary file), trimmed fastq files were then assembled using Unicycler (Wick *et al*., 2017), contigs of assembled fasta files were then ordered against the reference genome of Salmonella typhi CT18 strain (Accession number GCA_000195995.1) using Mauve (Darling *et al*., 2004) "move contigs". To evaluate genome completeness Quast (Gurevich *et al*., 2013) was used. The two genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova *et al*., 2016). The functional annotation of genes was also carried out using web-based Rapid Annotation Using Subsystem Technology (RAST) annotation server (Brettin *et al*., 2015). The genome assembly was then subjected to sequence type (ST) analysis using the Salmonella in silico Typing Resource platform (Yoshida *et al*., 2016). Salmonella pathogenicity islands (SPI), plasmids and incompatibility group using Res.Finder (Zankari *et al*., 2012) and Plasmid-Finder-1.3 (Carattoli *et al*., 2014). To observe conserved synteny blocks, we aligned all isolates and *S.* Typhi CT18 using Progressive Mauve (Darling *et al*., 2016). The circular image and circular comparisons between multiple genomes were done by BLAST Ring Image Generator (BRIG) (Alikhan *et al*., 2011). Each circular genomic map was drawn using the genome of one reference strain (henceforth referred to as "alignment reference genome") on a local BLAST + basis, with standard parameters (70% lower – 90% upper cut-off for identity and *E*-value of 10). For genome synteny and collinearity analyses, D-GENIES (Cabanettes and Klopp, 2018) dot plots was used to compare the reference CT18 genome with the Salmonella typhi 1(SO1) and Salmonella typhi 2 (SO2) genomes. The web-based tool which uses performing large genome alignments using the minimap2 software package and generating interactive dot plots was used. To identify Salmonella pathogenic Island, the web tool SPIFinder-1.0 Server (https://cge.cbs.dtu.dk/) was used.

Genotype determination of Salmonella

To determine the genotype of the *S. typhi* isolates using the whole genome. The genomes of SO1 and SO2 were mapped against *Salmonella typhi* strain CT18 using Harvest Varients obtained from the mapping was then used to compare against global database for the genotype of the isolates.

Data analysis

The data was analysed using simple descriptive statistics, chi square on statistical package for the social sciences (SPSS 23). P-value ≤ 0.05 was considered statistically significant. The prevalence rate for the study population was also obtained using the formula:

$$\text{Prevalence (\%)} = \frac{\text{No of Positive}}{\text{Total no of sample}}$$ (Oyeniran *et al*., 2014)

RESULTS AND DISCUSSION

The result showed that out of a total of 183 stool samples collected, two were positive for Salmonella enterica typhi (1.1%) and three were positive for other Salmonella specie (1.6%) while 178 (97.3%) samples were negative (Table 1). The serotyping of isolates using polyvalent somatic O antigen, O:4, O:12, O:9, O:6, flagellar antigen H phase 1 & 2 and VI Capsular antigen showed that two of the isolates were Salmonella typhi, two were Salmonella para typhi C and one Salmonella para typhi A (Table 2). The detection of InvA gene in isolated Salmonella by PCR method generated 284 bp amplified DNA fragment on 1.5% agarose gel for the five isolates including positive control while the negative control showed no amplified DNA fragment (Figure 1). Figures 2 to 5 shows the level of genetic variations between SO1, SO2 and the reference CT18 strain. A number of inversions, deletions where observed in the genome of SO1 and SO2.

The prevalence of *Salmonella enteric typhi* from internally displaced person in Jos metropolis, Plateau State, is as presented in Tables 1 & 2 and Figure 1. These shows a decline in prevalence with 1.1% and 1.6% for Salmonella enterica typhi and other Salmonella respectively. Other studies have reported similar decline in prevalence of the infection in Abuja and Kano (Akinyemi *et al*., 2018); contrary to the report of Essa *et al*. (2019) and Abakpa *et al*. (2015) with high prevalence of 17.61% and 13.9% respectively. The decline in percentage positive of typhoid may be attributed to the difficulty in distinguishing between typhoid fever and other febrile conditions mostly in endemic areas as many other viral, bacterial and protozoan infections resemble that of typhoid fever. Improved health care provided by the government to the IDPs by the deployment of health workers to monitor health condition and dispense drugs by the state government is an intervention geared towards the reduction of typhoid fever and other infectious diseases on camp. This proactive step on the part of the government might help explain the decline in the observed cases of typhoid fever in the study. The IDPs were already undergoing treatment with antibiotics and there were also healthcare givers attending to them.

Typhoid fever is common in places with poor sanitation and a lack of safe drinking water, water for cooking and bathing may have faecal contamination which may aid the transmission of infection and can have a substantial impact on the disease dynamics in the camp as isolates can survive for days in water. In this study, the prevalence rate with respect to source of drinking water shows significant difference (Table 5) and it was observed that individuals with typhoid fever were those with poor sanitation and those who lacked potable water. This may be as a result of non-conformity with WHO standards and Guidelines to National Agencies for Food and Drug Administration control in Nigeria as reported by Akinyemi *et al*. (2018). This work is also in agreement with Ajayi *et al*. (2015) who reported that the influence of water source on typhoid fever prevalence showed that patient who sourced their water from well had the highest frequency.

There is a high prevalence of infection in female than in male (Table 4) which may be as a result of more numbers of female (70%) on camp than male (30%). Mooney (2005) also reported that Women and children constitute over 70% of IDPs. Health care-seeking behaviour of female may also contribute to the increased number and poor personal hygiene may also contribute to the high prevalence. According to Butler *et al*. (1991) studies have suggested that severity between sexes can vary geographically and be more common in females. This study agrees with Abioye *et al.* (2017) and Ezeigbo *et al*. (2015) who reported high prevalence in female than in the male. Similar research was carried out in the Mahama refugee camp according to Nyamusore *et al.* (2018) who reported a high prevalence in females (56.4%) and males (43.6%) and that in African societies women are known to be at risk of infectious disease but disagrees with the finding of Okonko *et al.* (2010) who reported high prevalence in male than in the female. Zailani *et al* (2004) reported that the disparity of the infection prevalence among gender in different geographical areas across the globe is expected as several factors ranging from cultural to physiologic and immunologic can affect the disease status of each gender.

The main clinical symptoms were fever abdominal pain headache and chill for those who showed clinical symptoms (13.1%) with the prevalence of typhoid fever (1.1%) and other Salmonella isolates (1.6%) ($p<0.01$). There was no record of infection with the asymptomatic individual, this means the population may not have carriers and the spread of disease may be minimal (Table 7).

The finding in this study shows that the age group of 21-25 had the highest prevalence with 11.1% within the age of the respondent (Table 3). The prevalence of age for *S. typhi* showed no significant difference which agrees with Rabiu *et al*. (2018) who reported insignificant statistics with age.

Crowded living conditions were observed in one of the camps which accounted for the increase in isolation. Other conditions such as poor toilet facilities and the absence of sick bay may have contributed to the IDP camp with a prevalence of 1.1% typhoid and 1.6% other Salmonella isolates while the uncrowded had no case of infection

Table 1. Prevalence of *Salmonella* isolates among internally displaced persons in the two camps.

Names of IDP camp	No examined (%)	No of female (%)	No of male (%)	No of VI, O, H positive *Salmonella* (%)	No of O, H positive *Salmonella* (%)
House of Recab	27.9	11.5	16.4	0	0
Geoscience	72.1	58.5	13.6	1.1	1.6
Total	100	70	30	1.1	1.6

Table 2. Serotype of *Salmonella* isolates among internally displaced persons

Serotype	Polyvalent O	H phase 1&2	O:4	O:9	O:12	O:6	VI
S typhi 1	+	+	-	+	+	-	+
S para typhi A	+	+	-	-	+	-	-
S para typhi C 1	+	+	-	-	-	+	-
S typhi 2	+	+	-	+	+	-	+
S para typhi C 2	+	+	-	-	-	+	-

Key: Positive for antisera (+); Negative for antisera (-).

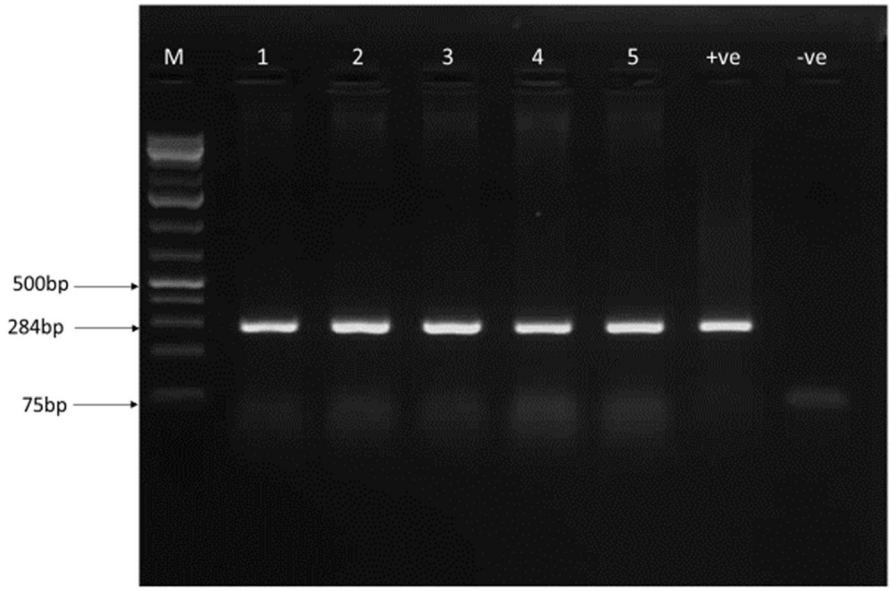

Figure 1. Molecular identification of Salmonella isolates using InvA gne. Lane M: 75bp marker, lane 1:*S typhi* 1, lane 2: *S typhi* 2, lane 3:S *para typhi* A, lane 4: *S para typhi* C 1, lane 5:*S para typhi C* 2, lane 6: *Salmonella typhi* as positive control, lane 7: nucleus free water as negative control.

(Table 6). There are also possibilities of sharing of utensils such as cups, spoons which were not properly cleaned and were shared between multiple individuals.

The antimicrobial susceptibility pattern showed a downward trend with Nalidixic acid and cefuroxime, and an upward trend was seen in the sensitivity pattern of ciprofloxacin, gentamicin, ceftriaxone, chloramphenicol and ofloxacin (Table 8). The downward trend shows intermediate which implies that the organisms are inhibited only by the maximum recommended dose. The upward trend showed by ciprofloxacin, ceftriaxone, gentamicin, ofloxacin and chloramphenicol shows sensitive which implies that the organism is inhibited by the serum concentration of the drug that is achieved using the usual dosage. Eibach *et al.* (2016) also reported fluoroquinolone susceptibility documented in West African countries. The variation in the susceptibility of *S. typhi* 1 (SO1) and *S. typhi* 2 (SO2) to some antibiotics such as ciprofloxacin, ofloxacin, cefuroxime and gentamicin may be as a result of host immune response, the virulence of the organism,

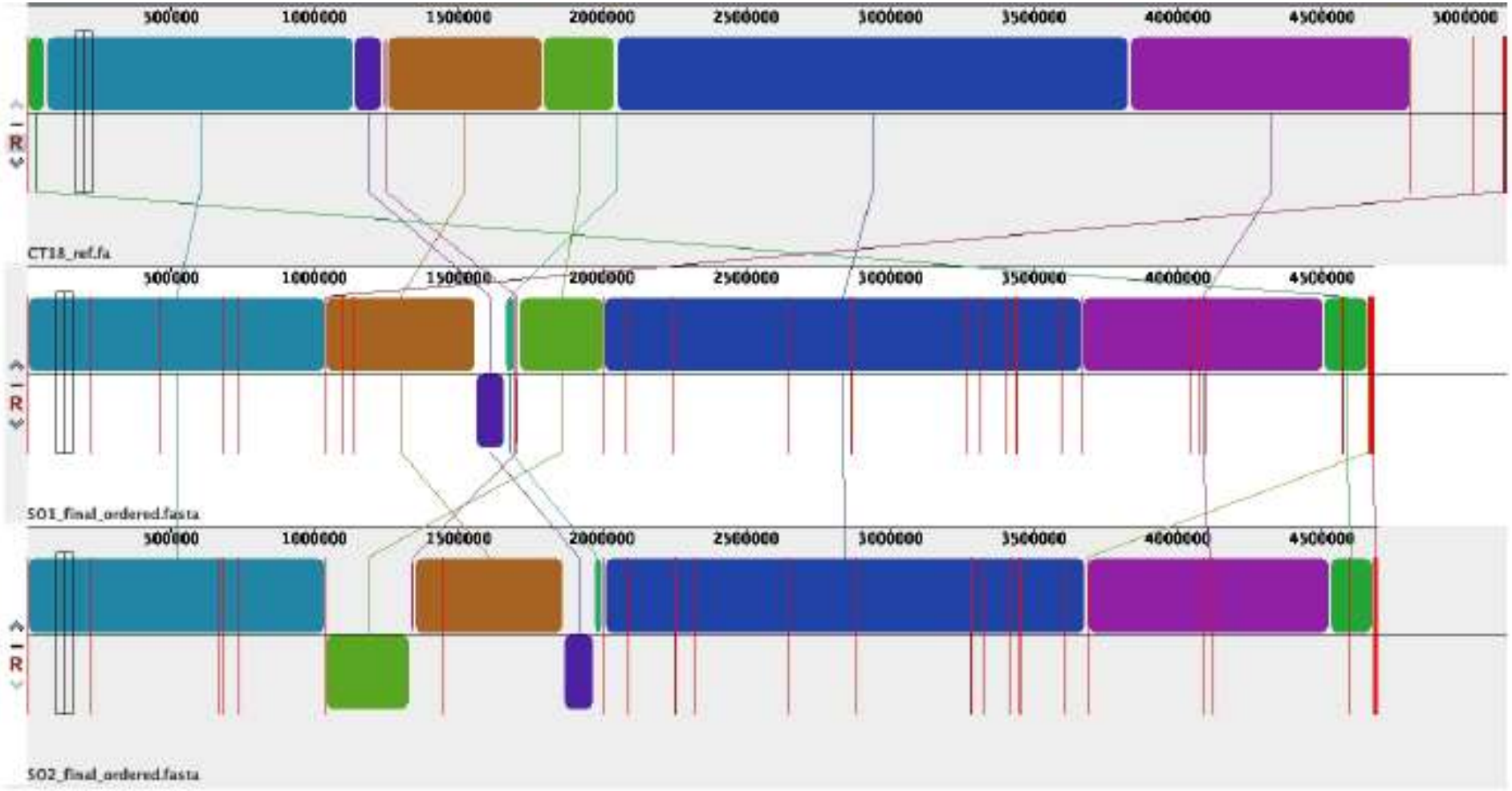

Figure 2. Mauve alignment of Reference isolate CT18 and isolates SO1, SO2. Coloured blocks indicates individual locally colinear blocks(LCB). Each coloured block represents similar sequences in the respective genomes. Homologous LCBs are connected with lines.

initial infective dose or age of the respondent. This high level of sensitivity by disc diffusion agrees with the findings of Gautam *et al*. (2002) who reported that in a search for improved treatment for enteric fever and multidrug resistance strain, in particular, attention has been focused on fluoroquinolone compounds and broad-spectrum cephalosporin because of their excellent properties. These highly active drugs reduce the duration of treatment and short treatment may reduce the period of hospitalization which will have obvious financial benefits particularly in developing countries. Antimicrobial susceptibility also affects the decision to use certain antimicrobial agents, it also determines when a patient does not respond to

Table 3**.** Prevalence of *Salmonella typhi* among internally displaced persons in Jos with respect to age.

Age	Count within status (%)	Positive for *S typhi* (%)	Count within status (%)	Positive for other *Salmonella spp* (%)	Negative (%)
0-5	0	0	0	0	10.9
6-10	0	0	0	0	11.5
11-15	1(2.7)	0.5	0	0	19.7
16-20	0	0	0	0	5.5
21-25	0	0	2(11.5)	1.1	8.7
26-30	0	0	0	0	9.3
30 above	1(1.7)	0.5	1(1.7)	0.5	31.7
Total	2	1.1	3	1.6	97.3
p-value	0.3				

The prevalence concerning age showed that age group 11-15 had the lowest prevalence of 2.7% out of 37, age group 21-25 showed the highest prevalence of 11.1% out of 18.

Table 4. Prevalence of *Salmonella typhi* among internally displaced persons in Jos with respect to sex

Sex	Count within status (%)	Positive for *S typhi* (%)	Count within status (%)	Positive for other *Salmonella* spp (%)	Negative (%)
Male	0	0	0	0	30.1
Female	2(1.6)	1.1	3(2.3)	1.6	67.2
Total	2(1.6)	1.1	3(2.3)	1.6	97.3
p-value	0.3				

The prevalence of *S typhi* concerning sex in the study population shows that S. typhi and other Salmonella isolate were only found in female. The result showed 1.6% was the rate of carriage of S typhi and 2.3% for other Salmonella spp.

Table 5. Prevalence of *Salmonella typhi* among internally displaced persons in Jos with respect to source of drinking water.

Source of water	Count within status (%)	Positive for *S typhi* (%)	Count within status (%)	Positive for other *Salmonella* spp (%)	Negative (%)
Pipe borne water	1(0)	0.5	0.0.	0.0	2.2
Well	1(0.8)	0.5	3(2.4)	1.6	67.2
Borehole	0	0.0	0.0	0.0	27.9
Total	2	1.1	3	1.6	97.3
p-value	0.001				

The prevalence rate concerning sources of drinking water showed a significant difference (P<0.05/0.001)) with the prevalence of 0.5% of *S typhi* in pipe-borne water,0.5% in well water and 1.6% of other *Samonella spp* with well water and negative for borehole water.

T**able 6.** Prevalence of *Salmonella typhi* among internally displaced persons in Jos with respect to population size.

Population size	Count within status (%)	Positive fo*r S. typhi* (%)	Count within status (%)	Positive for other *Salmonella* spp (%)	Negative (%)
Crowded	2(1.5)	1.1	3(2.3)	1.6	69.4
Uncrowded	0.0	0.0	0.0	0.0	27.9
Total	2(1.5)	1.1	3(2.3)	1.6	97.3
p-value	0.3				

The prevalence rate for population size showed no significant difference with a prevalence of S typhi 1.5% and other Salmonella spp 2.3% in the crowded population. The uncrowded population was negative for the infection.

treatment thought to be adequate, relapse while being treated or when there is immune suppression.

A visual inspection of the circular alignment of genomes SO1 and SO2 shows that the genomes are similar to the reference strain CT18. Genome wide re-arrangement was observed in the genomes of SO1 and SO2 (Figure 4). Coloured blocks in the first genome are connected by lines to similarly coloured blocks in the

Table 7. Prevalence of *Salmonella typhi* among internally displaced persons in Jos with respect to symptomatic and asymptomatic respondent.

Clinical manifestation	Count within status (%)	Positive for *S typhi* (%)	Count within status (%)	Positive for other *Salmonella* spp (%)	Negative (%)
Symptomatic	2(8.3)	1.1	3(12.5)	1.6	10.4
Asymptomatic	0.0	0.0	0.0	0.0	86.9
Total	2(8.3)	1.1	3(12.5)	1.6	97.3
P value	<0.001				

The clinical manifestation of symptomatic and asymptomatic individual shows that all asymptomatic individuals were negative and the prevalence rate showed significant difference (p<0.05/<0.001) with S typhi 8.3% and other Salmonella spp 12.5%.

Table 8. Antimicrobial susceptibility of *Salmonella* isolates from internally displaced persons in Jos Antimicrobial agents(ug)/ Zone of inhibition(mm).

Isolate	CXM (30)	NA (30)	OFX (5)	CN (10)	CRO (30)	CIP (5)	C (30)
S.typhi	17	17	24	19	24	26	23
S.typhi	15	17	27	16	27	29	21
Salmonella sp	22	19	24	21	28	30	20
Salmonella sp	17	17	24	18	24	30	18
Salmonella sp	16	15	22	21	28	32	16
Control *E. coli* ATCC 25922	22	22	29	22	31	31	16

The antimicrobial susceptibility pattern of the isolates against various antibiotics showed that all isolates were sensitive to ciprofloxacin, gentamicin, ofloxacin, ceftriaxone, and chloramphenicol while nalidixic acid and cefuroxime showed intermediate.

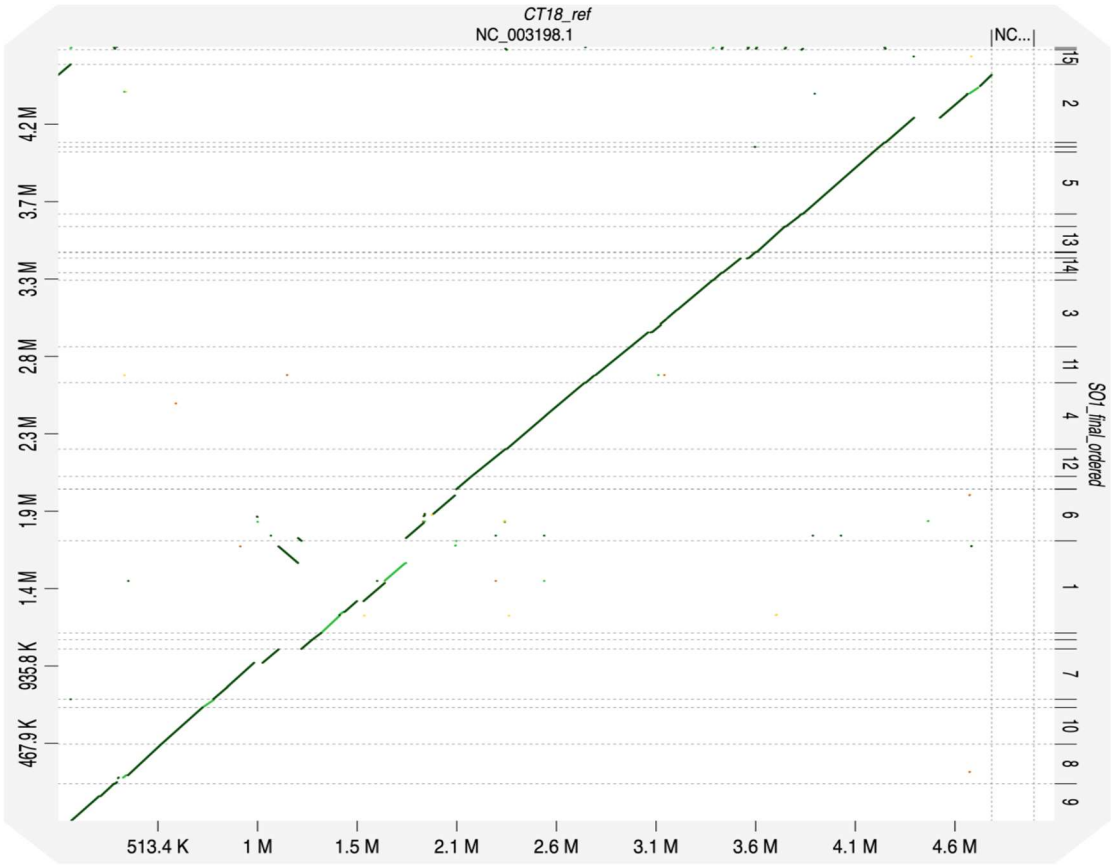

Figure 3a. DOT plot to SO1 shows the presence of genome breaks and inversions in the genome of SO1.

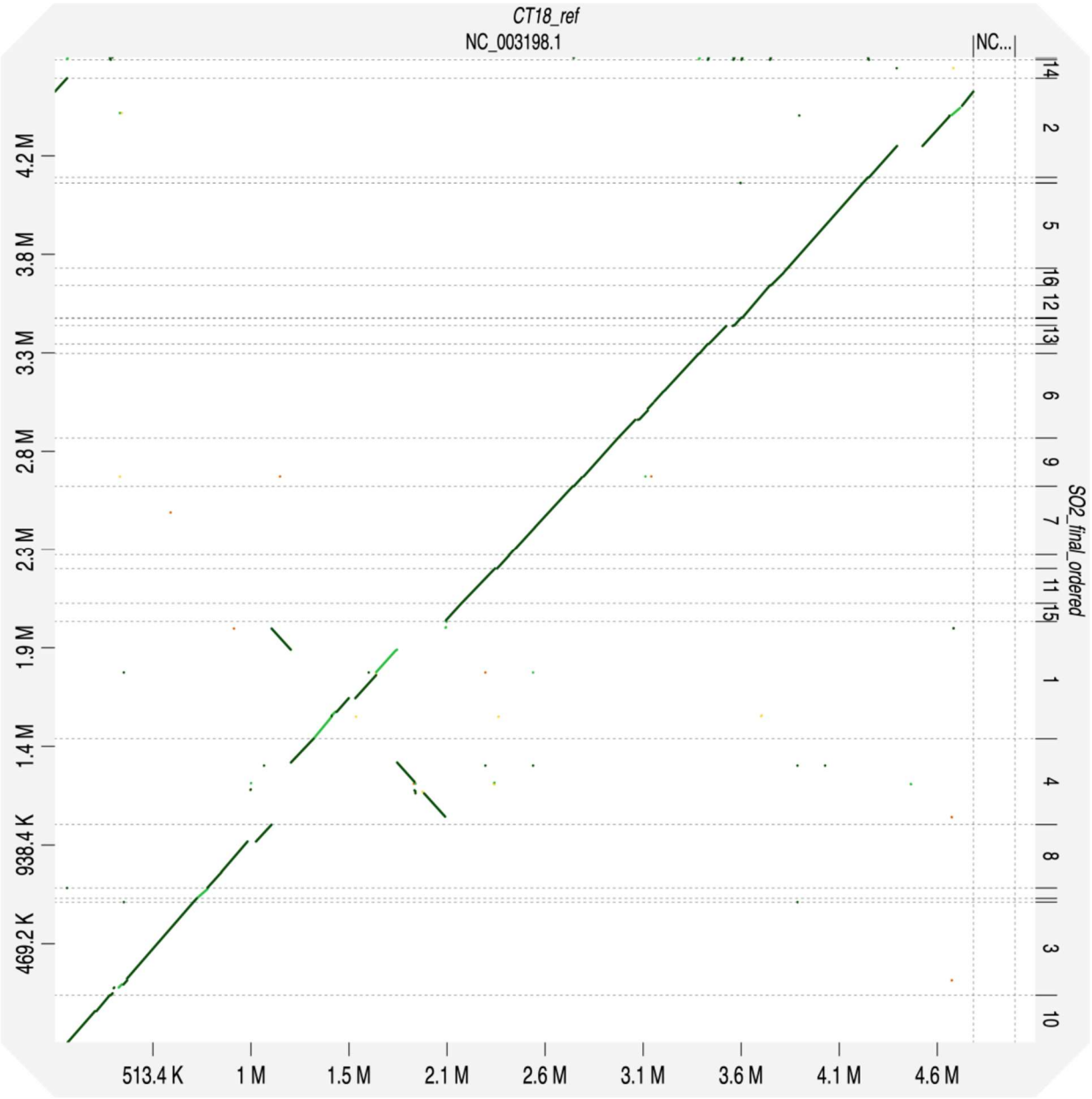

Figure 3b. DOT plot to SO2 shows the presence of genome breaks and inversions in the genome of SO2.

second and third genomes. These lines indicate which regions in each genome are homologous. Inversions and re-arrangements are indicated by the crossing lines (Figure 2). The genome of SO1 and SO2 has the presence of inversions that was further reinforced by the dot-plot result showing wide spread of genome gaps and inversions in the genomes of SO2 and SO1 (Figure 3a and 3b). The specific genes inverted and their impact on virulence was not evaluated in this study. Within the genomes of SO1 and SO2, gene deletions occur. Genomic rearrangement leading to gene loss or gain has been known to lead to genome evolution in *Salmonella* species.

Salmonella typhi SO1 and SO2 over the period of evolution have maintained conserved regions in the genome. An inspection of the BRIG image (Figure 4) shows the presence of conserved regions except for a few deletions in the genome of SO1 and SO2. Within the 5000 kbp region and the 4500 kbp region, the genomes of the three could not meet the lower limit of 70% similarity in the genome, showing a possibly high level of evolution in that area. Comparing the three genome proteins (Figure 5), the species form 4910 clusters, 1245 orthologous clusters (at least contains two species) and 3665 single-copy gene clusters. The three species shared 3683 gene clusters, between CT18 and SO1, only 16 gene clusters are unique to these two. SO1 and SO2 shared 1116 unique gene

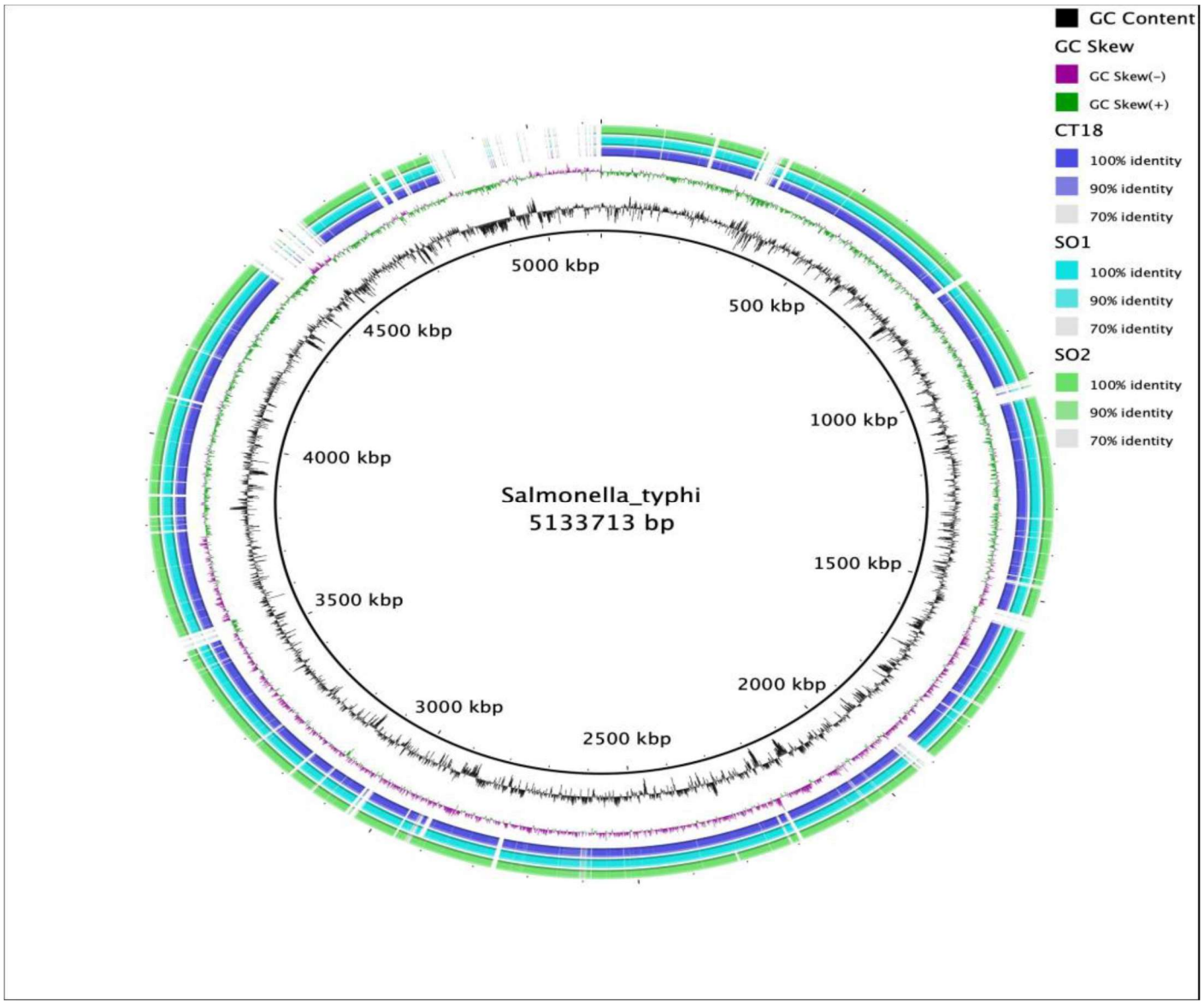

Figure 4. Comparative genomics of S. enterica QH and other serotype Salmonella strains. Shown are circular maps of genome sequences of Salmonella strains, generated using the BRIG package. BRIG image representation of SO1 and SO2 against the reference CT18 genome. The functions of the regions displaying low identities between different genome sequences are marked as bars at the outermost circle.

clusters. CT18 had 86 genes that were not found in both SO1 and SO2. In silico analysis of resistance profile showed that SO1 and SO2 were sensitive to 18 antimicrobials. However, the presence of resistance genes aac(6')-Iaa (aac(6')-Iaa_NC_003197) and gyrA (p.S83Y) that confers resistance against amikacin, tobramycin and nalidixic acid were identified both strains. Both strains possess the seven pathogenicity islands SPI-1 SPI-12, SPI-13, SPI-5, SPI-4, SPI-14 and centisome 63 pathogenicity island (C63PI). SPI-14 was detected in the study which according to Ikhimiukor *et al*. (2022) is common with non-typhoidal Salmonella.

General genomic features and comparative genomics on the two *S. typhi*, SO1 and SO2 obtained in this study were assembled. SO1 contained 38 contigs while SO2 had 35 contigs. The GC content of all chromosomes was 52%. SO1 and SO2 shared the same genome size of 4.6 mbp. SO1 has 4,838 protein coding sequences (CDS), 67 transfer RNA (tRNA) genes, and 4 ribosomal RNA (rRNA) genes, while SO2 genome has 4,841 protein coding sequences (CDS), 68 transfer RNA (tRNA) genes, and 4 ribosomal RNA (rRNA) genes. The sequence assembly was deposited to NCBI with Bioproject number PRJNA679969 and Biosample number SAMN16865206, SAMN16865207.

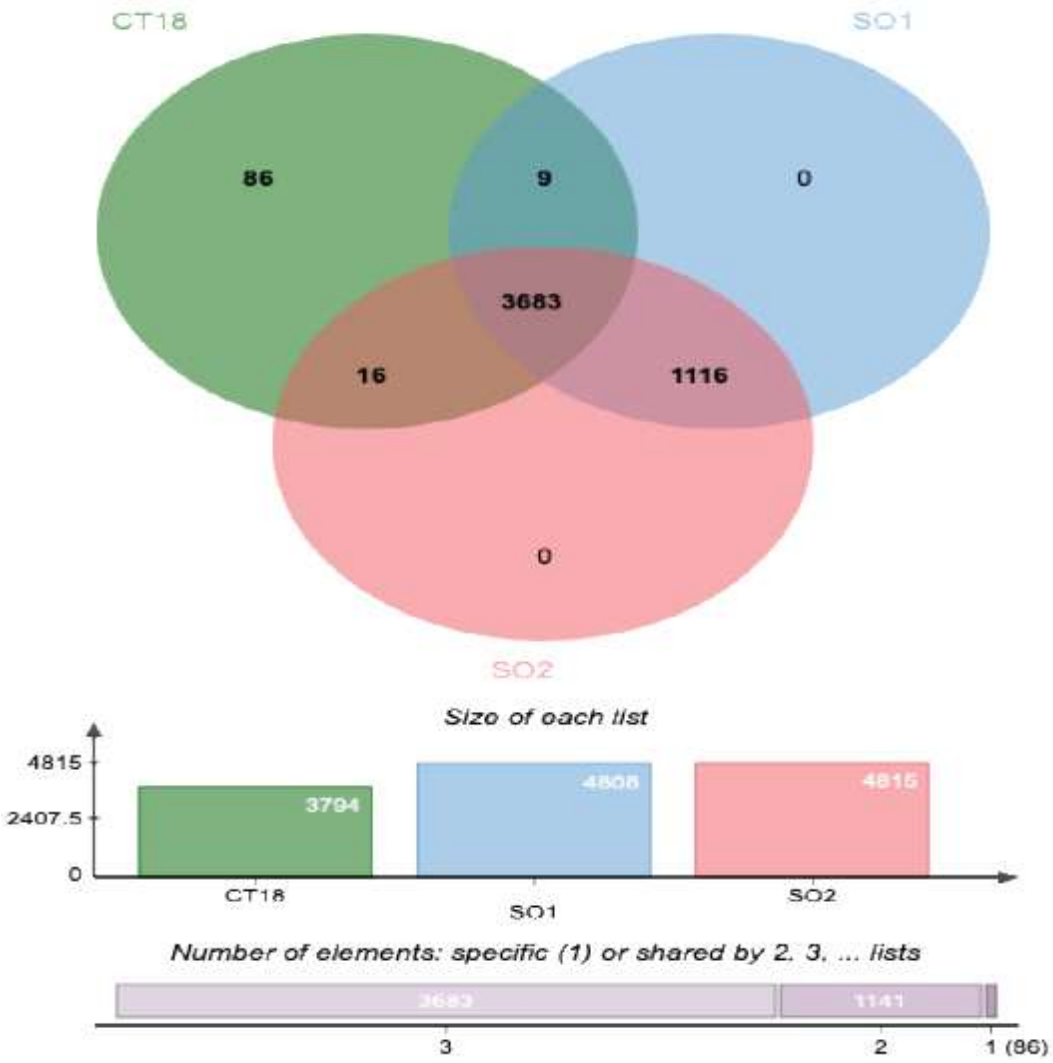

Figure 5. Venn diagrams showing shared and unique genes among SO1, SO2 and CT18. Shows the number of unique genes found in the annotated genomes of SO1 and SO2. No unique genes are found in SO1 and SO2 when compared to the reference CT18.

Conclusion

Salmonella enterica typhi is a leading cause of community-acquired infection in places such as IDP camps in which transmission is predominantly through water or food contaminated with human faeces. Culture remains the mainstay of laboratory diagnosis. There is an encouraging trend in antimicrobial susceptibility and effective antimicrobial therapy reduces mortality, complication and shortens the illness. A good approach strategy that would include the improvement of sanitation and safe water supply is essential. The government should increase the allocation of funds to the health sector and develop strategies for monitoring infectious diseases. The IDP camps should be provided with sickbay to meet health needs, clean sources of water and improve the sanitary condition of the camp.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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