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RESEARCH ARTICLE

In silico analysis of the whole genome of *Salmonella enterica*: genome assembly and annotation

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ABSTRACT

Aim: The goal is to classify *Salmonella enterica* using whole genome sequencing reads and explore their functional profiles. This approach simplifies resolving phylogenetic ambiguities in higher taxa compared to traditional methods.

Materials and Methods: Salmonella paired-end reads (SRA: SRR27334358) were obtained from the NCBI database and analyzed for quality using FastQC v0.12.1, with low-quality reads trimmed by Trimmomatic v0.36. De novo genome assembly was performed by using Unicycler v0.4.8, with subsequent gene annotation by using RAST. TYGS was utilized for taxonomic analysis. ResFinder v.2.1 identified antimicrobial resistance genes, and PathogenFinder v.1.1 was used for pathogenicity prediction. MLST analyzed the allele profile. CRISPR regions and proteins were identified by CRISPRCasFinder, while AntiSMASH 7.0.1 determined secondary metabolites. SPIFinder detected pathogenicity islands, and the genome map was created using the CGView server. RAST performed genomic functional classification.

Results: The genome, spanning 4,720,639 bp with 36 contigs, was analyzed by RAST, revealing 366 subsystems. TYGS showed a 100% dDDH with *S. enteritidis* ATCC 13076. The aac(6')-Iaa gene, conferring resistance to amikacin and tobramycin, was detected. PathogenFinder predicted *S. enterica* as a human pathogen with a 0.942 probability. MLST revealed 100% similarity with alleles of 7 housekeeping genes of Salmonella. CRISPRFinder identified eight Type I CRISPR-Cas proteins. AntiSMASH detected two secondary metabolites: enterobactin and O-antigen. SPIFinder identified 12 SPIs across the subspecies *S. Typhimurium*, *S. Typhi*, *S. Enteritidis*, *S. Choleraesuis*, and *S. Gallinarum*.

Conclusion: The genome showed 100% digital DNA-DNA hybridization (dDDH) with *Salmonella enteritidis* ATCC 13076 and was identified as a human pathogen. Recognizing pathogenic strains is crucial for timely intervention, control strategy design, and targeted vaccine development.

Keywords: *Salmonella enterica*, serovar, Salmonella Pathogenicity Islands, virulence

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INTRODUCTION

Salmonella is a type of Gram-negative bacteria that can infect humans and various animals, leading to self-limiting enteritis or systemic disease. It is a member of the Enterobacter family, close to Escherichia, and consists of rod-shaped bacteria that do not form spores (1). According to the latest nomenclature accepted by the Centers for Disease Control (CDC), the genus Salmonella consists of *Salmonella enterica* and *Salmonella bongori* species (2). Among these species, *S. enterica* comprises six subspecies: *S. enterica subsp. enterica* (I), *S. enterica subsp. salamae* (II), *S. enterica subsp. arizona* (IIIa), *S. enterica subsp. diarizona* (IIIb), *S. enterica subsp. houtenae* (IV) and *S. enterica subsp. indica* (VI). *S. bongori* is rarely isolated from clinical specimens, it is found in cold-blooded organisms. Therefore, almost all Salmonella organisms that cause disease in humans and domestic animals belong to the *S. enterica subspecies enterica* (I) (3). Salmonella infections are usually caused by the consumption of contaminated food or water. More than 2600 pathogenic serovars/serotypes have been identified for the six subspecies of *Salmonella enterica* using the Kauffmann-White scheme (4). Among these serotypes, *Salmonella typhimurium*, *enteritidis*, *typhi*, *newport*, *heidelberg* and *paratyphi*, have been identified as important pathogens for humans and domestic animals (5). *Salmonella typhi* (*S. typhi*) and *S. paratyphi* cause typhoid fever, a systemic febrile disease that affects only humans. Other NTS serovars such as *S. typhimurium* and *S. enteritidis* infect many different hosts and cause diarrhea (6). Following ingestion, *S. enterica* (*S. enterica serovar typhimurium* and *S. enterica serovar enteritidis*) invades the intestinal epithelium in the colon and ileum and causes sepsis or spreads to systemic sites and causes neutrophilic gastroenteritis (7). Serotype cholerasuis is a host-compatible pathogen causing swine paratyphoid fever. It is also highly pathogenic for humans, usually causing a septicemic disease with little intestinal tract involvement (8). *Salmonella enterica serovar gallinarum* is a pathogen that is responsible for acute and chronic chicken typhoid fever (9).

S. enterica species are usually transmitted orally and cause four main syndromes: enteric fever (typhus), enterocolitis/diarrhea, bacteremia and chronic asymptomatic carriage. Disease manifestation relies on both susceptibility of the host and infectious *S. enterica serovar* (3). Pathogenic Salmonella species attack non-phagocytosing intestinal epithelial cells by transporting a specialized set of agents through an advanced mechanism called the type 3 secretion system (T3SS). This mechanism plays an essential role in the pathogenesis of Salmonella (10).

The Salmonella chromosome contains multiple virulence mechanisms to execute the pathogenic process. The most essential virulence genes are found in regions called Salmonella Pathogenicity Islands (SPIs) (11). Salmonella species use different virulence programs to interact with host defense mechanisms during infection, causing significant host immunopathology, morbidity and mortality (1). The ability of Salmonella to cause disease is related to its ability to survive in host cells. The genes used for this ability are located on pathogenicity islands such as SPI-1 and SPI-2 and encode two independent type III secretion systems (called T3SSSPI-1 and T3SSSPI-2). These mechanisms introduce effector proteins, which are essential throughout different phases of infection, into host cells. Currently, 17 SPIs thought to be acquired by horizontal gene transfers have been identified (12). Clusters of genes that contribute to specific phenotypes are typically found in pathogenicity islands, which usually manifest at particular stages during the infection process. Some SPIs are present in all *Salmonella serovars*, while others are present only in certain serotypes (13).

Today, with the advancement of sequencing technologies, the detection of genetic diseases has become both faster and more reliable (14-20). Therefore, sequencing methods and bioinformatics studies in humans and other organisms are becoming more and more important as time progresses. This research conducted a thorough examination of the complete genome of Salmonella enterica bacteria using sequence data acquired from the Illumina MiSeq platform. Within the framework of in silico analyses, the *Salmonella enterica* genome was assembled and annotated, and various in silico analyses such as taxonomic analysis, identification of antimicrobial resistance genes, identification of pathogenicity islands and CRISPR regions, and detection of MLST alleles and secondary metabolites were carried out.

MATERIALS AND METHODS

Acquisition of whole genome sequencing reads and analysis of genomic features

Raw Illumina paired-end reads of Salmonella with accession number SRR27334358 SRA were downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/sra) in fastq format. Raw sequence reads were qualitatively evaluated using FastQC v0.12.1 (https://narrative.kbase.us). Low-quality reads were removed by Trimmomatic v0.36 (https://narrative.kbase.us). After this step, the quality of the reads was checked again with FastQC v0.12.1.

Genome assembly and annotation

The quality-controlled reads were de novo assembled by Unicycler v0.4.8 (https://narrative.kbase.us). Then, the quality of the sequence (genome size, contig number, N50 value, G+C content, etc.) was assessed using Quast v4.6.3 (https://narrative.kbase.us). Subsequently, the sequence was annotated using the Rapid Subsystem Technology (RAST) (https://rast.nmpdr.org/rast.cgi) tool.

Whole genome based taxonomic analysis

To conduct a comprehensive taxonomic analysis, the resulting assembled genome acquired by Unicycler v0.4.8 (https://narrative.kbase.us) was then submitted to the Type Strain Genome Server (TYGS) (https://tygs.dsmz.de). Then, the genome and phylogenetically related type strains were compared by Genome Blast Distance Phylogeny (GBDP). Inter-genome distances were estimated with 100 replicates utilizing the trim algorithm and the d5 distance formula. Using the recommended settings of the Genome-to-Genome Distance Calculator (GGDC) 2.1, digital DNA-DNA hybridization (dDDH) values and their associated confidence intervals were determined. The resultant inter-genome distances were utilized to build a balanced minimum evolutionary tree using FASTME 2.1.4 and SPR post-processing. The average nucleotide identity (ANI) of isolates was calculated using FastANI 0.1.2, utilizing the genomes of Enterobacter species that are closely related as reference genomes.

Identification of antimicrobial resistance determinants

Antimicrobial resistance genes in *Salmonella enterica* were identified from WGS data using ResFinder v.2.1 (https://www.genomicepidemiology.org).

Pathogenicity prediction

PathogenFinder v.1.1 (http://www.genomicepidemiology.org) was utilized to estimate the potential pathogenicity of *Salmonella enterica*.

Multiple locus sequencing typing (MLST) profile of *Salmonella enterica*

The MLST profile of *Salmonella enterica* was determined using MLST v.1.8 (http://www.genomicepidemiology.org) from WGS data using Illumina paired-end fastq files.

Predicting genomic islands and regularly interspaced short clustered palindromic repeats (CRISPR)

Genomic islands and regularly interspaced short clustered palindromic repeats (CRISPR) were predicted by CRISPRCasFinder (https://crisprcas.i2bc.paris-saclay.fr).

Secondary metabolite prediction

Gene clusters encoding secondary metabolites of known gene clusters were predicted by AntiSMASH 7.0.1 web server (https://antismash.secondarymetabolites.org/).

Prediction of pathogenicity islands

Salmonella Pathogenicity Islands (SPIs) were detected using SPIFinder (http://www.genomicepidemiology.org).

Circular genome mapping and gene prediction

The genome map of *Salmonella enterica* was predicted using CGView (https://cgview.ca). *Salmonella enteritidis* ATCC 13076 strain and its full sequence genome available in the NCBI database (S77744.1) were used as reference for comparison.

Genomic functional classification

The 4,720,639 bp draft genome was annotated with the help of the RAST (https://rast.nmpdr.org/rast.cgi) system. Genes involved in virulence, disease and defense mechanisms of Salmonella enterica genome and their functions were identified.

RESULTS

Genome assembly and annotation

The features of the draft complete genome sequence of *Salmonella enterica* were analyzed. The genome size of *S. enterica* is 4,720,639 bc with a G+C content of 52.1%. The draft genome consists of 4,744 coding sequences. The total number of RNAs is 79 and includes 366 subsystems (Table 1).

The RAST server provided information on 366 categorized subsystems, revealing the highest number of category features for carbohydrates (353), Amino acids and derivatives (346), protein metabolism (217) cofactors, vitamins, prosthetic groups, pigments (168), respiration (126), DNA metabolism and Stress response (88), Nucleosides and Nucleotides (82) (Figure 1).

Table 1. Genomic features of the genus *Salmonella enterica*

Description	Value
Chromosome size (bp)	4,720,639
GC content (%)	52,1
C366ontig (via PEG)	36
Contig N50	406,165
Contig L50	4
Number of Coding Sequences	4.744
Number of RNA	79
Number of Subsystems	366

Whole genome-based taxonomic analysis

According to the results of TYGS whole genome-based taxonomic analysis of *S. enterica*, the genome sequence analyzed is closer to *S. enteritidis* ATCC 13076 (Figure 2).

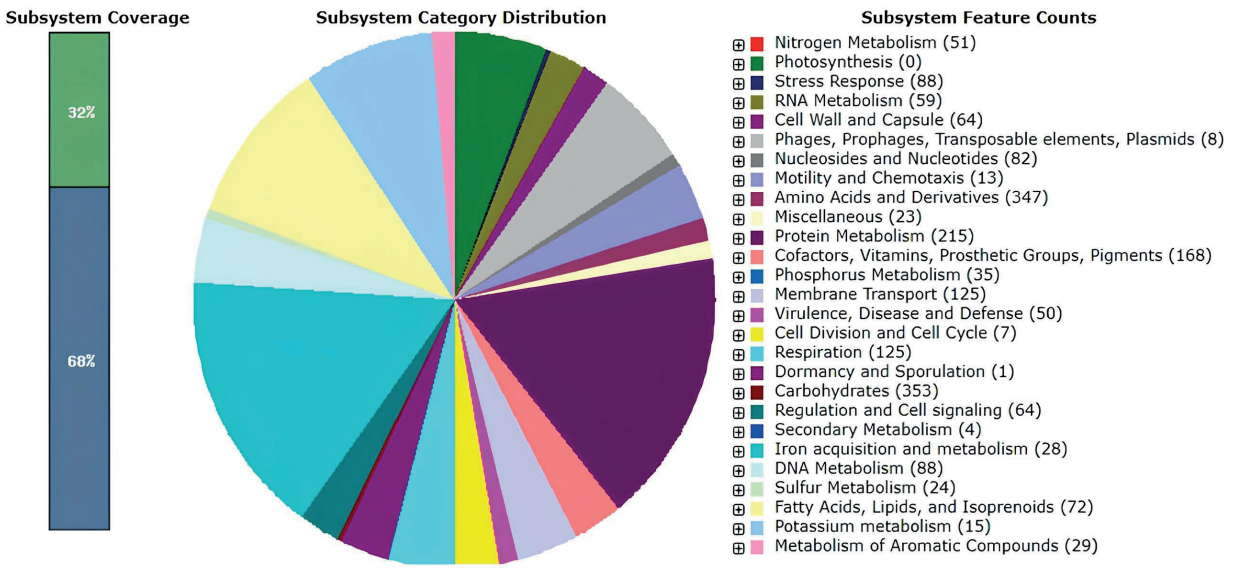

Figure 1. Subsystem distribution of the genus *Salmonella enterica* based on the RAST annotation server and functional classification of predicted genes in Salmonella.

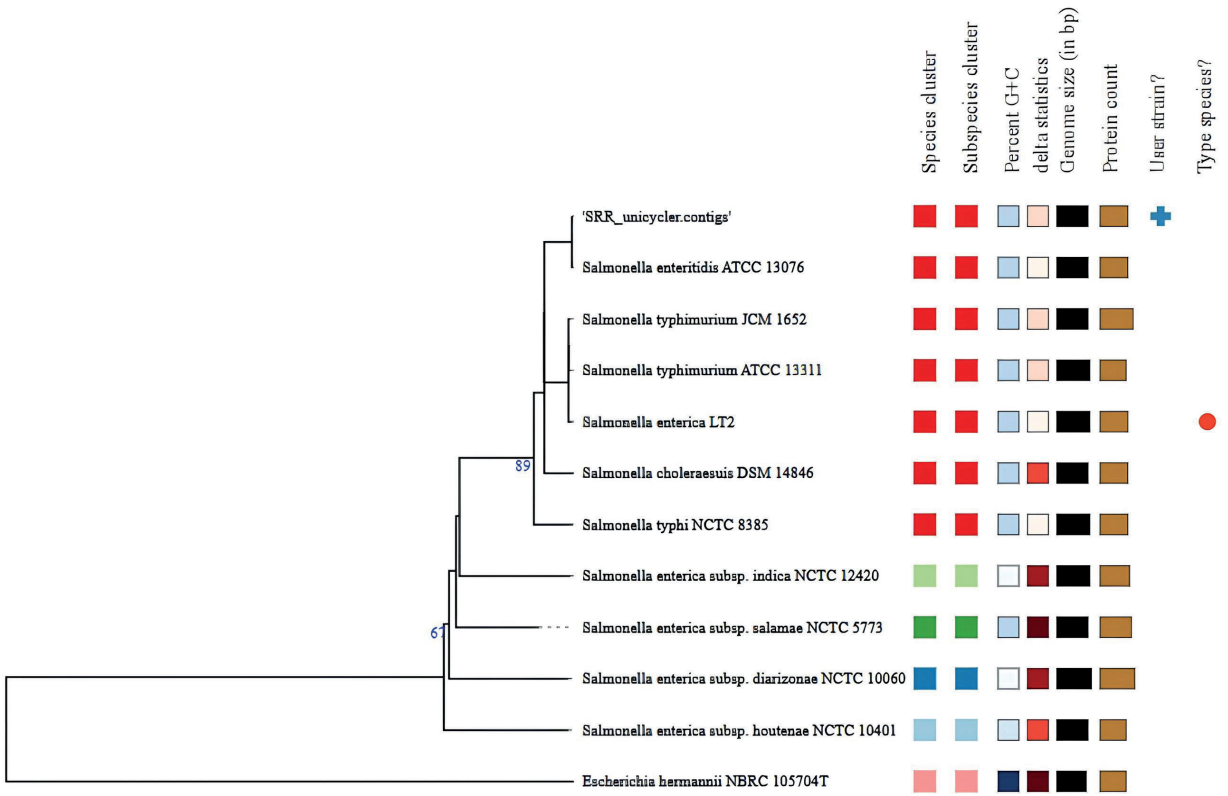

Figure 2. Tree inferred with FastME 2.1.6.1 from Genome Blast Distance Phylogeny (GBDP) distances calculated from whole genome sequences.

Table 2. Type Strain Genome Server (TYGS) calculation of digital DNA-DNA hybridization of *Salmonella enterica* and some closely related strains

Input Type	Output Type	dDDH (d4, %)	C.I. (d4, %)	G + C content difference (%)
SRR unicycler.contigs.fa	*Salmonella enteritidis* ATCC 13076	100.0	[99.9- 100.0]	0.02
SRR unicycler.contigs.fa	*Salmonella typhimurium* ATCC 13311	91.4	[89.3- 93.2]	0.01
SRR unicycler.contigs.fa	*Salmonella typhimurium* JCM 1652	91.2	[89.0- 92.9]	0.06
SRR unicycler.contigs.fa	*Salmonella enterica* LT2	90.9	[88.7- 92.7]	0.12
SRR unicycler.contigs.fa	*Salmonella choleraesuis* DSM 14846	90.3	[88.0- 92.2]	0.02
SRR unicycler.contigs.fa	*Salmonella typhi* NCTC 8385	86.1	[83.5- 88.4]	0.03
SRR unicycler.contigs.fa	*Salmonella enterica* subsp. salamae NCTC 5773	67.8	[64.8- 70.6]	0.04
SRR unicycler.contigs.fa	*Salmonella enterica* subsp. indica NCTC 12420	64.7	[61.7- 67.5]	0.55
SRR unicycler.contigs.fa	*Salmonella enterica* subsp. diarizonae NCTC 10060	62.0	[59.1- 64.8]	0.71
SRR unicycler.contigs.fa	*Salmonella enterica* subsp. houtenae NCTC 10401	60.5	[57.7- 63.3]	0.35
SRR unicycler.contigs.fa	*Escherichia hermannii* NBRC 105704T	21.1	[18.9- 23.6]	1.95

In addition to the resulting species and subspecies clusters, there is a taxonomic identification of *Salmonella enterica*. *Salmonella enterica* has 100% digital DNA-DNA hybridization (dDDH) and 0.02% G+C content difference with *Salmonella enteritidis* ATCC 13076, the closest strain genome in the TYGS database (Table 2).

Identification of antimicrobial resistance genes

Analysis with the ResFinder tool revealed aminoglycoside resistance genes. The aac(6')-Iaa gene, which has the potential to confer resistance to amikacin and tobramycin, was identified in *Salmonella enterica*.

Pathogenicity prediction

The pathogenicity of *Salmonella enterica* was estimated by comparing its proteins against a database of protein families associated with pathogenic and non-pathogenic bacteria. PathogenFinder predicted that *Salmonella enterica* is a human pathogen (probability of being pathogenic = 0.942). Proteins in *S. enterica* matched 1124 pathogenic families and 2 non-pathogenic families.

Multiple locus sequence typing (MLST)

It was revealed that Chorismate synthase (aroC_5), DNA polymerase III subunit beta (dnaN_2), uroporphyrinogen III synthase (hemD_3), histidinal dehydrogenase (hisD_7), phosphoribosylaminoimidazole carboxylase (purE_6), 2-oxoglutarate dehydrogenase decarboxylase (sucA_6), aspartokinase I (thrA_11) showed 100% similarity with alleles of the housekeeping gene in the *Salmonella enterica* genome.

Prediction of clustered regularly interspaced short palindromic repeats (CRISPRs)

The *Salmonella enterica* genome harbors two CRISPR sequences of 11 and 9 repeats, respectively, with a repeat length of 29 bc, matching two consensus sequences with evidence level 4 according to CRISPRFinder analysis. It also harbors 8 Type I CRISPR -Cas associated proteins (Table 3).

Secondary metabolites

In this study, two metabolite regions were detected using the AntiSMASH web server. Region 3.1 (location: 297,771- 352,985 nt) contained NRP-metallophore and NRP in contig 3; region 9.1 (location: 32,069-59,841 nt) contained thiopeptide in contig 6 (Table 4).

Table 3. Clustered regularly interspaced short palindromic repeats (CRISPR) sequences found in *Salmonella enterica* strain using CRISPRCasFinder

Element	CRISPR id/ Cas gene	Start	End	Spacer/ Gene	Repeat Consensus/Cas Gene	Direction	Level of Evidence
CRISPR	2_length_760858_1_14x_1	501789	502489	11	GTGTTCCCCGCGCCAGCGGGGATAAACCG	+	4
Cascluster	CAS-TypeIE	510092	518545	8	cas3_TypeI, cse1_TypeIE,cse2_TypeIE, cas7_TypeIE, cas5_TypeIE, cas6_TypeIE, cas1_TypeIE, cas2_TypeIE		
CRISPR	2_length_760858_1_14x_2	518642	519219	9	GTGTTCCCCGCGCCAGCGGGGATAAACCG	+	4

Table 4. Secondary metabolites identified by antiSMASH in *Samonella enterica*

Region	Type	Start	End	Most Similar Known Cluster	Similarity
Region 3.1	NRP- metalophor, NRPs	297,771	352,985	Enterobactin	100%
Region 9.1	Thiopeptide	32,069	59,841	O-antigen	14%

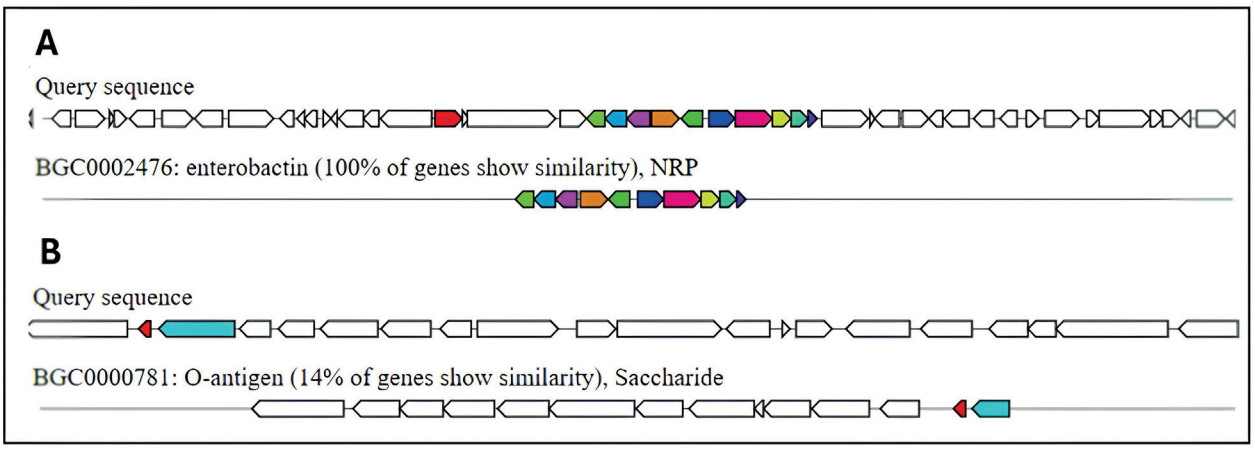

Figure 3. Biosynthetic gene clusters (BGCs) detected in the genome sequences of Salmonella enterica. A) Enterobactin biosynthetic gene cluster; B) O-antigen biosynthetic gene cluster.

Table 5. Pathogenicity islands of the *Salmonella enterica* genome analyzed with the SPIFinder tool

Subspecies	Serotype / Serovar	SPA	NCBI Number
Salmonella enterica - typhimurium	SL1344	C63PI	AF128999
	ATCC_14028	CS54_island	AF140550
	J4STEHO	SPI-1	JN982040
	SL1344	SPI-1	U16303
	SL1344	SPI-1	AF148689
	LT2	SPI-2	X99945
	Salmonella-enterica-Typhimurium-	SPI-2	Z95891
	14028s	SPI-3	AJ000509
	14028s	SPI-3	Y13864
	LT2	SPI-5	NC_003197
Salmonella-Typhi	CT18	SPI-9	NC_003198
Salmonella enterica- enteritidis	CMCC50041	Not_named	JQ071613
	C50041	SPI-2	JN673273
	Se11	SPI-2	JN673270
Salmonella enterica - choleraesuis	SC_B67	SPI-4	NC_006905
Salmonella enterica-gallinarum	SGB_1	SPI-1	AY956822
	SGE_2	SPI-1	AY956823
	SGB_4	SPI-1	AY956824
	SGB_8	SPI-1	AY956825
	SGE_3	SPI-10	AY956839
	SGD_3	SPI-13	AY956832
	SGA_10	SPI-13	AY956834
	SGA_8	SPI-14	AY956835
	SGC_8	SPI-14	AY956836
	SGB_10	SPI-2	AY956826
	SGC_2	SPI-2	AY956827
	SGC_9	SPI-2	AY956828
	SGH_1	SPI-2	AY956829
	SGD_8	SPI-2	AY956830

When the most similar gene clusters were analyzed by comparison with the MiBIG database, two cluster regions were identified. The first of these gene clusters was the NRP biosynthetic gene cluster from Escherichia coli (21) and the O-antigen biosynthetic gene cluster from Pseudomonas aeruginosa (Figure 3).

Salmonella pathogenicity islands (SPI)

As a result of the analysis performed with SPIFinder 2.0, 29 Pathogenicity Islands belonging to the total Salmonella genome were detected. As a result of the analysis, 6 pathogenicity islands belonging to 10 serovars of *S. typhimurium* subspecies, 1 belonging to one serovar of *S. typhi* subspecies, 2 belonging to 3 serovars of *S. enteritidis* subspecies, 1 belonging to one serovar of *S. choleraesius* subspecies and 5 belonging to 14 serovars of *S. Gallinarum* subspecies were identified (Table 5).

Circular genome mapping and gene prediction

The genome contains 4510 genes, 4427 protein-coding genes (CDS), 77 tRNA, 3 rRNA, 2 repetitive regions and 1 tmRNA copy. The physical genome map of *Salmonella enterica* was obtained by comparison with the reference strain *Salmonella enteritidis* ATCC 13076 (Figure 4). Prokka was used for position prediction, while BLAST was used to obtain information on function and identification in the nucleotide and protein sequence database against the assembled sequences.

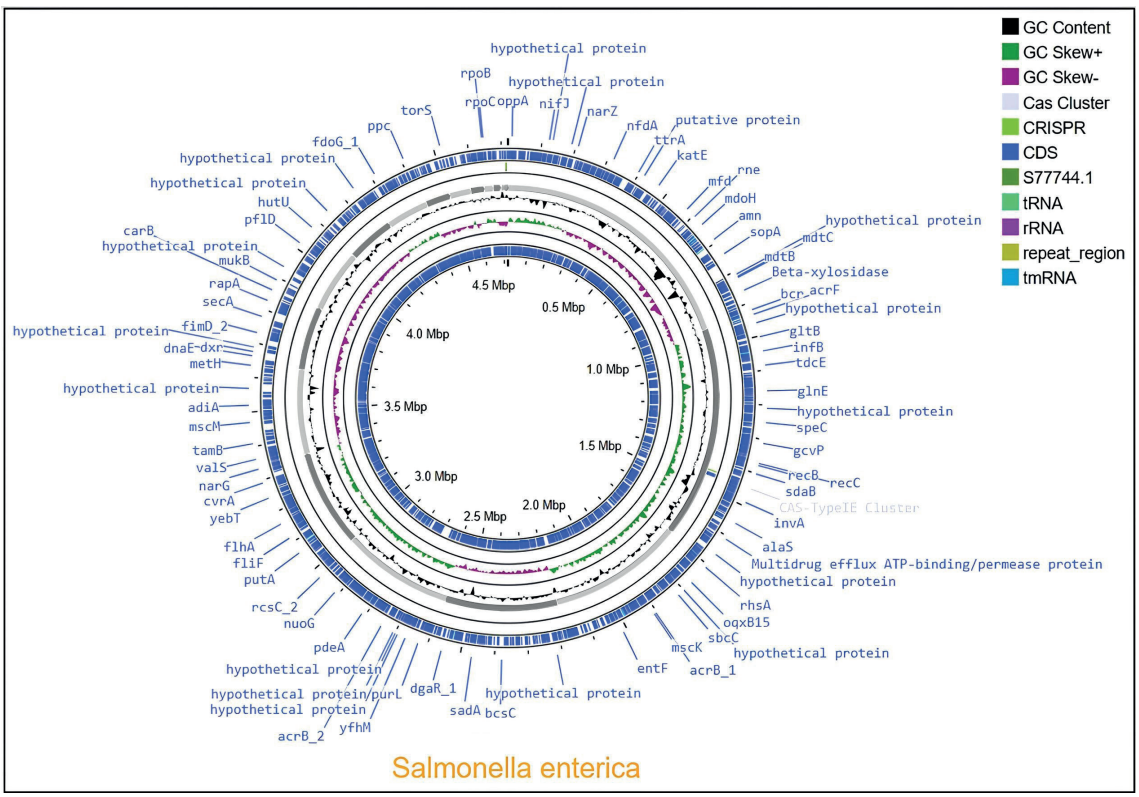

Figure 4. Circular map view of the *Salmonella enterica* genome generated using the CGView Server. The contents are organized starting from the outermost ring: the outermost first and last rings show the Prokka annotation (+/- helix) together with coding sequences (CDS), tRNA, rRNA, tmRNA and repetitive regions; the second ring represents the map of the reference taxon Salmonella enteritidis ATCC 13076; third ring shows CRISPRCasFinder anotation (+ helix); fourth ring shows contigs; fifth ring shows GC content; sixth ring shows GC skew information; seventh ring shows CRISPRCasFinder anotation (- helix).

Genomic functional classification

The genes of *Salmonella enterica* genome that play a role in virulence, disease and defense mechanisms and their functions were determined. It was determined that *S. enterica* has genes that function against bacteriocins, adhesion, invasion and intracellular resistance and antibiotics and toxic compounds (Table 6).

DISCUSSION

In this study, reads of Illumina sequencing of *Salmonella enterica* coded SRR27334358 in the Sequence Read Archive (SRA) database were merged and annotated. Salmonellosis is an essential public health problem caused by Salmonella bacteria and causes a significant increase in morbidity and mortality.

Table 6. Characteristics of Salmonella enterica that play a role in virulence, disease and defense mechanisms

Subcategory	Subsystem	Description				Function
		From	To (bp)	Size (bp:aa)	Contig	
Bacteriocins are antibacterial peptides that are synthesized by ribosome	Tolerance to Colicin E2	77300	76812	489;163	10	Conserved undefined protein CreA
		74628	73279	1350;450	10	Inner membrane protein CreD
Adhesion	YidE mediates hyperadherence in enterobacteria and in the conserved zone	403542	403114	429;143	4	16 kDa heat shock protein B
		404071	403652	420;140	4	16 kDa heat shock protein A
		405935	404709	1227;409	4	Uncharacterized YidR protein
		404417	404707	291;97	4	Outer membrane YidQ lipoprotein
Invasion and intracellular resistance	Mycobacterium virulence operon involved in DNA transcription.	4199	8227	4029;1343	15	DNA-directed RNA polymerase beta subunit
		8304	12527	4224;1408	15	
	Mycobacterium virulence operon likely involved in quinolinate biosynthesis	480054	481157	1104;368	3	Kinolinat sentetaz
		15968	14346	1623;241	5	L-aspartate oxidase
		123211	124104	894;298	8	Quinolinate phosphoribosyltransferase [decarboxylating]
	Mycobacterium virulence operon involved in protein synthesis (SSU ribosomal proteins)	3405	3031	375;125	4	SSU ribosomal protein S12p (S23e)
		2935	2465	471;157	4	SSU ribosomal protein S7p (S5e)
		2368	254	2115;705	4	Translation Elongation factor G
		2	82	81;27	15	Translation Elongation factor Tu
		26072	25992	81;27	16	
		1103	3	1101;367	18	
		182	3	180;60	4	
	Mycobacterium virulence operon involved in protein synthesis (LSU ribosomal proteins)	451004	450570	435;145	1	Translation initiation factor 3
		450474	450277	198;66	1	LSU ribosomal protein L35p
		450226	449870	357;119	1	LSU ribosomal protein L20p
Resistance to antibiotics and toxic compounds	Copper homeostasis	78317	80620	2304;768	3	ATPase transporting lead, cadmium, zinc and mercury; P-type ATPase transporting copper
		240386	237885	2502;834	3	
		142376	144574	2199;733	4	

Table 6. Continued

Subcategory	Subsystem	Description				Function
		From	To (bp)	Size (bp:aa)	Contig	
Resistance to antibiotics and toxic compounds	Copper homeostasis: copper torelance	95582	93972	1611;537	8	CueO precursor to blue copper oxidase.
		935	3	933;311	17	Cytochrome c heme lyase subunit CcmL /
		3417	1486	1932;644	17	
		406164	406057	108;36	4	
		2	109	108;36	6	Cytochrome c heme lyase subunit CcmH
		210893	211267	375;125	6	Copper resistance protein CopC
		211268	212143	876;292	6	Copper resistance protein CopD
		64440	64802	363;121	6	Suppression of copper sensitization: putative copper-binding protein ScsA.
		64851	66737	1887;629	6	Membrane protein, copper sensitivity suppressor ScsB
		66734	67357	624;204	6	The secreted protein is the copper sensitization suppressor ScsC.
		67347	67853	507;169	6	Membrane protein, copper sensitivity suppressor ScsD
		151391	151738	348;116		Periplasmic divalent cation tolerance protein CutA
		179718	180464	747;249	6	Cytoplasmic copper homeostasis protein CutC
		7186	6485	702;234	8	Copper homeostasis protein CuffF precursor / Lipoprotein NlpE involved in surface adhesion
		404058	402469	1590;530	3	Apolipoprotein N-acyltransferase / Copper homeostasis protein CutE
		404905	404027	879;293	3	Magnesium and cobalt streaming protein CorC
	Mercury reductase	300227	301552	1326;442	3	Putative Dihydrolipoamide dehydrogenase; Mercury ion reductase; PF00070 family, FAD-dependent NAD(P)-disulfide oxidoreductase.
	Resistance to fluoroquinolones	377955	380591	2637;879	5	DNA gyrase subunit A
		22754	20340	2415;805	13	DNA gyrase subunit B
	Cobalt-zinc-cadmium resistance	482836	481898	939;313	3	Zinc carrier ZitB
		80617	81081	465;155	3	Transcriptional regulator, MerR family
	Zinc resistance	26727	28124	1398;466	15	Sensor protein of the zinc sigma-54-dependent two-component system
		28130	29455	1326;442	15	
	Adaptation to D-cysteine	138465	139451	987;329	6	D-cysteine desulfhydrase

There are more than 2600 serovars of Salmonella species and they are transmitted by fecal or oral route through contaminated water and food (4). Salmonella serotyping has an important function in diagnosis and detection. Serovar prediction by conventional serotyping may be limited due to lack of surface antigen expression or autoagglutination (22). Recently, serovar identification can be performed with the advancement of whole genome sequencing platforms. Many research have used whole genome sequencing-based genomic comparison to identify serovar-specific genes or DNA segments for serotyping (23). In this study, we tried to show that serovar identification by genome sequencing technology is more efficient than traditional methods.

To affiliate the species, the DNA-DNA hybridization threshold should be 70% as a main criteria (24). Digital DNA-DNA hybridization (dDDH) calculated by the Genome-Genome Distance Calculator (GGDC) between the analyzed genome and *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* ATCC 13311, *Salmonella typhimurium* JCM 1652, *Salmonella enterica* LT2, *Salmonella cholerasuis* DSM 14846 and *Salmonella typhi* NCTC 8385 species was well above the threshold of 70%. In particular, the dDDH value obtained for *Salmonella enteritidis* ATCC 13076 was 100%. GGDC results have high average branch support and low delta values, indicating high phylogenetic accuracy. With whole genome sequencing, the functional profiles of taxonomic groups can be elucidated and species can be defined to a large extent and uncertainties in the phylogeny of high taxa, which may be difficult with traditional approaches, can be easily resolved (25).

The aac(6')-Iaa gene, which has the potential to confer resistance to the aminoglycoside antibiotics amikacin and tobramycin, was detected in the analyzed Salmonella genome. Enzymes that alter the drugs through acetylation, adenylation, or phosphorylation mediate resistance to these antibiotics. AAC(6′) enzymes make their subsrtaes inactive by acetylating them at the 6′ position of aminoglycosides. The aac(6′)-Iaa gene is also responsible for aminoglycoside resistance. Antimicrobial resistance genes to aminoglycosides are the most diverse and most frequently identified genes in Salmonella strains (26).

Identification of pathogenic bacterial strains and understanding their biological processes related with pathogenicity are essential for timely intervention, design of control strategies and development of targeted vaccines. The pathogenicity of the analyzed genome was predicted with the PathogenFinder algorithm (27). The analysis predicted that *Salmonella enterica* is a human pathogen (probability of pathogenicity = 0.942).

For Salmonella to exert pathogenicity, virulence genes assembled in its genome, called SPIs, must be expressed in a coordinated manner (28). As a result of the analysis performed with SPIFinder, a total of 29 pathogenicity islands belonging to the Salmonella genome were detected. The detected pathogenicity islands belong to *S. typhimurium*, *S. typhi*, *S. enteritidis*, *S. cholerasuis* and *S. gallinarum* subspecies. Among these subspecies, the highest number of pathogenicity islands (6) was detected in *S. typhimurium* serovar. *Salmonella enterica* serovar *typhimurium* is the primary enteric pathogen that infects both humans and animals (11). In addition, horizontal gene transfer is important for *S. typhimurium* serovars. Therefore, they can infect many cell types and cause severe infections with small changes in virulence genes by using pathogenicity islands (29). C63PI, CS54_island, SPI-1, SPI-2, SPI-3 and SPI-5 pathogenicity islands were detected in *S. typhimurium* serovars analyzed in silico. C63PI is the iron transport system in SPI-1 and mediates the entry of Salmonella into the host cell (30). The presence of C63PI in this serovar indicates that iron is indispensable for its survival. The CS54 island has an important role in intestinal colonization and persistence. The Type 3 Secretion System (T3SS) encoded by SPI-1 and SPI-2 (30) is required for Salmonella invasion of intestinal epithelial cells and is therefore essential for intestinal colonization causing to enteritis (13). Thus, the T3SS has an essentila function in Salmonella pathogenesis (10). So far, five SPIs (SPI-1-SPI-5) have been determined that are clearly included in *S. typhimurium* virulence. SPI-2 and SPI-3 are essential for the growth

and survival of bacteria within the host, which occurs during the systemic phase of the disease. The recently identified virulence factors encoded by SPI-5 appear to have a role in the inflammation and chloride secretion that characterize the enteric phase of the disease (31). SPI-9, detected in *S. typhi* serovars, is included in the transport and metabolism of specific nutrients essential for Salmonella survival in the host (32). SPI-10 is presumed to play a role in host specificity (33). SPI-13 and SPI-14 were first determined in avian-adapted *S. gallinarum*, the causative agent of typhoid fever in poultry (34).

The advantages of CRISPRs as a subtyping tool in Salmonella have been demonstrated in studies. Serotyping has been used as the reference method for Salmonella typing for almost 80 years (35). CRISPRs have been identified in the genomes of many archaea and bacterial species, including Salmonella (36). Salmonella has a type I E CRISPR-Cas system consisting of two CRISPR sequences (CRISPR-I and CRISPR-II) and a cas operon (37). Salmonella has two CRISPR loci, CRISPR1 and CRISPR2, separated by ~16 kb and sharing the same consensus direct repeat (DR) sequence (29 nt) and 32 nt long spacer (38). There are eight cas genes characteristic of the type I-E CRISPR-Cas system: cas3, cse1, cse2, cas7, cas5, cas6, cas1 and cas2. These genes are located at the CRISPR1 locus (39). Furthermore, all Salmonella analyzed to date harbor only the type I-E system (40). As a result of analysis with CRISPRFinder, the type I-E CRISPR-Cas system was identified in the Salmonella genome. In addition, cas1, cas2, cas3, cas3, cas7, cas5, cas6, cse1 and cse2 genes were identified.

The secondary metabolite analysis identified two gene clusters, non-ribosomal peptide synthase (NRPs) and thiopeptide, potentially related to the biosynthesis of secondary metabolites. Enterobactin from the NRPs gene cluster and O-antigen compounds from the thiopeptide gene cluster were identified. Since iron is an essential cofactor for processes such as energy production and DNA replication, iron retention offers an effective antimicrobial defense. Salmonella-infected macrophages increase iron export, thus Salmonella proliferation is limited by low iron levels in macrophages, highlighting the importance of identifying the iron uptake mechanisms of Salmonella in these iron-deficient environments. Enterobactin, a class of catecholate siderophores, are small iron chelators secreted by Salmonella that facilitate the transport of iron into bacterial cells (41). Lipopolysaccharides (LPS), the main part of the outer membrane of gram-negative bacteria, are important virulence factors of bacteria (such as Salmonella species) that are pathogenic in animals and humans. O-antigens are structures that are an important component of LPS that contribute to the diversity of the cell wall of gram-negative bacteria (42). O-antigen diversity is a fundamental criterion in Salmonella serotyping. O-antigen identification has resulted in the serologic identification of more than 2000 Salmonella strains. The presence of O-antigen is also important for the survival of bacteria in their natural environment and has a role in bacterial virulence. There is direct evidence that loss of the O-antigen sensitizes many pathogens to serum or otherwise severely impairs virulence (43).

Accurate typing and monitoring is important for microbial epidemiological research, food safety and public health. Bacterial typing methods are divided into phenotyping and genotyping. Among these, serotyping and multilocus sequence typing (MLST) are the most commonly utilized despite low resolution (35). MLST is an attractive method because it is an easily implemented protocol and is well associated with most lineages and serovars through eBGs. The advantages of MLST are more uniformity, better association with serotypes and accessibility to databases (44). MLST analysis resulted in 100% similarity with seven Salmonella housekeeping genes (aroC, dnaN, hemD, hisD, purE, sucA and thrA) (45). This indicates that the analyzed genome belongs to *Salmonella enterica*.

It was also identified 50 gene clusters of the Salmonella enterica genome are responsible for virulence, disease and defense mechanisms. Genes with functions such as bacteriocins, adhesion, invasion and intracellular resistance and resistance to antibiotics and toxic compounds were identified. In the subcategory of bacteriocins, ribosomally synthesized antibacterial peptides, the conserved, uncharacterized protein CreA, included in tolerance to colicin E2, needs to be characterized. Bacteriocins produced by enterobacteria (*E. coli*, Salmonella and relatives) are called colicins.

Colicins are bacterial protein toxins that has strong activity against sensitive strains in vitro (46). Such bacteriocins kill other bacteria with a high specificity (47). The tolerance of Salmonella to colicin E2 by RAST analysis indicates that the bacterium has the ability to withstand or resist the inhibitory effects of colicin E2. Thus, Salmonella bacteria can survive and continue to grow in the presence of bacteriocin. In addition, overexpression of creD protein, an inner membrane protein, is responsible for colicin E2 tolerance (48). Genes responsible for adhesion were also identified by RAST annotation. The mediator of hyperadhesion YidE in enterobacteria and its conserved region were predicted in the isolate. The pathogenesis of infections caused by *S. enterica* requires adhesion to various host cell surfaces (49).

Genomic functions associated with resistance to antibiotics and toxic compounds have also been determined. These functions include copper homeostasis and copper tolerance. Bacteria must maintain complete copper homeostasis to prevent copper-mediated toxicity while ensuring copper supply for copper-demanding proteins. In order to do this, copper-sensing transcriptional regulators must differentiate copper from other metal ions and, in reaction to levels above or below a threshold, initiate the proper physiological response, such as copper import, export, and detoxification. (50). Another defined function is mercury reductase. Mercury ion reductase (MerA), a mercury detoxification enzyme used by microorganisms, has high specificity for mercury ions (Hg2+) and functions to catalyze their reduction to a more volatile, less toxic elemental form (51). MerA (mercury reductase) is found in the mer operon responsible for the detoxification of inorganic (Mg(II)) mercury in many bacteria growing in Hg-contaminated environments (52). Fluoroquinolone resistance in bacteria arises as a result of changes in DNA gyrase enzymes found in gram-negative bacteria (53). Especially mutations in gyrA and gyrB genes contribute to fluoroquinolone resistance. Thus, some serovars continue to show their virulence effects by developing resistance or becoming less susceptible to fluoroquinolones, the antibiotics used against Salmonella (54). Resistance genes to cobalt, cadmium and zinc have also been found in the Salmonella genome. These heavy metals show toxic effects when used in high concentrations. Genes related to these heavy metal resistance have been detected in many isolates of Salmonella (55). Finally, there is adaptation to d-cysteine in the genome. Genes involved in adaptation to D-cysteine, a potent growth inhibitor, were obtained as a result of the analysis.

As a result, Illumina sequence reads of the *Salmonella enterica* genome were analyzed by in silico tools and annotation was performed. As a result of RAST analysis, 366 subsystems were identified. 50 of these subsystems are responsible for virulence, disease and defense. TYGS analysis showed that it has 100% digital DNA-DNA hybridization with *Salmonella enteritidis* ATCC 13076. The aac(6')-Iaa gene, which has the potential to confer resistance to amikacin and tobramycin, was found in the analyzed genome. The pathogenicity prediction predicted that Salmonella enterica is a human pathogen (probability of pathogenicity = 0.942). MLST analysis revealed 100% similarity with alleles of the Salmonella enterica housekeeping gene. CRISPR analysis identified 8 Type I CRISPR -Cas associated proteins. In addition, two secondary metabolites (enterobactin and O-antigen) were found as a result of secondary metabolite analysis. Finally, 6 pathogenicity islands belonging to 10 serovars of *S. typhimurium* subspecies, 1 serovar of *S. typhi* subspecies, 2 serovars of 3 serovars of *S. enteritidis* subspecies, 1 serovar of *S. cholerasuis* subspecies and 5 pathogenicity islands belonging to 14 serovars of S. *gallinarum* subspecies were identified.

Our primary goal in this work is to clarify the taxonomic classification and functional characteristics of *Salmonella enterica*, a pathogenic bacteria that causes serious illness in humans, using reads of whole genome sequencing. We also aim to easily resolve uncertainties in the phylogeny of higher taxa that are difficult to resolve by traditional methods. Identification of pathogenic bacterial strains and understanding the biological mechanisms associated with pathogenicity are also essential for timely intervention, design of control strategies and development of targeted vaccines.

Ethical approval

Our study is not in-vitro, in-vivo or survey, it was designed through a bioinformatics perspective. Therefore, there is no need for ethical approval.

Author contribution

Consept: YŞ; Data Collection or Processing: YŞ; Analysis or Interpretation: YŞ, CY; Design: YŞ, CY; Literature researh: YŞ, CY, RE. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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