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Population structure of the *Salmonella enterica* serotype Oranienburg reveals similar virulence, regardless of isolation years and sources

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ABSTRACT

Salmonella enterica serotype Oranienburg is a multi-host, ubiquitous, and prevalent Non-typhoidal *Salmonella* (NTS) in subtropical rivers, particularly in sediments; little studied so far possible the adaptation and establishment of this microorganism based on its genetic content. This study was focused on the first five genomes of *S.* Oranienburg in sediments through whole-genome sequencing (WGS) and 61 river water genomes isolated in previous studies. Results showed an open pangenome with 5,594 gene clusters (GCs), and the division of their categories showed; 3,303 core genes, 741 persistent genes, 1,282 accessory genes, and 268 unique genes. Additionally, it showed three main subclades within the same serotype and showed a conserved genetic content, suggesting the display of different adaptation strategies to its establishment. Nine genes for antimicrobial resistance were detected: *aac (6′) - Iy*, *H-NS*, *golS*, *marA*, *mdsABC*, *mdtK*, and *sdiA*, and a mutation in the *parC* gene p. T57S generating a resistance. In addition, virulence genes and pathogenicity islands (SPI's) were analyzed, finding 92 genes and an identity above 80 % in the SPI's 1 to 5, and the centisomes 54 and 63. The

Abbreviations: aac (6′) - Iy, gene,encoded aminoglycoside acetyltransferase; a*crAB*, gene,efflux transmembrane transporter activity; AMR, antimicrobial resistance; CDC, Centers for Disease Control and Prevention; *cdtB*, gene,cytolethal distending toxin B; *csa3*, CRISPR-associated CARF protein Csa3; *csgA-G*, gene,curli production assembly; *cspH*, gene,encoding CspH,one of the cold shock proteins; *ClpP*, gene,essential for growth and survival at low temperature; COGs, clusters of orthologous groups; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; CS63, centisome 63; GCs, gene clusters; EHEC, enterohemorrhagic *Escherichia coli*; *entAB*, enterobactin biosynthesis gene; EPEC, enteropathogenic *Escherichia coli*; *faeC*, gene,adherence fimbrial *Escherichia coli*; *fepACD*, gene,ferrienterobactin outer membrane transporter; *flgGH*, gene,flagellar L-ring protein precursor FlgH; *fliAGMP*, gene,flagellar biosynthetic protein FliP; *fimCDFHI*, gene, fimbrial adherence; *golS*, gene,regulator activated by the presence of gold and promotes the expression of the MdsABC efflux pump; *gtrB*, gene,bactoprenol glucosyl transferase; *H-NS*, gene,involved in global gene regulation in Gram-negative bacteria; *invA-C*, *E-J*, gene,invasive; *iroBCN*, gene,iron transporter; *marA*, gene,multiple antibiotic resistance; MFS, major facilitator superfamily; *mig-14*, gene,macrophage-inducible gene-14; *misL*, gene,extracellular matrix adhesin involved in intestinal colonization; MDR, multidrug resistance; *mdsABC*, gene,outer membrane channel of the multidrug and metal efflux complex; *mdtK*, gene,multidrug and toxic compound extrusions; *mgtBC*, gene,magnesium uptake; NTS, non-typhoidal *Salmonella*; *orgABC*, gene,oxygen-regulated invasion protein OrgA; *parC*, gene,Fluoroquinolone Resistance; *pipB*, gene,translocated effector of the SPI-2- encoded TTSS; *pltAB*, gene,*Pertussis* like toxin subunit; *pmrAB*, gene,regulate resistance to several AP,including polymyxin B; *prgHIJK*, gene,inner MS ring; *ratB*, gene,involved in intestinal colonization and persistence; SCV, *Salmonella* cell vacuole; *sdiA*, gene,positive regulator of AcrAB; SGI, genomic island; *shdA*, gene,mediated binding of the extracellular matrix contributes to persistent intestinal carriage; *sicAP*, gene,chaparone for *SipC* and *SipB*; *sifAB*, gene,secreted effector protein; *sinH*, gene,Involved in intestinal colonization and persistence; *sipA-D/sspA-c*, gene,cell invasion protein SipA; *slrP*, gene,efector SlrP E3 ubiquitin ligase; *sopABDE*, gene,cell invasion; *spaOPQRS*, gene,minor export apparatus protein SpaQ; *spiC*, gene, activation of signal transduction pathways in macrophages; SPI's, pathogenicity islands; *sptP*, gene,tyrosine phosphatase and GTPase-activating protein; *ssaB-V*, gene, secretion system apparatus; *sscA-B*, gene,chaperone for the translocon component; *sseA-G,J-L*, gene,chaperone for the tranlocon; *steABC*, gene,control of membrane dynamics of SCV; *tcpC*, gene,Tir domain containing protein TcpC; TSB, tryptic soy broth; TSS, type secretion system; UV, ultraviolet; VBNC, viable-but-nonculturable; VFDB, virulence factors database; WGS, whole-genome sequencing.

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environmental strains of *S*. Oranienburg do not represent a concern as multidrug resistance (MDR) bacterium; however, virulence genes remain a potential health risk. This study contributes to understanding its adaptation to aquatic environments in Mexico.

1. Introduction

Non-typhoidal *Salmonella* represents one of the primary causes of gastrointestinal infection worldwide, causing approximately 153 million enteric infections and 57 thousand deaths annually; it is spread by the fecal-oral route of contamination and transmitted mainly by food and water (Brunette, 2019). Although Salmonellae NTS in most cases is self-limiting, it has also been described as potentially pathogenic, and it can also deal in fatal cases. The severity depends on specific factors from the host and *Salmonella* serotype (Levantesi et al., 2012; WHO, 2018). *Salmonella* can persist for several years in aquatic environments and has been detected in different countries, in diverse water sources; rivers, lakes, estuarine and coastal waters, is a potential crop contaminant. Its concentration in rivers may increase due to feces released from infected humans or animals and sewage discharge (Martinez-Urtaza et al., 2004; Haley et al., 2009; Gorski et al., 2011; Levantesi et al., 2012). Likewise, *Salmonella* has also been found in soil and sediments as part of a micro ecological niche within lakes, allowing its survival and fresh produce contamination as these waters might be used for crop irrigation (Chandran et al., 2011; Gorski et al., 2011). For example, *S*. Oranienburg has been responsible for human infections in food outbreaks of fruit salads, chia powder, egg, and onion, as reported by the CDC between 2006 and 2021 (CDC, 2006; CDC, 2014; CDC, 2016; CDC, 2021), illustrating the relationship with the water used in field irrigation.

In the central region of Sinaloa state, previous studies have demonstrated a high prevalence of the Oranienburg serotype in the Humaya, Tamazula, and Culiacan rivers; this prevalence has been related to stables nearby rivers, running water in the rainfall season, and at the temperature of the subtropical region (López-Cuevas et al., 2009; Estrada-Acosta et al., 2013; Jimenez et al., 2014). The use of this water is a great concern since river water is used for crop irrigation and could be an important vehicle for the spread of *Salmonella* and food contamination (Simental and Martinez-Urtaza, 2008).

Salmonella survival in river water and sediment is a selective process favored by preconditioning abilities. This process is performed through strategies to deal with external factors such as pH, temperature changes, desiccation, UV radiation, stress osmotic, salinity, oxygen levels, and nutrient deprivation. Interestingly, *Salmonella* can develop resistance to one certain factor or multiples factors resistance through modulation of gene expression, and metabolic changes also may enter a dormancy or viable-but-nonculturable (VBNC) state, in which they can subsist for long-term. Additionally, inside the host, *Salmonella* can display strategies to evade humoral responses and being inside macrophages to resist intestinal factors (Finlay and Falkow, 1989; Spector and Kenyon, 2012; Ryan et al., 2015; Medrano-Félix et al., 2017; Contreras-Soto et al., 2019). Although inside of the cell virulence will depend largely on the expression and regulation of *Salmonella* genes, as well as the nutrients available for the involvement of metabolic pathways (Jaiswal et al., 2016).

Another alternative for *Salmonella* to persist in non-host environments is the establishment in sediments. Microorganisms tend to self-accumulate in sediment particles over time and it has been reported that proteobacteria prevail in 85 % of the total bacterial communities in freshwater, estuarine and marine sediments by weak Van der Waals forces or secretion of polymeric substances (Marshall et al., 1971; Behera et al., 2017;Singh et al., 2017). Pathogenic bacteria can survive longer in sediments than in surface water due to available nutrients (organic and inorganic compounds), environmental factors such as biotic interactions with benthic organisms, sediment grain size (small), physical–chemical conditions (oxygen availability), besides, the coexistence of different microbial communities in the sediment, carry out efficient cycling of carbon, nitrogen, and sulfur (Chandran et al., 2011; Vignaroli et al., 2013; Behera et al., 2017). Recreational activities in natural water bodies can become a health problem since resuspended sediments can reach up to 10^5 cells per gram of sediment (Luna et al., 2012).

Interestingly, the interaction of microorganisms with the environment and their adaptation could significantly influence its virulence when returning to a new host and cause disease (Chakroun et al., 2017; Ramírez et al., 2018). Also, a suitable environment allows the expression of its virulence factors; an example of this is the use of iron present in river water, which is involved in the expression of genes in adaptation (Dos Santos et al., 2021). In addition, Ramírez et al. (2018) demonstrate that *S*. Oranienburg, isolated from the aquatic environment, is able to invade the host cell, survive intracellularly, and cause severe damage. Also, a study performed by Medrano-Félix et al. (2017) showed that *S*. Oranienburg from an environmental source has pre-conditioning abilities that allow greater adaptation and a broader genetic content for stress response, metabolism, and transport compared to *S*. Typhimurium 14,028 exposed to the same conditions.

Virulence genes are responsible for the main mechanism of pathogenesis of *Salmonella*, found in the pathogenicity islands (SPI's) and their type III secretion system (T3SS), and mobile elements (Dos Santos et al., 2021). In addition, the genes associated with antimicrobial resistance are fundamental to understand their influence on resistance, which can be conferred by the presence of plasmids, active efflux pumps, and chromosomal mutations, even increasing MDR (Ferrari et al., 2013). Although MDR is not only limited to human pathogenic serotypes, which are commonly exposed to antimicrobials, it is also observed in strains that predominate in non-host environments, as described by López-Cuevas et al. (2009). For this reason, the implementation of different bioinformatic tools brings us closer to knowing more about the virulence and resistance of any microorganism.

The whole-genome sequencing (WGS) allows us to know the genetic characteristics of *S*. Oranienburg with a high resolution and provide the guideline to know its biological evolution, virulence, and antimicrobial resistance profile (Antony et al., 2020; Kipper et al., 2021). There are several studies overtime on the population structure of *Salmonella* in a specific region through WGS (Antony et al., 2020; Pearce et al., 2021); however, in the Culiacan Valley, there is still no available detailed information about genomic data on *Salmonella* isolated from sediments beyond those obtained through biochemical tests. Therefore, this study aimed to evaluate the genomic repertoire of the population of *S*. Oranienburg isolated from water sources in 11 years and recently in sediments in the Culiacan Valley region, and identify the variability of genes that may influence their adaptation to river water and sediments. In addition, is important to know the virulence potential of this serotype isolated from an environmental source and the impact it could have when entering the host.

2. Materials and methods

2.1. *Salmonella Oranienburg isolates and sequencing*

In this study, a total of 66 *S*. Oranienburg genomes from the Culiacan Valley were used, listed in Supplementary Table 1. Five strains of *S*. Oranienburg from river sediments were isolated, sequenced and were deposited in GenBank under accession numbers JALPLR000000000, JALPLS000000000, JALPLT000000000, JALPLU000000000, and JALPLV000000000 in the BioProject PRJNA831307 (Table 1). These

strains belong to the collection of the Laboratorio Nacional para la Investigación en Inocuidad Alimentaria (LANIIA) from Centro de Investigación en Alimentación y Desarrollo (CIAD).

The recovered *S.* Oranienburg isolates of sediments were grown for 24 h in TSB at 37 °C under aerobic conditions, and DNA extraction was performed using the kit DNeasy Blood & Tissue kit (QIAGEN, Mexico City, Mexico) according to the manufacturer's instructions and quantified with a NanoDrop 2000c Spectrophotometer. After extraction, libraries were prepared with Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The Whole-genome sequencing for JCS-05, JCS-30, JCS-31 and JCS-32 strains was performed with the Illumina Miseq platform, for JCS-38 strain was performed with the Illumina MiniSeq platform.

Additionally, 44 WGS of *S.* Oranienburg strains of river water were downloaded from National Center for Biotechnology Information (NCBI). The selection criteria in the search were: *S.* Oranienburg (river water, sediment, and Culiacan Sinaloa). Conducting previously reported studies in the region: 17 strains by González-López et al. (2022), 17 strains by Casteñeda-Ruelas et al. (2017), S-76 by Medrano-Félix et al. (2013), and 26 unpublished strains by Chaidez 2008–2010, Supplementary Table 1.

2.2. Assembling and annotation

The reads quality was analyzed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc), and cleanup was performed with fastp v0.22.0 (Chen et al., 2018). After filtering, the remaining reads were assembled into contigs using SPAdes v3.0.0 (Bankevich et al., 2012). Before further analysis, the serotype Oranienburg was verified in all strains through *Salmonella In Silico* Typing Resource (SISTR) v1.1.1 (Yoshida et al., 2016). The generated assemblies were annotated using Prokka v1.11.

2.3. Pangenome construction

In this analysis, the 66 genomes of *S.* Oranienburg were used to construct the pangenome, performed with anvi'o v7 (Eren et al., 2015), following the workflow of (https://merenlab.org/2016/11/08/pangenomics-v2/). The scripts ran were the following: anvi-gen-contigs-database, employed for database creation and identify open reading frames in contigs using Prodigal (Hyatt et al., 2010); anvi-run-ncbi-cogs, used to obtain gene annotations using NCBI's Clusters of Orthologous Groups (Tatusov et al., 2000); anvi-run-kegg-kofams used to gene annotations from KOfam profiles (Aramaki et al., 2020) from the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2017). Finally, the database was created with anvi-gen-genomes-storage and anvi-pan-genome to run the pangenomic analysis for visualization. The pangenome is an illustrative representation of the distribution of gene families, so each genome has a set of gene families, and these could be present in other genomes (Jacobsen et al., 2011).

2.4. Antimicrobial resistance (AMR) genes

To identify antimicrobial resistance genes in the genome of *S.* Oranienburg strains, AMR genes and chromosomal mutations were searched in ResFinder v3.2 (Zankari et al., 2012). For this analysis, a criterion of mutations above 90 % identity with a minimum alignment of 70 % was considered. Additionally, with the ABRicate program v0.8.13 (https://github.com/tseemann/abricate), the search for resistance genes was compared with the Comprehensive Antimicrobial Resistance Database (CARD, https://card.mcmaster.ca/home). The graphic was made with the R software package (Team, 2015).

2.5. Virulence genes

The identification of virulence genes in the genome of *S.* Oranienburg strains was performed using the ABRicate program v0.8.13 and compared with the virulence factor database VFDB (Liu et al., 2019). The criterion used considered the presence of genes above 90 % identity and a minimum alignment of 70 %. The graphic was made with the R software package (Team, 2015).

2.6. Salmonella pathogenicity islands (SPI's) identification

The SPI's, centisomes, and the genomic island (SGI) of *S. enterica* were downloaded from the Pathogenicity Island Database (PAI DB) (Yoon et al., 2007) (Table 2), and they were subjected to a re-annotation using Prokka v1.11. The proteomes of each island were blasted against the *S.* Oranienburg proteomes using Geneious v9.1 to determine the presence or absence of each SPI protein. The identity score for each SPI protein was multiplied by the ratio of the alignment length to the total sequence length, averaged for all proteins in each SPI to arrive at an overall identity for each island (Jacobsen et al., 2011). The heatmap was made with an R software package (Team, 2015).

3. Results and discussion

S. Oranienburg persists as a relevant public health and food safety risk due to it has recently been implicated in food outbreaks and this serotype persists in water and sediment under adverse environmental conditions, in this study, it also proves to be a well-adapted microorganism, in addition, to showing potential for pathogenicity.

The use of new tools such as whole-genome sequencing is an excellent alternative for surveillance and characterization of *Salmonella* through the analysis of pangenome, serotyping, virulence, and antimicrobial resistance; this is a faster and more efficient alternative compared to traditional methods of molecular biology (Ben Hassena et al., 2021). In addition, we suggest that results in this study contribute to better understand about the characteristics of an environmental serotype such as *S.* Oranienburg, which has been little studied.

In Culiacan Sinaloa, the isolation, incidence, and high prevalence of *S.* Oranienburg in river water have been continuously documented, while in sediments, the information is almost scarce. Nonetheless, its genomic characteristics had not yet been fully studied during these 11 years (2008–2019). One of the most important traits to know about this microorganism is to elucidate the gene content related to adapting process and stress response, which may contribute to the long-term survival in an environmental niche. Martinez-Urtaza et al. (2004) mention that *Salmonella* can prevail for long periods in coastal environments, while Behera et al. (2017) mention that proteobacteria prevail in 85 % of the total bacterial communities in freshwater sediments, estuaries, and marine areas. Nonetheless, genomes among strains,

Table 1
Statistics of five newly sequenced *S.* Oranienburg genomes isolated from sediments

Strain	Bioproject	Biosample	Accession	Assembler	Seq technology	Contigs	Bases genome	N50	Coverage
JCS-05	PRJNA831307	SAMN27751245	JALPLR000000000	A5-miseq	Illumina MiSeq	245	5,006,385	184,668	147.9
JCS-30	PRJNA831307	SAMN27751246	JALPLS000000000	A5-miseq	Illumina MiSeq	80	4,642,522	127,264	186.2
JCS-31	PRJNA831307	SAMN27751247	JALPLT000000000	A5-miseq	Illumina MiSeq	54	4,788,112	273,360	156.5
JCS-32	PRJNA831307	SAMN27751248	JALPLU000000000	A5-miseq	Illumina MiSeq	53	4,791,515	225,752	150.2
JCS-38	PRJNA831307	SAMN27751249	JALPLV000000000	SPAdes	Illumina MiniSeq	590	4,812,111	22,919	45.6

Table 2
Salmonella pathogenicity islands used in this study.

SPI	Host strain	Insertion site	Accession	Size (kb)
SPI-1_Typhi Ty2	*S.* Typhi Ty2	fhlA/mutS	NC_004631_P2	41.9
SPI-2_Typhi Ty2	*S.* Typhi Ty2	tRNA-val	NC_004631_P1	41.6
SPI-2_Typhimurium LT2	*S.* Typhimurium LT2	tRNA-val	AJ224892	8.5
SPI-2_Choleraesuis SC-B67	*S.* Choleraesuis SC-B67	tRNA-val	NC_006905_P3	41.8
SPI-3_Choleraesuis SC-B67	*S.* Choleraesuis SC-B67	tRNA-selC	NC_006905_P6	12.8
SPI-3_Dublin	*S.* Dublin	tRNA-selC	AY144490	10.1
SPI-3_Typhi CT18	*S.* Typhi CT18	tRNA-pro	NC_003198_P7	16.9
SPI-3_Typhimurium LT2	*S.* Typhimurium LT2	tRNA-selC	NC_003197_P4	16.6
SPI-4_Choleraesuis SC-B67	*S.* Choleraesuis str. SC-B67	ssb/soxSR	NC_006905_P7	26.7
SPI-4_Typhi CT18	*S.* Typhi CT18	ssb	NC_003198_P8	23.4
SPI-4_Typhimurium LT2_2	*S.* Typhimurium LT2	ssb/soxSR	AF060869	27.3
SPI-5_Choleraesuis SC-B67	*S.* Choleraesuis str. SC-B67	tRNA-ser	NC_006905_P1	5.7
SPI-5_Dublin	*S.* Dublin	tRNA-serT	AF060858	9.7
SPI-5_Typhi CT18	*S.* Typhi CT18	tRNA-serT	NC_003198_P2	7.5
SPI-5_Typhimurium LT2	*S.* Typhimurium LT2	tRNA-serT	NC_003197_P1	9.1
SPI-6_Typhi CT18	*S.* Typhi CT18	tRNA-asp	NC_003198_P1	58.7
SPI-7_Typhi CT18	*S.* Typhi CT18	tRNA-phe	NC_003198_P9	133.6
SPI-8_Typhi CT18	*S.* Typhi CT18	tRNA-phe	NC_003198_P6	6.9
SPI-9_Typhi CT18	*S.* Typhi CT18	Not published	NC_003198_P4	15.7
SPI-10_Typhi CT18	*S.* Typhi CT18	tRNA-leu	NC_003198_P10	32.9
SPI-11_Choleraesuis SC-B67	*S.* Choleraesuis SC-B67	Gifsy-1 prophage	NC_006905_P2	15.7
SPI-12_Choleraesuis SC-B67	*S.* Choleraesuis SC-B67	tRNA-pro	NC_006905_P4	11.1
CS54_island_Typhimurium ATCC14028	*S.* Typhimurium ATCC14028	xseA/yfgK	AF140550	25.3
CS63_island_SL1344	*Salmonella enterica* SL1344	fhlA	AF128999	4.0
SGI1_Typhimurium DT104	*S.* Typhimurium DT104	thdF	AF261825	47.7

regardless of the year of isolation did not show a marked pattern in the population structure of *S.* Oranienburg concerning their isolation source (water and sediment), geographic origin, and date of isolation.

3.1. Pangenome

According to open pangenome analysis of the 66 genomes of *S.*

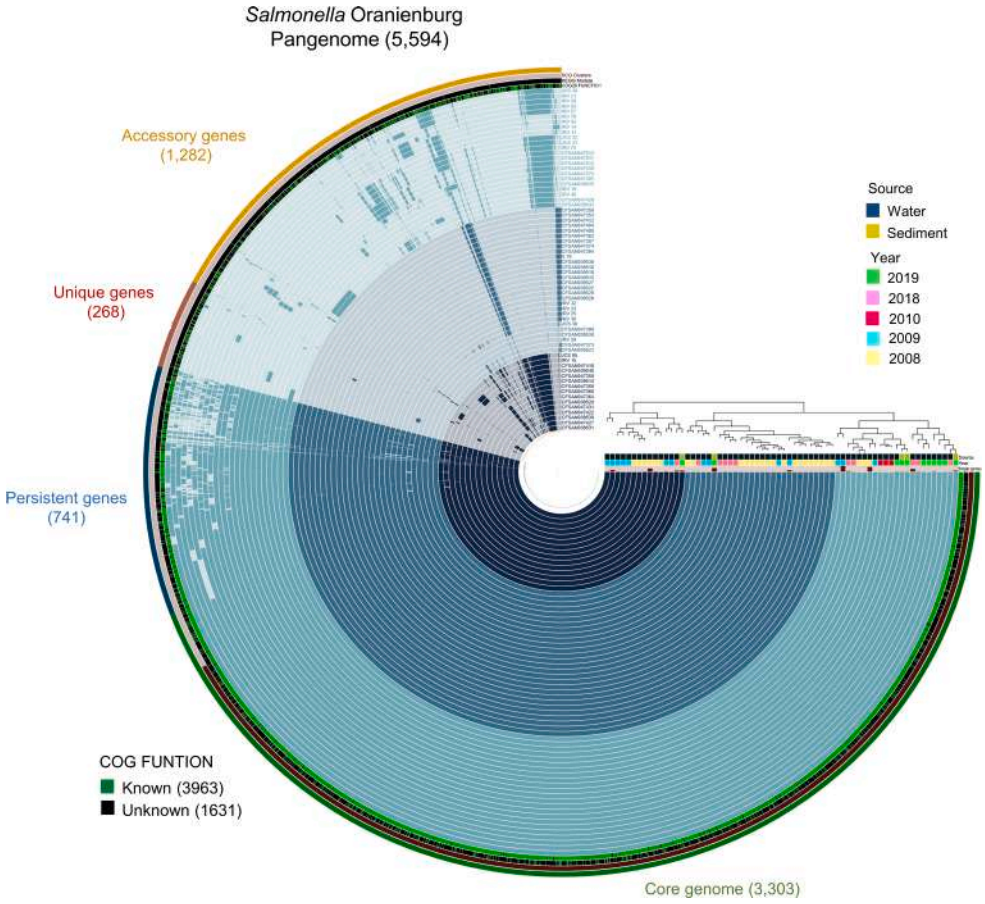

Fig. 1. Pangenome analysis generated with anvi'o for 66 S . Oranienburg genomes isolated from river water and sediment. The dark–colored regions indicate the presence of a group of genes and the light–colored ones its absence.

Oranienburg in the present study, the accessory and core genomes of sediment strains and water strains showed the gene distribution throughout the chromosome, indicating a total of 5,594 gene clusters (GCs). We grouped these GCs into four bins based on their occurrence across the genomes: a core genome containing 3,303 GCs (100 % occurrence), persistent genes containing 741 GCs (99–90 %), accessory genome containing 1,282 GCs (<90 %), and unique genes GCs, containing 268 present in a single genome (1 %), (Fig. 1, Supplementary Table 2).

In the cladogram based on the presence/absence of GCs across the genomes, there were three main clades where the strains isolated from water and river sediments were distributed. Besides, nearly 70 % of clusters of orthologous groups (COGs) were functionally annotated; the majority belonged to highly conserved genes and were found in the core genome. The core genome mainly consists of a highly conserved region of essential genes, detecting a great number of genes related to carbohydrates, lipids, energy, nucleotide, amino acids, cofactors, and vitamins; as well as for c-starvation. Results concur with Spector and Kenyon (2012) and a minimal gene content associated with resistance and virulence, explaining the low-occurrence rate of cases caused by this environmental serotype when reaching a host. The rest of the gene content has been associated with metabolism, DNA process, cell wall and envelope biogenesis, intracellular transport, secretion, and vesicular transport, which are part of *Salmonella* basic biology (Seif et al., 2018).

In the accessory genome, there are groupings in the cluster of genes, thus maintaining their genetic characteristics despite the time of isolation and its source, which is evidence of genetic conservation, agree with Lapierre and Gogarten (2009). Interestingly, were shown the presence of prophages, transposases, integrases/recombinases, CRISPR-associated protein Csa3, the *fimCDFHI* genes for biofilm formation and antitoxin genes, virulence factors such as the toxin (EHEC/EPEC, Pertussis, *Vibrio Cholerae*, *Helicobacter pylori*), and cytolethal distending toxin subunit B, that according to the author Pons et al. (2019), these promotes infection persistence. Furthermore, SOS-response transcriptional repressors and the *ClpP* gene that plays an important role in the growth of the microorganism under stress conditions such as low pH, high salinity, and high temperature (Thomsen et al., 2002), as well as fimbrial protein, and efflux pump complex, iron, and magnesium acquisition systems ABC type.

Among the unique genes, the analysis showed the presence of type II, III, IV, and VI secretory system, iron, and magnesium acquisition systems ABC type, which are important for enteropathogenic bacteria to catalyze oxide-reduction reactions by enzyme cofactor (Drago-Serrano, 2009), cell motility such as pili, restriction endonucleases, associated with mobilome (prophages, transposons, transposases, integrases) and hypothetical proteins. Moreover, the number of unique genes (268) had not fluctuated between the isolation years and sources. Hence, there has not been a noticeable evolutionary change among strains. This condition could be linked to the selective or specific establishment of *Salmonella* serotypes in the niche, suggesting the conservation of the physicochemical and nutrient characteristics of the river and sediments of the region, being common in subtropical environments, this agrees with Jacobsen et al. (2011) who mention, that a greater number of unique genes will be associated with adaptation to a new niche. Interestingly, the content of hypothetical proteins was higher in the single genes than in other categories. Therefore, the functional characterization of these proteins is fundamental to understanding if these unique genes provide an adaptative advantage acquired through the mobilome.

3.2. Antimicrobial resistance genes

The burden of excessive use of antibiotics is a global health concern; this is a consequence of the use of antibiotics in medicine and agriculture, with the spread of antibiotics exposing bacteria to different non-lethal concentrations of different drug classes (Dawan and Ahn, 2020). For this reason, the presence of antimicrobial resistance genes and their characteristics, as well as punctual mutations that induce resistance in *Salmonella*, were extremely important to address.

The resistance genes of *S.* Oranienburg were analyzed with the ResFinder and CARD databases and were consistently detected in the 66 genomes. The AMR factors consist of nine resistance genes; *aac (6′) - Iy*, *H-NS*, *golS*, *mdtK,* and *sdiA* found in all genomes, *marA* gene, only present in the IRV-57 and IRV-83, and JCS-38 genomes, and *mdsABC* genes were absent only in IRV-48 genome (Fig. 2, Supplementary Table 3). Also, five types of mutations in genes *acrB*, *pmrAB*, *parC*, and *16S_rrsD*, and the only mutation that could generate antibiotic resistance to nalidixic acid and ciprofloxacin detected among the genomes was *parC* p. T57S.

The resistance genes found in this study belong to the AMR family of aminoglycoside resistance, major facilitator superfamily (MFS), nodulation cell division RND-type multidrug exporters, gold stress response, porin with reduced permeability to beta-lactams, MDR, and efflux pump complex, with probable resistance to antibiotics; aminoglycoside (amikacin, tobramycin), macrolide, monobactam, fluoroquinolone, carbapenem, cephalosporin, cephamycin, penam, penem, glycylcycline, rifamycin, phenicol, triclosan, tetracycline, and nitroimidazole.

Genes present in all genomes were found, such as *aac (6′) - Iy*, involved in chromosomal-encoded aminoglycoside acetyltransferase. This gene is responsible for the enzymatic detoxification of drugs and has been described as part of plasmids or transposons associated with the spread of resistance. The presence of *H-NS* is a histone-like protein associated with gene regulation of RND-type multidrug exporters and negatively affects transcription (Atlung and Ingmer, 1997). The *golS* gene function in stress response, while the *mdsABC* genes are grouped in the MDR along with the RND family. Both genes encode membrane fusion proteins of the multidrug and metal efflux (Al-Ansari et al., 2021). Furthermore, the genes *golS*, and *mdsAB,* were present in a previous study by Li et al. (2021) and were part of outbreaks and non-outbreak of *Salmonella* and classified as conserved genes. The *marA* gene can cause multiple antibiotic resistance by activating two membranes with a dependent mechanism. Gene activation in the log phase has been observed when bacteria are in contact with the antimicrobial ciprofloxacin and tetracycline (Jaktaji and Ebadi, 2013).

The *mdtK* gene encodes the multidrug and toxic compound extrusion (MATE-type efflux protein), conferring resistance to fluoroquinolones norfloxacin, doxorubicin, and acriflavine (Nishino et al., 2006). The *sdiA* gene is part of beta-lactamases and has been involved in chickens and humans, representing a threat to public health; it is a positive regulator of a*crAB* only when expressed from a plasmid. On the contrary, when the *sdiA* gene is located within the chromosome, it does not affect the expression of *acrAB*, and it is also a regulator of cell division (Al-Ansari et al., 2021).

The results of this study allowed us to compare the resistance profile among strains belonging to *S.* Oranienburg isolated from the aquatic environments with probable resistance to tobramycin, amikacin, and imipenem, in contrast to the clinical *S*. Oranienburg isolates obtained by Vazquez-Garciduenas et al. (2014), that showed resistance to ampicillin, carbenicillin, and cephalothin. However, all strains belonged to the same serotype, and their abilities for the use of genes or acquisition in a niche or host change, in addition, the concentrations of antibiotics that reach the water probably are not sufficient to generate resistance, this is due to changes of physicochemical properties of the antibiotics as well as the biogeochemical conditions in aquatic systems as pH, redox potential, temperature, and salinity that can degrade or lose their shape (Gwenzi et al., 2021). In this regard, *S.* Oranienburg, isolated from water and sediments, did not show resistance to multiple antibiotics, and the treatment would not be complicated since its AMR gene repertoire is limited.

3.3. Virulence genes

We analyzed 115 virulence genes throughout the whole genomes of

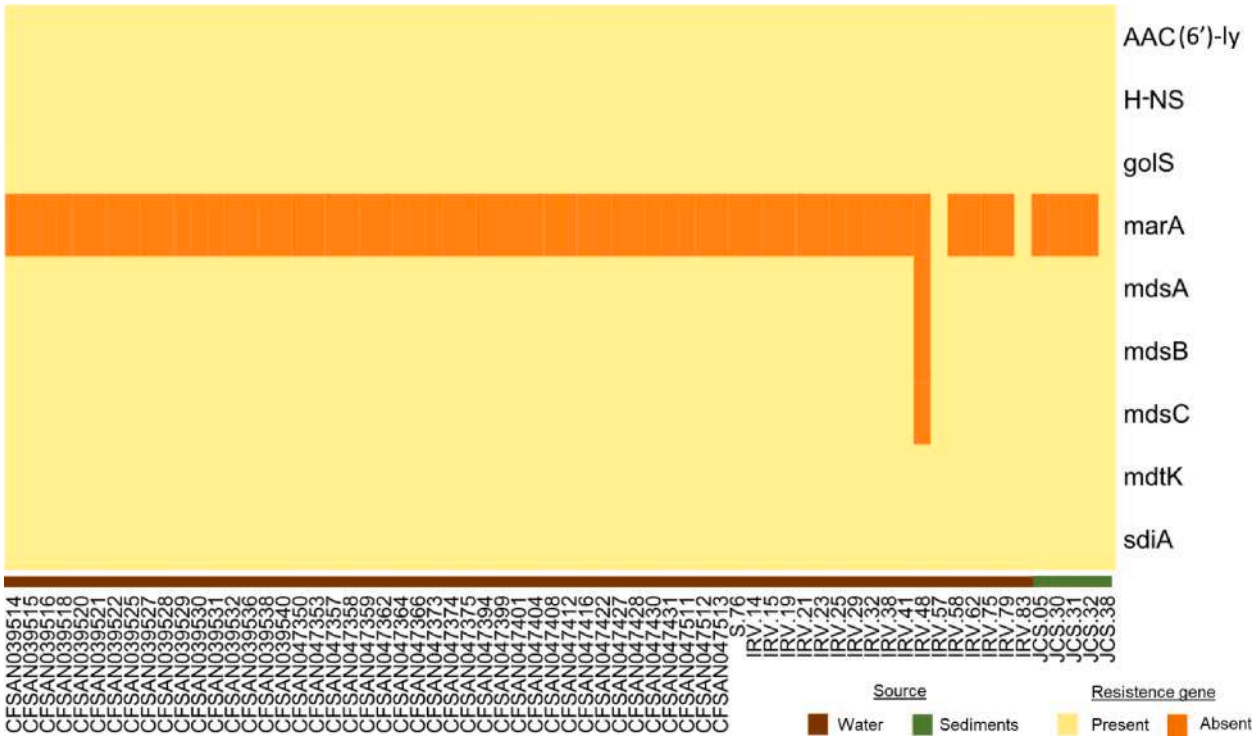

Fig. 2. Heatmap of MRA genes presence in 66 *S.* Oranienburg strains. Present genes are in khaki and absent genes are in orange. 2-column fitting image. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Salmonella using the Virulence Factors Database (VFDB). The gene pattern investigated represented the current knowledge to elucidate pathogenic mechanisms and virulence potential of *S.* Oranienburg infection, which is not yet characterized and could pave the way to develop new strategic approaches to prevent infections in a host by water use in the region. A total of 92 virulence genes were present in all genomes (Fig. 3A and 3B, Supplementary Table 4). Genes that were present and complete in all genomes were: *faeC* (adherence *Escherichia coli*), *mig-14* (inducible macrophage), *mgtBC* (magnesium uptake), *iroBCN* (ABC transporter), *fepACD* (iron uptake), *cspH* (stress adaptation), *flgGH* and *fliAGMP* (flagellum), and *entAB* (enterobactin biosynthesis).

Furthermore, some genomes isolated from surface water IRV-14, 15, 19, 21, 29, 41, 57, 58, 62, 75, 79, 83, and sediment JCS-38 showed the lack of genes in several virulence categories in at least one genome. The absence of the *ratB* gene in 20 strains and *shdA* gene for 15 strains were the two genes with the greatest absence (nonfimbrial adherence). On the other hand, *pegB* and *steC* (fimbrial adherence), *invGJ* and *sipD* (T3SS SPI-1), *ssaEJRSTU* (T3SS SPI-2), *slrP*, *sipA/sspA*, *sopA*, *sptP*, *sifA*, *sopD2*, *sseK1* and *sseL* (translocated effectors), *gtrB*, *pltA* and *tcpC* (toxin) were also absent in at least one genome.

The genes involved in virulence are important as each one plays a specific role in *Salmonella*, these are regulated and coordinated to facilitate adaptation and colonization to various hosts and environments (Ilyas et al., 2017). Genes associated with adherence will allow *Salmonella* to survive in a specific niche depending on its ability to adhere. This adherence is mediated by fimbrial and nonfimbrial adhesins, recognizing receptors on host cells, and attachment to epithelial cells (enterocytes) (Ochoa and Rodríguez, 2005). The presence of *Escherichia* fimbrial adherence could have been probably acquired by horizontal transfer and are a clear example of the ability of different *Salmonella* serovars to adapt to different hosts (Mooi et al., 1986).

Likewise, the *csgABCDFG* cassette was associated with biofilm production, auto-aggregation, increased antibiotic resistance, inflammation, and fluid accumulation in calves (Webber et al., 2019; Dos Santos et al., 2021). The *steA* effector has been associated with peritoneal infection for a long time; it controls the dynamics of the *Salmonella* cell vacuole (SCV) membrane (Geddes et al., 2005; Domingues et al., 2014).

The *mig-14* gene is an inner membrane-associated protein necessary for bacterial proliferation in the liver and spleen (Valdivia et al., 2000). The *iroB* and *iroC* genes cause host cell damage and have been associated with the severity of *Salmonella* outbreaks (Li et al., 2021). The *iroN* gene is used for bacterial survival after the invasion because the iron becomes scarce, and it is necessary for *Salmonella* survival and growth within the cell (Webber et al., 2019).

Also, the *invACG* genes acts with host recognition and internalization during cell invasion, which belong to the T3SS-1, while *orgABC*, *sipBD*, *prgHIJK,* and *spaOPQRS*, mediate invasion. On the other hand, *spa* plays an important role in apoptosis and activating MAP kinase-dependent phosphorylation cascades. The *ssaCGHJNV* and *sseBCD* genes encode T3SS-2, which is involved in intracellular survival and replication in phagocytes, as for the SCV trafficking and maturation to fulfill the cycle within the host cell (Skyberg et al., 2006; Van der Heijden and Finlay, 2012; Webber et al., 2019). Moreover, a study by Ryan et al. (2015), mentions that these genes belonging to T3SS-1 and T3SS-2, as well as those involved in SVC, will be differentially regulated under acid-tolerant response (ATR) conditions.

The *mgtB* is an Mg^{2+} transporter co-transcribed with *mgtC*; both genes are required for intramacrophage survival, help *Salmonella* find its location both intracellularly and extracellularly, and growth in low concentrations of Mg^{2+}. Also, as the ability to produce biofilm and prevent chemical and physical stresses (Groisman, 1998; Dos Santos et al., 2021). Genes such as *sopB/sigD* and *pipB,* are involved in the inflammatory reaction in intestinal mucose and fluid secretion (Ochoa and Rodríguez, 2005). The genes *entA* and *entB* encode an integrase and indicate the mobile capability of this element (Wang et al., 2020).

Only the *cspH* gene, one of the cold shock proteins, was present for adaptation to stress which is important in decreased temperatures; thus, *S.* Oranienburg could survive the environmental stress during its exponential phase (Kim et al., 2001). Furthermore, genes associated with

Fig. 3. A) and B). Heatmap of virulence genes presence in 66 *S.* Oranienburg strains. Present genes are in green and absent genes are in yellow. Virulence genes and the virulence category labels are indicated next to the heatmap. 2-column fitting image. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

toxin production; cytolethal distending toxin *cdtB,* which induces typhoid toxin, intoxicate the cell and induce apoptosis, and *pltA* and *pltB* homologous to the pertussis-like toxin (Fuentes et al., 2008; Rodriguez-Rivera et al., 2015). Finally, the *flgG* and *flgH* genes responsible for the flagella formation, can be highly downregulated under the ATR and FliA factor responsible for motility and chemotaxis, with variations in pH can have low mobility (Ohnishi et al., 1994; Ryan et al., 2015). We notice a homogeneity between the first isolates (2008–2010) and the last isolates that included the strains: IRV-23, 25, 32, 38, and 48, and JCS-05, 30, 31, and 32. Nonetheless, different gene profiles among *S.* Oranienburg strains may be due to an evolutionary adaptation to a new niche or livestock practices that have changed in recent years and may influence

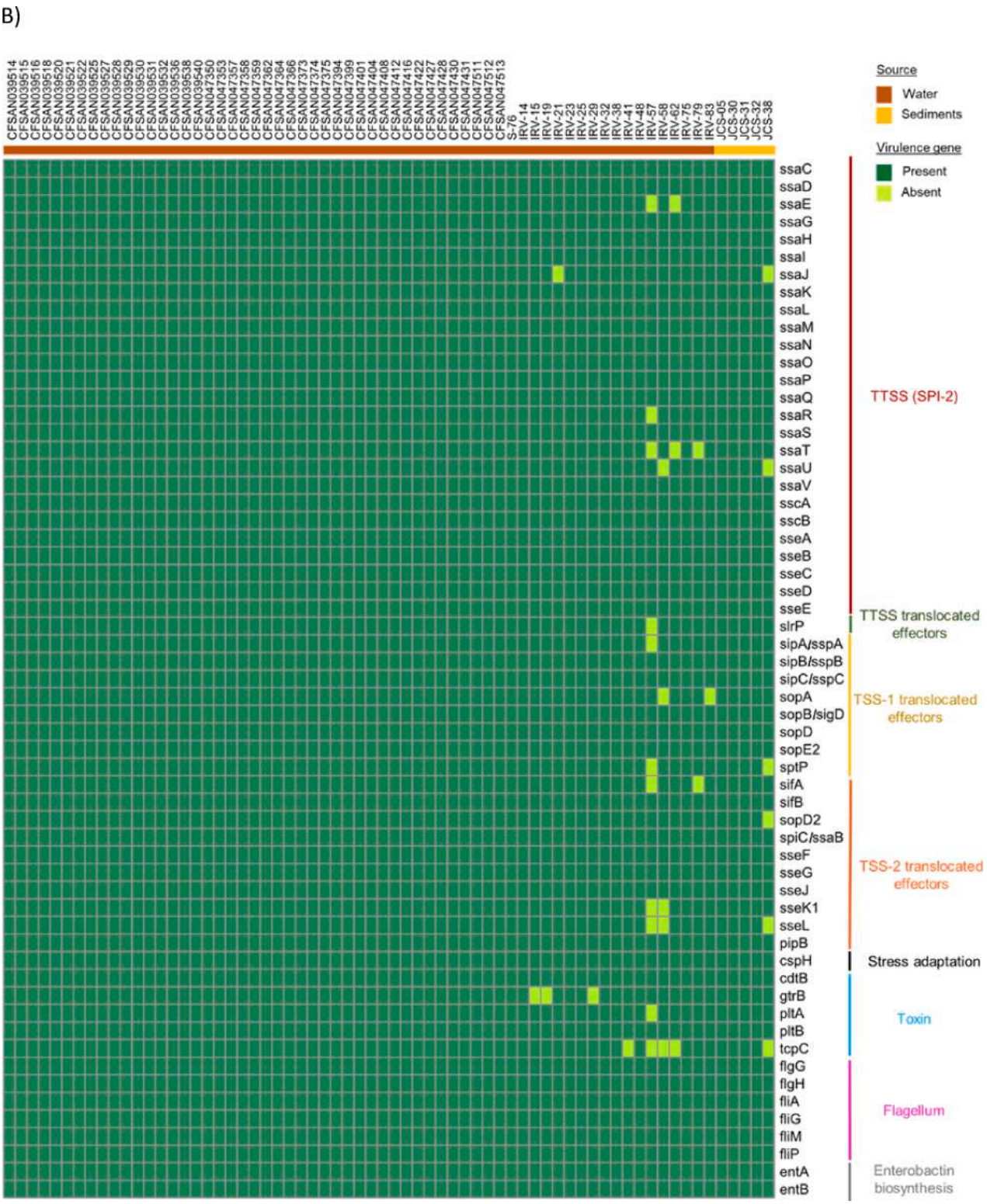

Fig. 3. (*continued*).

survival and multiplication over the years (Webber et al., 2019; Dos Santos et al., 2021).

In addition, we can elucidate that *S.* Oranienburg, when found in an environment, makes use of the gene repertoire of the categories; adherence, biofilm and aggregation producers, iron and magnesium uptake, and stress response, with the purpose of survival and growth until returning to a new host. Although *S.* Oranienburg is an environmental microorganism adapted to both water and sediments, it has the primordial virulence genes that allow it to adhere, invade and to survive intracellularly, being able to carry out an infection in the host, however, studies by Jaiswal et al. (2016) report that the virulence of *S.* enteritidis is altered depending on the environment where it is found and the composition of nutrients, also mention that when growing up in a new environment, *S.* enteritidis acquire new virulence factors and

modulate their pathogenicity. Although it has also been described that the virulence is influenced by the metabolism and the metabolic pathways involved, as well as the stress that *Salmonella* faces within the host cell (Bumann and Schothorst, 2017), thus being a challenge for *S.* Oranienburg to face in subsequent cell line analyzes.

3.4. Distribution of SPI's across genomes

We analyze 22 SPI's, two centisomes, and one genomic island from PAI DB. There are proteins in SPI's exclusive for *Salmonella enterica*, which can vary among species and even within subspecies. It is important since this gives a genetic identity; likewise, this variance will be a function of the presence or absence of proteins (Jacobsen et al., 2011). The SPI's heatmap (Fig. 4, Supplementary Table 5) of *S.* Oranienburg strains had a percentage of identity above 80 % in most of the SPI's evaluated; having a high identity with different serotypes which probably come from the neighboring farms in the rivers of Culiacan Valley, and this coincides with Ilyas et al. (2017), where confirm that evolution of *Salmonella* is mediated by several horizontal transfer events.

The antibiotic resistance island SGI-1 showed an identity of less than 21 %. The two SPI's that had an identity of less than 30 % were the SPI-8, which contained bacteriocin pseudogenes and proteins that code for bacteriocin resistance (Wang et al., 2020), and the SPI-10, involved in modulating intra-macrophage survival. Nonetheless, the two SPI's that had an identity of less than 70 % were; the SPI-11 and SPI-12. SPI-11 has been involved in typhoid pathology, the production of toxins, with significantly higher rates of invasive disease and macrophages survival. SPI-12 regulates genes in the adaptability *in vivo*, providing a better approach to estimating the scenario for the infectious process in mice (Tomljenovic-Berube et al., 2013; Wang et al., 2020). The SPI-6 Typhi showed identities ranging between 62 and 70 % and encoding for a type VI secretion system (T6SS) that allows the establishment in the host during infection (Wang et al., 2020).

On the other hand, the different versions of SPI-2 (Typhi, Choleraesuis, and Typhimurium), cluster perfectly together in the dendrogram and had a similar distribution also SPI-4 (Typhi, Typhimurium, and Choleraesuis), cluster together in the dendrogram. The two islands, SPI-2 and SPI-4, had identities above 90 %; however, the changes were observed in the JCS-38 and nine IRV strains, showing a decrease in identity. Besides, the SPI-2 is one of the most important due to encodes for the T3SS-2 and the effectors of translocation of proteins into the cytoplasm of the host that is required for the intracellular survival and replication in phagocytes, as for the SCV trafficking, maturation and prevent acidification of the medium, this gives it the conditions to fulfill its cycle within the host cell (Skyberg et al., 2006; Van der Heijden and Finlay, 2012; Cheng et al., 2019; Webber et al., 2019). In addition, SPI-4 modulates adhesion, invasion, intracellular adaptation, toxins, and apoptosis and also has high identity with the islands of *S.* Choleraesuis and *S.* Typhi (Yoon et al., 2007).

The SPI-9 carries genes related to T1SS. Interestingly, although this island has been associated with *S.* Typhi, it has a 93 % of identity with *S.* Oranienburg strains (Hensel, 2004; Vazquez-Garciduenas et al., 2014). In addition, SPI-1 is associated with the structure of T3SS-1, essential for the export of effector proteins required for invasion of host cells; we hypothesize that *S.* Oranienburg would be successful in the early stages of infection, considering the identity of 81 % to 94 %, but only three recent strains had a decrease (Hensel, 2004; Ochoa and Rodríguez, 2005; Seribelli et al., 2021).

The centisome CS63 was one of the highest conserved signatures with 99 % of identity. The different versions of SPI-5 were grouped (Choleraesuis, Typhimurium, and Dublin) since they had similar behavior; they coincided with SPI-3 Dublin, and the notorious changes showed in the strains IRV 41 and 83, and JCS-38. However, the SPI-5 is involved in the inflammatory reaction in the intestinal mucose and its fluid secretion (Ochoa and Rodríguez, 2005), while SPI-3 is involved in intra-macrophage survival, biofilm production, and prevention chemical and physical stress (Groisman, 1998; Dos Santos et al., 2021).

Another grouping based on dendrogram was SPI-3 (Typhi and

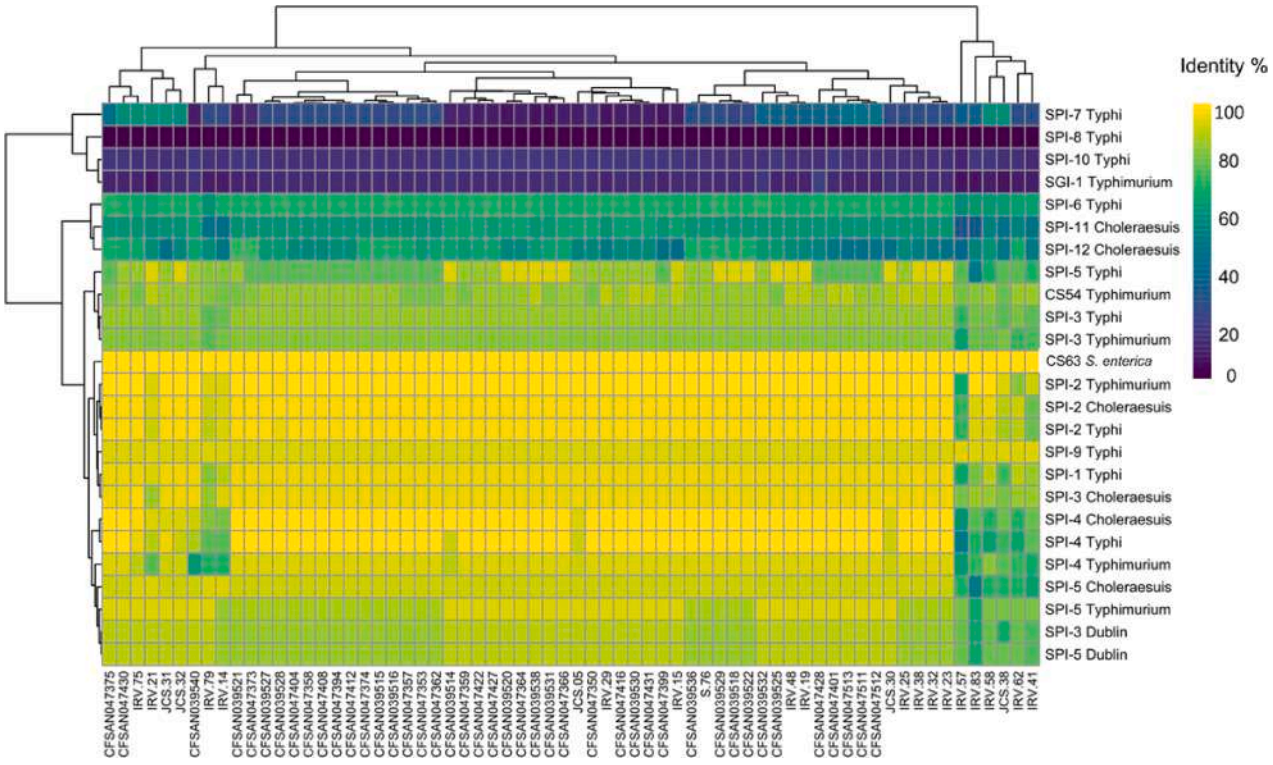

Fig. 4. Heatmap of average percent identity of SPI's in 66 *S.* Oranienburg strains. SPI's from the Pathogenicity Island Database are indicated in the vertical axis, and genomes are horizontal. The average percent identity scale is represented in yellow for 100 % and blue for the 0 % range. 2-column fitting image. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Typhimurium) with CS54. SPI's with differential variation was SPI-5 and SPI-7. The SPI-7 had an identity of less than 40 % since this island is almost exclusive to *S*. Typhi, whereas the strains CFSAN047430, IRV-21, 58, 75, and JCS-31, 32, 38 were distinguished for presenting an increase in the identity of 60–61 %. Also, encoding Vi capsule synthesis and modulates the innate immune response (Hensel, 2004; Wang et al., 2020). The SPI-5 Typhi presented variations from 53 to 93 %. Interestingly, there are strains of IRV and JCS isolates that show noticeable changes in most SPI's, while the rest of the strains does not show significant changes concerning isolates from 2008 to 2010.

The prevalence and conservation of SPI's throughout the strains allow us to better understand the behavior of *S.* Oranienburg due to the concordance with the virulence genes related to SPI's of other serotypes. For example, *S. Choleraesuis* is a swine serotype; however, it has been implicated in human infections and has general integrity above 92 % in SPI's 2 to 5. *S.* Dublin, being a bovine serotype, presented integrity above 85 %, which indicates that *S*. Oranienburg, when in contact with other serotypes from farms and wastewater, has possibly acquired this virulence. A high identity in most of the SPI's evaluated, either in the water and sediment strains, coincides with the repertoire of virulence genes and the probable aptitude of *S*. Oranienburg against a cell line and the potential pathogenicity in the host, in addition, this high similarity to the SPI's of the different serotypes will imply a probable multiplicity of hosts (humans, pigs, cows); although for this, studies that demonstrate their virulent capacity would be lacking. However, it is important to note that S. Oranienburg contains the two most important islands SPI-1 and SPI-2 to cause pathogenesis, from invasion to survival and intracellular replication.

4. Conclusions

In this study we determined the genetic content of *S*. Oranienburg isolated from water and river sediments in Culiacan, Sinaloa, using WGS and we observed that there is little gene fluctuation in the pangenome, resistance and virulence analyses, thus determining that it is a well conserved and adapted serotype to these environmental niches and did not show differential changes in its genetic repertoire regardless source of isolation and the 11 years involved, moreover, had not generated MDR, which is good before the use of antibiotic treatment in the infected host. Considering the sediments as a niche with the necessary nutrients and oxygen viability for their survival, when these are resuspended represents a potential risk for more than mild gastroenteritis if water is used without treatment, allowing an intracellular invasion and survival in epithelial cells and infection in different hosts. In order to characterize the pathogenicity more extensively, further studies based on cell line assays are suggested to analyze adhesion, invasion, and survival, of *S*. Oranienburg, as well as quantitatively evaluate the metabolic and virulent functions during the invasion and perform the correlation of both.

Data availability

The genomes obtained from this study are available in GenBank under BioProject PRJNA831307.

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CRediT authorship contribution statement

Berenice González-Torres: Formal analysis, Investigation, Writing – original draft, Visualization. **Jean P. González-Gómez:** Conceptualization, Methodology, Software, Data curation, Writing – review & editing. **Karina Ramírez:** Supervision, Writing – review & editing. **Nohelia Castro-del Campo:** Supervision, Writing – review & editing. **Irvin González-López:** Methodology, Data curation, Writing – review & editing. **Lennin I. Garrido-Palazuelos:** Software. **Cristóbal Chaidez:** Resources, Writing – review & editing. **José A. Medrano-Félix:** Conceptualization, Investigation, Writing – review & editing, Supervision.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2022.146966.

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