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Multiple Introductions of *Salmonella enterica* Serovar Typhi H58 with Reduced Fluoroquinolone Susceptibility, Chile

Appendix 1

Computational Methods

Read Alignment and SNP Analysis

Seven *Salmonella* Typhi isolates collected from Chile during 2012–2016 were identified as being members of genotype 4.3.1, formerly known as H58 under the haplotyping scheme of Roumagnac et al. 2006 (*1*) by using Pathogenwatch (https://pathogen.watch). Two isolates were typed as H58 lineage 1 (genotype 4.3.1.1), and another further 5 were determined to be members of H58 lineage II (genotype 4.3.1.2). Because *Salmonella* Typhi genotype 4.3.1 has not been reported in South America to date, we contextualized these 7 isolates with a global collection of 2,326 previously published genotype 4.3.1 sequenced to date (*2–11*; Rahman et al. unpub. data, https://www.biorxiv.org/content/10.1101/664136v1) to determine if these might be recent introductions to the region. Subsequently, *Salmonella* Typhi raw read data from all isolates were mapped to the CT18 reference sequence (accession no. AL513382) by using the RedDog mapping pipeline version 1β.11 (https://github.com/katholt/RedDog). RedDog uses Bowtie version 2.2.9 (*12*) to map reads to the reference genome and SAMtools version 1.3.1 (*13*) to identify SNPs that have phred quality scores >30, and to filter out SNPs supported by <5 reads or with 2.5× the average read depth that represent putative repetitive sequences, or those with ambiguous base calls. For every SNP that passed these criteria in any 1 isolate, the consensus base calls for the SNP locus were extracted from all mapped genomes and those with phred scores <20 were treated as unknown alleles and represented with a gap character. These SNPs were used to confirm the 7 *Salmonella* Typhi genomes from Chile were members of genotype 4.3.1 according to an extended global *Salmonella* Typhi framework (*4*,*14*; Rahman et al. unpub.

data, https://www.biorxiv.org/content/10.1101/664136v1) with the GenoTyphi python script (https://github.com/katholt/genotyphi).

Chromosomal SNPs with confident homozygous allele calls (phred score >20) in all genomes mapped were concatenated to form an alignment of alleles at 15,434 variant sites. SNPs called in both prophage and repetitive sequences of 354 kbp; »74% of bases in the CT18 reference sequence, as defined previously (*2*,*3*; Rahman et al. unpub. data, https://www.biorxiv.org/content/10.1101/664136v1), were excluded along with recombinant regions detected by Gubbins version 2.3.2 (*15*) giving a final alignment length of 11,145 chromosomal SNPs out of a total alignment length of 4,462,203 bp for 2,334 H58 isolates (Appendix 2 Table 1, https://wwwnc.cdc.gov/EID/article/26/11/20-1676-App2.xlsx). SNP alleles from 60 *Salmonella* Typhi genomes, and *Salmonella* Paratyphi A AKU1_12601 (accession no. FM2000053) were included as an outgroup for phylogenetic tree rooting (Appendix 2 Table 2). SNP distances were calculated using snp-dists (https://github.com/tseemann/snp-dists).

Phylogenetic Analysis

Maximum likelihood phylogenetic trees were inferred from the chromosomal SNP alignments with RAxML version 8.2.9 (*16*). A generalized time-reversible model and Gamma distribution were used to model site-specific rate variation (GTR+ Γ substitution model) by using GTRGAMMA in RAxML with 100 bootstrap pseuoreplicates to assess branch support for the maximum likelihood phylogeny. Resulting phylogenies were visualized with Microreact (*17*) (https://microreact.org/project/ktISRBvRz) and iTOL (*18*). Raw read data were deposited in the European Nucleotide Archive and individual accession numbers are listed in Appendix 2 Table 1.

Molecular Determination of AMR Genes and Plasmids

Whole sequences where screened using SRST2 version 0.2.0 (*19*) with the ARG-ANNOT (*20*) and PlasmidFinder (*21*) databases to determine molecular determinants of AMR and known plasmid replicon genes

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Appendix Table. Metadata and SRST2 results from isolates of *Salmonella* Typhi H58 from Chile and nearest neighbors

Accession no.	Original publication	Genotype	Country	Year	QRDR mutations		Resistance genes	Plasmids
					gyrA	*parC*		
SRR1957928	Ingle et al. 2019 (2)	4.3.1.2	India	2014	D87N, S83F	S80I	–	–
ERS3348134	This study	4.3.1.2	Chile	2012	D87N, S83F	S80I	–	–
ERS3348156	This study	4.3.1.2	Chile	2015	D87N, S83F	S80I	–	–
ERS3348160	This study	4.3.1.2	Chile	2015	D87N, S83F	S80I	–	–
ERS3348158	This study	4.3.1.2	Chile	2015	D87N, S83F	S80I	–	–
ERS3348157	This study	4.3.1.2	Chile	2015	D87N, S83F	S80I	–	–
DRR071039	Matono et al. 2017 (*7*)	4.3.1.2	India	2012	D87N, S83F	S80I	–	–
DRR071040	Matono et al. 2017 (*7*)	4.3.1.2	India	2012	D87N, S83F	S80I	–	–
DRR071041	Matono et al. 2017 (*7*)	4.3.1.2	India	2012	D87N, S83F	S80I	–	–
SRR5990036	Ingle et al. 2019 (2)	4.3.1.1	India	2015	S83F	–	–	pHCM2
ERS3348167	This study	4.3.1.1	Chile	2015	S83F	–	–	pHCM2
ERS362834	Wong et al. 2016 (*3*)	4.3.1.1	India	2009	D87N	–	–	pHCM2
SRR5982990	Ingle et al. 2019 (2)	4.3.1.1	India	2016	D87N	–	–	pHCM2
ERS1867197	Britto et al. 2020 (*5*)	4.3.1.1	India	2016	–	–	–	pHCM2
ERS248938	Wong et al. 2016 (*3*)	4.3.1.1	India	2006	–	–	–	pHCM2
ERS326284	Wong et al. 2016 (*3*)	4.3.1.1	India	2011	–	–	–	pHCM2
ERS326476	Wong et al. 2016 (*3*)	4.3.1.1	India	2011	–	–	–	pHCM2
ERS3348176	This study	4.3.1.1	Chile	2016	S83F	–	–	–
DRR071000	Matono et al. 2017 (*7*)	4.3.1.1	India	2001	S83F	–	–	–
SRR5193310	Ingle et al. 2019 (2)	4.3.1.1	India	2015	S83F	–	–	–
ERR2663466	Tanmoy et al. 2018 (*9*)	4.3.1.1	Bangladesh	1999	S83F	–	–	–
ERS168231	Wong et al. 2016 (*3*)	4.3.1.1	India	2006	S83F	–	–	–
ERR2663468	Tanmoy et al. 2018 (*9*)	4.3.1.1	Bangladesh	2001	S83F	–	–	–

*QDR, quinolone resistance determining region.