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

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*Salmonella enterica* serovars Typhi, Paratyphi A, and Paratyphi B are the etiologic agents of typhoid and paratyphoid fever. Each year, ≈11–21 million cases and 128,000–161,000 typhoid-related deaths occur, making typhoid a continued health concern in many low- and middle-income countries, particularly among populations without access to clean water or improved sanitation (1). *Salmonella* Typhi H58 lineage, genotype 4.3.1, commonly is associated with multidrug resistance, including resistance to chloramphenicol, ampicillin, and trimethoprim/sulfamethoxazole. In addition, isolates exhibiting resistance to fluoroquinolones have been linked to emergent clades of genotype 4.3.1 in South Asia (2), the spread of which could cause major challenges for disease management.

*Salmonella* Typhi H58 4.3.1 is the dominant genotype in many parts of Southeast and South Asia and in East Africa (3) and has spread globally but has not been reported in Latin America. Recent data on typhoid fever in South America are limited, and little is known about the population structure and antimicrobial susceptibility profiles of *Salmonella* Typhi on the continent. However, a report of 402 *Salmonella* Typhi isolates collected in Colombia during 2012–2015 showed that only 2.2% were resistant to fluoroquinolones (4). In 2016, Colombia reported collecting 204 *Salmonella* Typhi isolates, 12.7% of which exhibited decreased susceptibility to fluoroquinolones (5). Because these reports did not include whole-genome sequencing (WGS) data, determining whether isolates were genotype 4.3.1 is not possible.

Before the 1970s, typhoid fever was endemic in parts of South America and hyperendemic in Chile. However, water quality and sanitation improvements across the continent, partly in response to a major cholera epidemic in 1991, likely have contributed to a steep decline in the incidence of typhoid fever (6). During 1982–1992, Chile implemented interventions to reduce typhoid fever, including immunizing schoolchildren, prohibiting use of untreated sewage to irrigate crops, and detecting and treating chronic carriers. These interventions drastically reduced transmission and typhoid incidence has declined to 0.2 cases/100,000 persons (7), including in the greater Santiago metropolitan region (8).

Chile’s epidemiologic surveillance system tracks suspected typhoid fever. Two thirds of cases are confirmed by pathogen isolation from ordinarily sterile body fluids, such as blood or bone marrow. *Salmonella* Typhi isolates from Chile typically are susceptible

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to antimicrobial agents, but ciprofloxacin resistance has been reported. Among isolates collected during 2009–2016, nearly 2% were ciprofloxacin resistant and 14% displayed intermediate resistance (9). We used WGS and bioinformatic analyses to characterize Salmonella Typhi isolates from Chile to determine if antimicrobial-resistant H58 4.3.1 isolates have been introduced into South America.

The Study
We used a HiSeq WGS platform (Illumina, https://www.illumina.com) to generate 150 bp paired-end reads from Salmonella Typhi isolates collected during 2011–2017 by Chile’s National Typhoid Surveillance System. We assigned sequences to previously defined genotypes and identified 7 genotype 4.3.1 isolates (Appendix 1, https://wwwnc.cdc.gov/EID/article/26/11/20-1676-App1.pdf). Isolates were obtained from clinical cases in the Santiago metropolitan region: 1 in 2012, 5 in 2015, and 1 in 2016. For global context, we analyzed these 7 genomes and 2,386 publicly available sequences (Appendix 2 Table 1, https://wwwnc.cdc.gov/EID/article/26/11/20-1676-App2.xlsx). Among publicly available sequences, 2,326 were genotype 4.3.1 and 60 were non–4.3.1 genotypes (Appendix 1 Table 2). We used the non–4.3.1 genotypes and a Salmonella Paratyphi A sequence as an outgroup for phylogenetic tree rooting. We produced clean and filtered SNP alignments (Appendix 1) and used these alignments to

Figure 1. Global context of Salmonella enterica serovar Typhi genotype 4.3.1 from Chile. Salmonella Typhi H58 genotype 4.3.1-based phylogenetic tree. Branches are colored by genotypes labeled in the tree. A, B, and C arrows indicate isolates from the Chile and the 3 independent introductions. The inner circle indicates country of isolation. The middle circle indicates AMR, excluding reduced susceptibility to fluoroquinolones caused by QDRD SNPs; MDR, including resistance to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole; or XDR, multidrug resistance plus resistance to third-generation cephalosporins and reduced susceptibility to fluoroquinolones. The outer circle indicates number of SNPs, 0, 1, 2 or 3, in the quinolone resistance determining region of gyrA and parC genes. Scale bar indicates nucleotide substitutions per site. AMR, antimicrobial resistance; MDR, multidrug-resistant; QRDR, quinolone-resistance determining region; SNP, single-nucleotide polymorphism; XDR, extremely drug-resistant.
Phylogenomic and SNP analyses confirmed 7 Salmonella Typhi genotype 4.3.1 isolates from Chile. Contact tracing implies that 4/5 isolates from 2015 were part of a localized outbreak. We found that the 7 isolates were members of 2 different sublineages, lineage I (4.3.1.1) and lineage II (4.3.1.2), suggesting multiple introductions into Chile. The 2 isolates of lineage I carried a single gyrA-S83F mutation predicted to confer reduced susceptibility to fluoroquinolones. The 5 lineage II isolates carried 3 quinolone-resistance determining region mutations, 2 in gyrA genes, S83F and D87N, and 1 in parC-S80I. Genotype 4.3.1 triple mutants were predicted to be resistant to fluoroquinolones, and isolates of this sublineage with identical mutations have been observed on the subcontinent of India and have been associated with treatment failure (2,11,12). None of the lineage II triple mutants in Chile carried detectable horizontally acquired AMR genes.

To provide a global contextualization of Salmonella Typhi genotype 4.3.1 in Chile, we analyzed the novel sequences alongside 2,326 existing sequences from 31 countries (Figure 1). The 4.3.1.2 triple...
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mutants from Chile formed a closely related phylogenetic cluster (median distance 2 SNPs) with sequences that have the same antimicrobial susceptibility profile isolated from India during 2012-2014, indicating an introduction from South Asia (Figure 2, panel A).

The two 4.3.1.1 isolates from 2015 and 2016 in Chile were in distinct subclades of the tree and were separated by 19 SNPs, suggestive of 2 separate introductions. Of these, 1 introduction was closely related to a 2015 isolate from India (5 SNPs apart) (Figure 2, panel B) and the other was nested in a cluster of sequences from Southeast and South Asia and most closely related (median distance of 20 SNPs) to sequences from India and Bangladesh (Figure 2, panel C).

Conclusions

Our study confirmed Salmonella Typhi H58 genotype 4.3.1 in South America. Phylogenomic and SNP analyses indicate ≥3 separate genotype introductions into Chile; 5/7 isolates carried 3 distinct mutations, 2 in the gyrA gene, at D87N and S83F, and 1 in the parC gene at S80I, which are associated with ciprofloxacin resistance. For a high-income country with adequate surveillance, like Chile, the presence of fluoroquinolone-resistant genotype 4.3.1 Salmonella Typhi has no immediate implications. However, if this genotype is transferred to low- or middle-income countries in South America, it could have major consequences. Therefore, these data should be of concern to other countries in the region where potential typhoid fever transmission remains high and adequate sanitation might be lacking (5,6,10). Ciprofloxacin is a first-line drug for typhoid fever in much of Latin America, and fluoroquinolone-resistant genotype 4.3.1 would reduce its long-term efficacy.

Most diagnostic laboratories across South America are using pulsed-field gel electrophoresis to study Salmonella Typhi epidemiology (13), but efforts are underway to implement WGS for epidemiologic surveillance in several countries (14,15). However, WGS-based approaches for detecting genotype 4.3.1 and understanding trends in genotype population, circulating lineages, and AMR dynamics have not been adopted widely across the region. Our work highlights the need for a uniform WGS platform for global Salmonella Typhi monitoring and the need to elucidate the current epidemiology of typhoid fever in South America.

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Ms. Maes is a PhD student at the University of Cambridge. Her research interests include the intracellular pathogen Salmonella Typhi, phylogenetics of Salmonella Typhi in Latin America, and the behavior of Salmonella Typhi in macrophages and the gallbladder.

References


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**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.)

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 pseudoreplicates 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

**References**


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

<table>
<thead>
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<th>Accession no.</th>
<th>Original publication</th>
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<th>Year</th>
<th>QRDR mutations</th>
<th>Resistance genes</th>
<th>Plasmids</th>
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*QRDR, quinolone resistance determining region.*