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Salmonella enterica serovar Typhi H58 clone has been endemic in Zimbabwe from 2012 to 2019

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Background: Typhoid fever, caused by *S. enterica* ser. Typhi, continues to be a substantial health burden in developing countries. Little is known of the genotypic diversity of *S. enterica* ser. Typhi in Zimbabwe, but this is key for understanding the emergence and spread of this pathogen and devising interventions for its control.

Objectives: To report the molecular epidemiology of *S. enterica* ser. Typhi outbreak strains circulating from 2012 to 2019 in Zimbabwe, using comparative genomics.

Methods: A review of typhoid cases records from 2012 to 2019 in Zimbabwe was performed. The phylogenetic relationship of outbreak isolates from 2012 to 2019 and emergence of antibiotic resistance was investigated by whole-genome sequence analysis.

Results: A total 22 479 suspected typhoid cases, 760 confirmed cases were reported from 2012 to 2019 and 29 isolates were sequenced. The majority of the sequenced isolates were predicted to confer resistance to aminoglycosides, β-lactams, phenicols, sulphonamides, tetracycline and fluoroquinolones (including *qnrS* detection). The *qnrS1* gene was associated with an IncN (subtype PST3) plasmid in 79% of the isolates. Whole-genome SNP analysis, SNP-based haplotyping and resistance determinant analysis showed that 93% of the isolates belonged to a single clade represented by multidrug-resistant H58 lineage I (4.3.1.1), with a maximum pair-wise distance of 22 SNPs.

Conclusions: This study has provided detailed genotypic characterization of the outbreak strain, identified as *S.* Typhi 4.3.1.1 (H58). The strain has reduced susceptibility to ciprofloxacin due to *qnr*S carried by an IncN (subtype PST3) plasmid resulting from ongoing evolution to full resistance.

Introduction

Salmonella enterica serovar Typhi (*S. enterica* serovar Typhi), the aetiological agent of typhoid fever, affects approximately 10.9 million people annually worldwide.[1] Typhoid fever remains a major public health problem in Zimbabwe with recurrent outbreaks reported since 2009.[2] Between 2009 and 2017, 16 398 suspected cases and 550 confirmed cases of typhoid fever were reported in Zimbabwe.[2] In sub-Saharan Africa, the burden of typhoid fever, caused by *S. enterica* ser. Typhi, remains largely unknown, in part because of a lack of facilities for its isolation,

identification and discrimination from infection with invasive non-typhoidal *Salmonella*.[3]

The emergence of antibiotic-resistant *S. enterica* ser. Typhi strains, particularly MDR H58 typhoid strains in Africa (including Zimbabwe[2]) and Asia, highlights the necessity of monitoring the antibiotic resistance profiles and molecular epidemiology of this invasive pathogen.[4] In Southern Africa, MDR typhoid is mainly due to determinants encoded on IncHI1 plasmids.[5] Such resistance genes are clustered on composite transposons and include *catA, sul1, sul2, dfrA, bla*$_{TEM-1}$, *strA, strB, tetA, tetB, tetC* and *tetD*. These MDR-associated genes can also be found integrated in the chromosome of H58 *S. enterica* ser. Typhi in isolates from countries including India and Bangladesh.[6] The situation is somewhat different in Western Africa, where H58 is still uncommon and MDR typhoid is spread via non-H58 clades[7] with both IncH1 and IncY plasmids being present in the circulating population. Other plasmid incompatibility types identified in *S. enterica* ser. Typhi are IncH and IncN, but these are relatively uncommon.[5]

Due to the continued emergence of resistance to antibiotics used for therapy (ampicillin, amoxicillin, co-trimoxazole and chloramphenicol),[8] the empirical use of fluoroquinolones for the treatment of these infections has become commonplace[9] and isolates showing antibiotic resistance are recommended to be treated with ceftriaxone or azithromycin.[3] Genetic signatures associated with fluoroquinolone resistance are mainly found in the quinolone resistance determining region (QRDR) in the *S. enterica* ser. Typhi genome, as well as fluoroquinolone resistance-conferring plasmids containing *qnrB2, qnrB4* and *qnrS1* genes.[5]

The relationship of antimicrobial susceptibility, *S. enterica* ser. Typhi genotype and patient outcome is poorly understood[10] and a better understanding is essential for effective typhoid management. However, antimicrobial resistance (AMR) gene abundance strongly correlates with socio-economic, health and environmental factors, which are used to predict AMR gene abundances in all countries in the world.[11] Improving sanitation, increasing access to clean water, and ensuring good governance, as well as increasing public healthcare expenditure and better regulating the private health sector are all necessary to reduce global antimicrobial resistance.[12]

There is a paucity of data regarding the geographical distribution, incidence and phylogenetics of MDR *S.* Typhi in Zimbabwe.[13] To investigate the phylogenetic relationship and emergence of antimicrobial resistance of *S. enterica* ser. Typhi in Zimbabwe the whole genome sequences of a collection of *S. enterica* ser. Typhi clinical isolates from outbreaks from Harare (2012 to 2019) and Gweru (2018) were analysed.

Methods

Outbreak data collection

Suspected and confirmed typhoid cases were detected and reported to public health authorities through the national surveillance system. Demographic variables collected included symptoms, onset date, age, management and treatment outcome for Gweru outbreak (2018). In the Harare outbreaks (2012 to 2019) only suspected and confirmed cases statistics were collected for each year.

Bacterial isolates collection and ethics considerations

Ethics approval for the study was granted by the University of Pretoria, South Africa (779/2018) and Medical Research Council of Zimbabwe (MRCZ/A/2369). Twenty-nine isolates ($n = 29$) were selected for whole genome sequencing from the National Microbiology Reference Laboratory *Salmonella* Biobank using phenotypic antimicrobial susceptibility testing results from the National *Salmonella* Surveillance Program to provide representation of antimicrobial resistance phenotypes, different specimen types (stool and blood isolates), and different years of isolation [2012 (1), 2014 (1), 2016 (6), 2017 (4), 2018 (12) and 2019 (5)] (Table 1). The selected isolates were as follows: eight from Budiriro [2014 (1), 2016 (3), 2019 (4)], five from Glenview [2012 (1), 2016 (1), 2018 (3)], three from Kuwadzana [2016 (1), 2018 (2)], three from Mbare [2016 (1), 2017 (2)], one from Mufakose (2019), one from Stoneridge (2018), two from Dzivarasekwa, one from Hopley (2018) in Harare, and five from Mkoba in 2018 [Mkoba 14 (1), Mkoba 20 (2) and Mkoba 15 (2)] in Gweru (Table 1). The demographic data (age, sex, etc.) were retrieved from Gweru Provincial Hospital and Beatrice Road Infectious Diseases hospital records. Antimicrobial susceptibility testing was done using Kirby–Bauer disc diffusion assay as described previously (Table 1).[14]

Whole-genome sequencing and analysis

In brief, 1 mL of an overnight *Salmonella* culture was harvested. Genomic DNA was extracted from the 29 *S. enterica* ser. Typhi isolates using the Easy-DNA kit (Invitrogen). The DNA concentrations were measured with a Qubit fluorometer (Life Technologies) and standardized to 0.2 ng/μL, the extracted DNA were stored at −20°C prior to library preparation. Library preparation was performed using the Nextera XT DNA Library Preparation Kit (Illumina). Subsequently, sequencing was performed with a MiSeq benchtop sequencer (Illumina). Raw sequence data were submitted to the European Nucleotide Archive (ENA) (http://www.ebi.ac.uk/ena) under study accession PRJEB41494. The raw reads were *de novo* assembled using SPAdes 3.7.0.[15] The assembled genomes were analysed to identify the MLST ST for *Salmonella enterica*, plasmid replicons and acquired antimicrobial resistance genes and chromosomal point mutations including mutations in the *gyrA* and *gyrB* DNA gyrase genes and in the *parC* and *parE* DNA topoisomerase IV genes using the pipelines MLST (version 1.7),[16] PlasmidFinder (version 1.2)[17] and ResFinder (version 3.1).[18] The subtype of the IncN plasmid was further investigated using publicly available plasmid MLST (pMLST) scheme for IncN,[19] available at https://pubmlst.org/plasmid/. The haplotype of each genome was determined using an in-house bioinformatics script[4] which identifies biallelic polymorphism positions (BiPs) and assigns the haplotype according to the dendrogram by Roumagnac *et al.*,[20] such as haplotype H58 being defined by BiP36, BiP48, BiP56, and BiP33. Additionally, node B of haplotype H58 lineage I was determined based on SNP position 1193220 as defined by Kariuki *et al.*[21]

SNP-base genotyping was performed with GenoTyphi (first described in Wong *et al.*[22] and available from https://github.com/katholt/genotyphi). The 4.3.1.1 clade identified in this study was added to GenoTyphi following studies by Britto *et al.*[23] as well as Rahman *et al.*[24]

Phylogenetic reconstruction

SNPs were determined using the CSI Phylogeny (version 1.4) pipeline.[25] Briefly, raw reads were aligned against the reference *S. enterica* ser. Typhi CT18 genome using Burrows-Wheeler Aligner (BWA) version 0.7.2.[26] The SNPs were identified from the alignments using the 'mpileup' module in SAMTools version 0.1.18.[27] Subsequently, SNPs were selected that met the following criteria: (i) a minimum distance of 15 bp between each SNP; (ii) a minimum of 10% of the average depth; (iii) the mapping quality was above 25; (iv) the SNP quality was more than 30; and (v) all INDELs were excluded. The qualified core SNPs were concatenated to a single alignment

Table 1. Epidemiological features and antimicrobial susceptibility testing of the 29 whole-genome-sequenced isolates

Isolate	Patient sex	Patient age	Specimen	Year of isolation	Township	City	CIP	TET	CRO	AMP	CHL	AZM	SXT
HG3-1	F	34	Stool	2012	Glenview 3	Harare	S	S	S	R	R	S	R
HB1-2	M	8	Blood	2014	Budiriro 1	Harare	S	S	S	R	R	S	R
HG8-3	F	26	Blood	2016	Glenview 8	Harare	I	R	S	R	R	S	R
HK3-4	F	7	Blood	2016	Kuwadzana 3	Harare	I	R	S	R	R	S	R
HB1-5	M	3	Blood	2016	Budiriro 1	Harare	I	R	S	R	R	S	R
HB5-6	M	21	Blood	2016	Budiriro 5	Harare	I	R	S	R	R	S	R
HB1-7	M	21	Blood	2016	Budiriro 1	Harare	I	R	S	R	R	S	R
HM-8	F	5	Blood	2016	Mbare	Harare	I	R	S	R	R	S	R
HM-11	F	10	Blood	2017	Mbare	Harare	S	S	S	S	S	S	S
HD-10	F	7	Blood	2017	Dzivarasekwa Ext	Harare	S	S	S	R	R	S	R
HM-9	M	20	Stool	2017	Mbare	Harare	S	S	S	S	S	S	S
HD-12	F	13	Blood	2017	Dzivarasekwa Ext	Harare	S	S	S	R	R	S	R
HG3-15	M	18	Blood	2018	Glenview 3	Harare	I	R	S	R	R	S	R
HG1-14	M	6	Stool	2018	Glenview 1	Harare	I	R	S	R	R	S	R
HG3-13	F	16	Stool	2018	Glenview 3	Harare	I	R	S	R	R	S	R
HK3-16	M	19	Blood	2018	Kuwadzana	Harare	I	R	S	R	R	S	R
HS-17	F	64	Blood	2018	Stoneridge	Harare	I	R	S	R	R	S	R
HK3-18	F	23	Blood	2018	Kuwadzana 3	Harare	I	R	S	R	R	S	R
HH-19	M	21	Stool	2018	Hopley	Harare	I	R	S	R	R	S	R
GM14-20	F	9	Blood	2018	Mkoba 14	Gweru	R	R	S	R	R	S	R
GM15-21	M	48	Blood	2018	Mkoba 15	Gweru	R	R	S	R	R	S	R
GM20-22	M	44	Blood	2018	Mkoba 20	Gweru	R	R	S	R	R	S	R
GM20-23	M	17	Blood	2018	Mkoba 20	Gweru	R	R	S	R	R	S	R
GM15-24	M	34	Blood	2018	Mkoba 15	Gweru	R	R	S	R	R	S	R
HM-25	F	15	Blood	2019	Mufakose	Harare	R	R	S	R	R	S	R
HB-26	F	13	Blood	2019	Budiriro	Harare	I	R	S	R	R	S	R
HB-27	F	13	Blood	2019	Budiriro	Harare	I	R	S	R	R	S	R
HB-28	F	6	Blood	2019	Budiriro	Harare	I	R	S	R	R	S	R
HB-29	M	14	Blood	2019	Budiriro	Harare	I	R	S	R	R	S	R

M, male; F, female; CIP, ciprofloxacin; TET, tetracycline; CRO, ceftriaxone; AMP, ampicillin; CHL, chloramphenicol; AZM, azithromycin; SXT, co-trimoxazole.

corresponding to the positions of the reference genome. A maximum likelihood (ML) phylogenetic tree was built, with RAxML version 8.0.20 under the GTR model with 200 bootstrap.[28]

Results

Epidemiological data

A total of 22 479 typhoid cases were recorded in Zimbabwe from 2012 to 2019, with the highest number of cases in Harare city [20 288/22 479 (90.3%) suspected and 737/760 (97%) confirmed]. The burden of typhoid disease is underestimated due to lack of diagnostic laboratories and we estimate that the cases are more than the reported figures. Gweru, a city 275 km from Harare recorded 2191 suspected typhoid cases in 2018 for the first time, of which 23 were confirmed and 10 were fatal (Figure 1b). The outbreak affected five suburbs: Mkoba, Woodlands, Senga, Ascot and Windsor Park (Figure 1). Retrospective review of patient records in Gweru revealed that the first suspected case occurred in July 2018 (Figure 1c) and four deaths occurred prior to the declaration of the outbreak. The deaths were due to severe complications such as acute renal and hepatic failure. During the initial days of the outbreak the recommended empirical treatment was administered, which consisted of 500 mg of ciprofloxacin twice daily for a period of 5 to 7 days. The treatment regimen was changed to a 1 g daily dose of ceftriaxone as a starting dose followed by 500 mg of azithromycin daily for 7 days (for severe cases) and 500 mg of azithromycin once daily for 7 days (outpatient management), following identification of resistance to fluoroquinolones (Figure 1d). Due to lack of diagnostic capacity, only 23 cases were confirmed for the entire Gweru outbreak and all isolates were ciprofloxacin resistant. Based on our analysis of this subset, and because they were from different suburbs in the city, we speculate that the majority of the outbreak was likely to have been caused by a ciprofloxacin-resistant strain. Paediatric patients received a dose of 20 mg/kg azithromycin daily for 14 days. A sharp increase in reported cases from 02 August 2018 that peaked in early August 2018 was followed by a gradual decline by the end of August 2018 (Figure 1). The majority of infected patients where $\leq$24 years old. Following detailed case investigation, faecal contamination of treated municipal drinking water was implicated as the cause of the outbreak. The overall case fatality ratio (CFR) was estimated

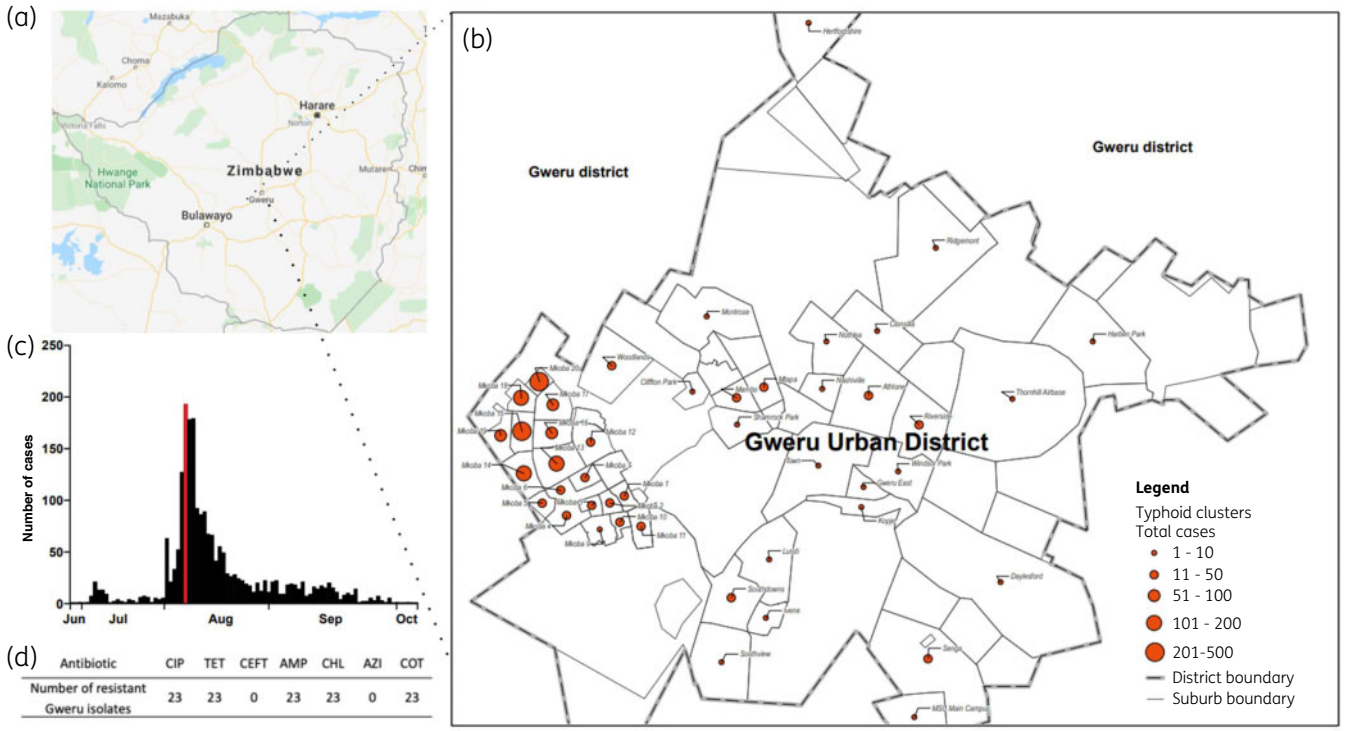

Figure 1. Typhoid distribution by time and location and susceptibility. (a) Zimbabwe map. (b) Geographic distribution of suspected and confirmed cases of typhoid per suburb. (c) Daily number of suspected typhoid cases over time. (d) Antimicrobial susceptibility pattern of 23 confirmed cases. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

to be 0.5% (10/2191) during the outbreak period 03 July to 12 October 2018 in Gweru.

Antimicrobial resistance patterns, genes, sequence type and plasmid replicons

The majority of the *S. enterica* ser. Typhi isolates (27/29; 93%) belonged to genotype 4.3.1.1 (H58), two isolates from 2017 belonged to genotype 3.1.1 (ST2) (Figure 2). The genotype 3.1.1 (non-H58) strains were susceptible to all the antibiotics tested (Table 1). The *S.* Typhi 4.3.1.1 (H58) isolates from Harare (18/24; 75%) collected during the years 2016, 2017, 2018 and 2019 harboured the *aph(6)-Id* and *aph(3″)-Ib* genes that confer resistance to aminoglycosides. Additional genes such as $bla_{\text{TEM-1B}}$ (associated with resistance to β-lactam antibiotics), *catA1* (associated with resistance to phenicols), *sul1* and *sul2* (associated with resistance to sulphonamides) and *dfrA7* (associated with resistance to trimethoprim) were detected. The *tet*(A) (associated with resistance to tetracycline) and *dfr*A14 (associated with resistance to trimethoprim) genes were absent in only four Harare isolates (Figure 2). The two *S.* Typhi 3.1.1 strains harboured no resistance genes. The isolates from the 2018 Gweru outbreak were positive for aminoglycoside, β-lactams, phenicol, sulphonamide, trimethoprim and tetracycline resistance genes and the *gyrA* mutation (Figure 2). It was not possible to resolve the precise locations of the acquired AMR genes due to the limitations of short read assembly. However, according to the results we obtained (Figure 2) MDR genes are likely to be carried on the typical composite transposon comprising Tn*6029* (encoding $bla_{\text{TEM-1}}$, *sul2* and *strAB*), inserted in Tn*21* (carrying a class 1 integron encoding *dfrA* alleles in the gene cassette and *sul1* at the end), which is in turn inserted within Tn*9* (encoding *catA1*).[29] The other MDR genes *qnrS, dfrA14* and *tet*(A), are likely to have been associated with an IncN plasmid (subtype PST3).

The *qnrS1* gene was common amongst MDR *S. enterica* ser. Typhi 4.3.1.1 isolates with reduced ciprofloxacin susceptibility (79%; 18 from Harare and 5 from Gweru) (Table 1 and Figure 2). The five Gweru isolates and one from Harare had a single mutation in the *gyrA* QRDR that led to an amino acid substitution in codon 87 (Asp87Asn), with one strain (HM-25) showing a mutation of codon 464 of *gyrB* (TCT to TTC), leading to the amino acid substitution of a phenylalanine for a serine, further underpinning the importance of *gyrA*-associated SNPs, likely in response to antimicrobial selection pressure[5] (Figure 2). The presence of the *qnrS1* gene and mutation in the *gyrA* QRDR was associated with complete resistance to ciprofloxacin (Table 1 and Figure 2). The outbreak strain appears to have evolved by acquiring the *gyrA* mutation and this resulted in the Gweru cluster being fully resistant to ciprofloxacin.

None of the 29 *S. enterica* ser. Typhi strains were positive for the globally dominant IncHI1 plasmid. However, the analysis revealed that 23 [2016 (6), 2018 (12) and 2019 (5)] of the *S.* Typhi 4.3.1.1 strains contained an IncN plasmid (subtype PST3) instead of the IncHI1 plasmids that often harbour the resistance genes responsible for MDR typhoid. Six isolates from Harare [2012 (1), 2014 (1), 2017 (2) and 2018 (2)] contained no IncN plasmid.

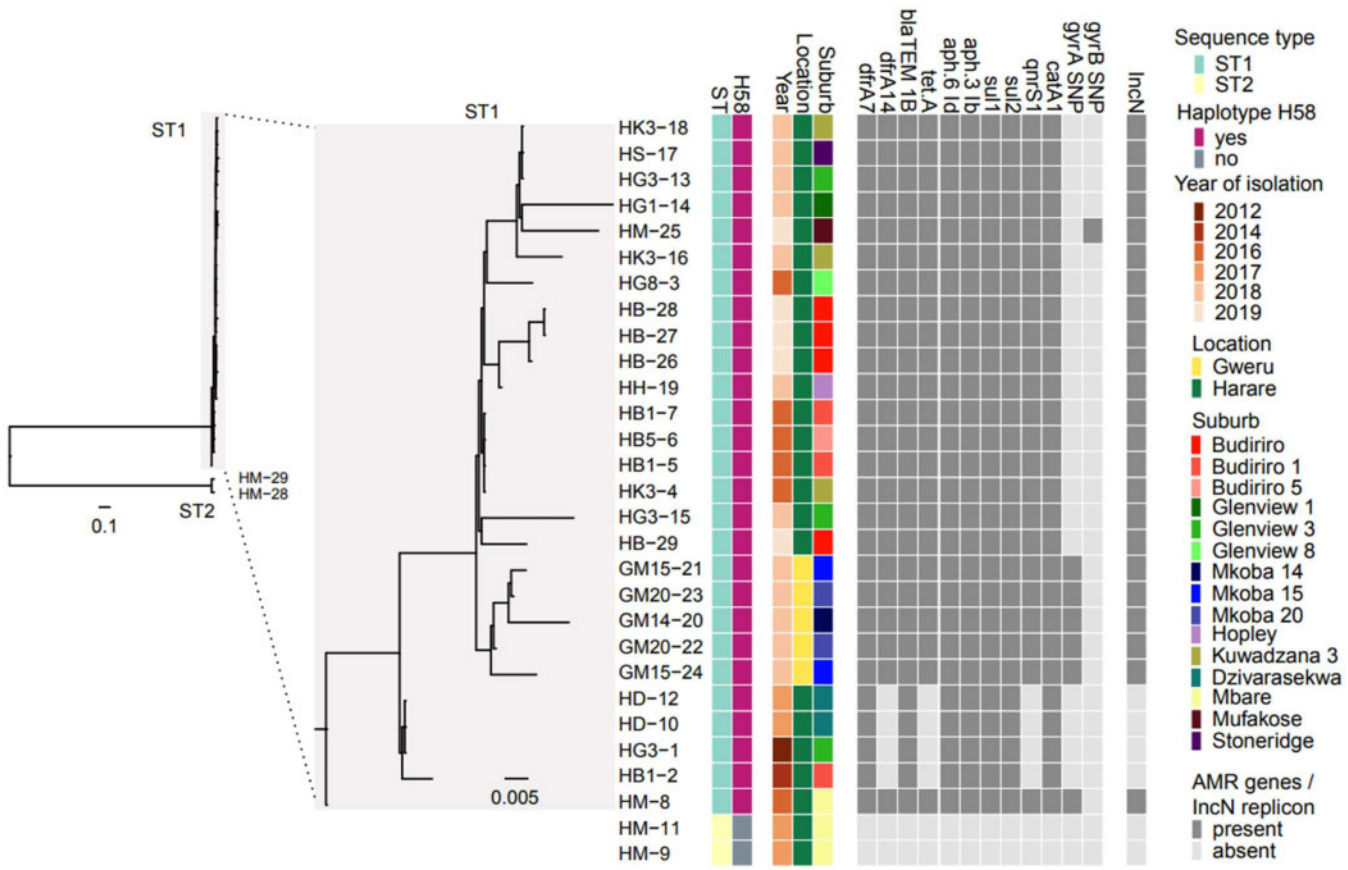

Figure 2. Phylogenetic relatedness and genomic characteristics of 29 *S. enterica* ser. Typhi isolates from Harare and Gweru outbreaks (2012 to 2019) in Zimbabwe. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Phylogenetic relationship of S. enterica ser. Typhi isolates from Harare (2012–19) and Gweru (2018)

A maximum likelihood phylogenetic tree was constructed using the concatenated SNPs with reference to *S. enterica* ser. Typhi CT18 genome sequence (Figure 2). The 27/29 *S. enterica* ser. Typhi isolates (22 from Harare: 2012, 2014, 2016, 2017, 2018, 2019 and all 5 from 2018 Gweru) of haplotype H58 genotype 4.3.1.1 formed a distinct clade with a maximum pair-wise distance of 22 SNPs. Older isolates from Harare from 2012 and 2014 were more deeply rooted and exhibited less sequence divergence from the most recent common ancestor of the clade compared with more-recent isolates. Five isolates from Gweru in 2018 formed a distinct subclade within the main Harare epidemic clade (Figure 2).

Discussion

Harare, the capital city of Zimbabwe, has been the epicentre of typhoid outbreaks in recent years, and serves as a hub for transport systems connecting with other cities in the country. The first typhoid outbreak in Harare was recorded in 2009.[2] In 2014, Harare recorded its first ciprofloxacin-resistant typhoid isolate.[2] Ciprofloxacin resistance of typhoid isolates in Harare rose from 4.2% in 2014 to 22% in 2017[2] and 75% in Kuwadzana, one of the Harare suburbs, in 2018. In 2018, Gweru (a city 275 km southwest of Harare) also recorded a 100% ciprofloxacin-resistant typhoid outbreak. It was the first report of a typhoid outbreak in Gweru. Investigations implicated poor drinking water quality as the source of the outbreak, similar to the previously reported outbreak in 2009 in Zimbabwe.[30] Initial empirical treatment with ciprofloxacin[31] was not successful and resulted in the death of four people (through severe complications such as renal failure). Based on the AST profiles of the isolates, the treatment was changed to azithromycin and ceftriaxone. The complications faced with initial empirical treatment due to MDR typhoid were similar to those reported by Bano-Zaidi *et al.*[32] during a study conducted in a major outbreak in Mexico. We investigated the source of this MDR strain by checking the genetic mechanisms associated with the MDR phenotype. Our study showed that the new ciprofloxacin-resistant Gweru subclade H58 lineage 1 (Gweru cluster) has been introduced into Gweru and was associated with a lack of ciprofloxacin treatment efficacy. Whole genome analysis of 2012 to 2019 Harare *S. enterica* ser. Typhi suggests that the strains have undergone microevolution from reduced susceptibility to complete resistance to ciprofloxacin within Harare. Full resistance to ciprofloxacin is quite rare in *S. enterica* ser. Typhi and is mainly linked to QRDR triple mutants found in India.[33] We speculate that these organisms have been transferred, maintained and selected through the sustained movement of people between the two cities. Given the national significance of ciprofloxacin for the treatment of typhoid fever, our findings have a major implication for the long-term use

of this antimicrobial in Zimbabwe. The sudden emergence and rapid spread of MDR *S. enterica* ser. Typhi with complete resistance to ciprofloxacin, underline the importance of AMR surveillance for typhoid and other priority pathogens in Zimbabwe.

Previous large outbreaks in Zimbabwe recorded during the period 2012 to 2017 were caused by *S. enterica* ser. Typhi isolates of MDR H58 haplotype.[2] Since 2012, there has been a rapid increase in the incidence of MDR, H58-haplotype typhoid in Zimbabwe.[2] Despite the known circulation of H58 *S.* Typhi in Zimbabwe, there is a paucity of data regarding the exact lineages circulating and their phylogenetic structure. Of note, genome 4.3.1 lineage 1 (H58), which has become common in sub-Saharan Africa and accounts for the majority of AMR typhoid globally, was the dominant strain in Zimbabwe. Genomes previously reported by Ingle *et al.*[34] from travellers returning to the United Kingdom from Zimbabwe in 2014, 2015 and 2016 were genotyped as 4.3.1.1 with IncN plasmids (subtype PST3), consistent with the current study. Hence, these isolates were from the same outbreak. The MDR H58 haplotype was introduced from Asia into Africa, displacing the H58 antibiotic-susceptible lineage and transforming the global population structure of this pathogen.[6] Phylogenetic analyses further suggest that the MDR *S. enterica* ser. Typhi genotype 4.3.1 lineage 1 dominates and circulated across Southeast Asia (lineage I: Vietnam, Cambodia, and Laos) before introduction in Africa.[6] Strains from the Gweru 2018 outbreak formed a distinct subclade that was closely related to earlier and contemporary H58 genotype 4.3.1.1 strains from Harare, suggesting recent transmission. In other studies by Park *et al.*,[13] analyses of 249 *S. enterica* ser. Typhi isolates showed that genotype 4.3.1 was found only in East Africa and genotype 3.1.1 was found only in West Africa. The genotype 3.1.1 is relatively common across Africa, predominantly in western and central countries.[7] Park *et al.*[13] found out that Ghana was the most likely recent source of the MDR 3.1.1 *S. enterica* ser. Typhi population in West Africa. Conversely, Zimbabwe genotype 3.1.1 (ST2) strains, were susceptible to all the antibiotics tested. We speculate that importation of these organisms occurred from West or Central Africa.[7,13]

Resistance of *S. enterica* ser. Typhi to fluoroquinolones is of public health importance, hence understanding the mechanisms of such resistance is of paramount importance. Mutations in the QRDR of *gyrA* at positions Ser83 and Asp87 and *parC* at position Ser80, as well as outside of the QRDR region of *parE* at Ser458 and Glu460, are known to lead to ciprofloxacin resistance.[35] This mechanism of resistance was not common among isolates analysed. The *qnrB2, qnrB4* and *qnrS1* resistance determinants encoded on plasmids in *S. enterica* ser. Typhi are still rare.[5] However, whole genome sequence analysis showed that *S. enterica* ser. Typhi isolates from Harare and Gweru contained a *qnrS* gene that is responsible for reduced susceptibility to ciprofloxacin due to plasmid-mediated fluoroquinolone resistance, as previously reported.[34] The 2018 Gweru and Harare (2012 to 2019) outbreaks were caused by the H58 genotype 4.3.1 lineage 1 of *S. enterica* ser. Typhi containing *qnrS* genes, and hence had reduced susceptibility to ciprofloxacin. However, complete phenotypic ciprofloxacin resistance was found in isolates from Gweru and Harare with *qnrS* genes and mutations in the QRDR. Our data suggests that there was a significant association between disease outcome and susceptibility profile of the infecting organism. The presence of the IncN plasmid replicon in the H58 haplotype was rare, as it is usually associated with a single distinct plasmid type known as IncHI1.[36] IncHI1 is the most commonly implicated plasmid group found in *S. enterica* ser. Typhi.[37] Bayesian analysis suggests that this plasmid was first acquired by H58 and some other haplotypes of *S. enterica* ser. Typhi in Asia around the early 1990s.[6] The Gweru (2018) and Harare (2012 to 2019) typhoid outbreak isolates were resistant to ampicillin, chloramphenicol, cotrimoxazole, tetracycline and had reduced or complete resistance to ciprofloxacin, which was associated with the presence of the IncN plasmid and an MDR composite transposon (with *dfrA7* in the integron) as previously reported by Ingle *et al.*[34] and Wong *et al.*[6] Consequently, according to the MDR pattern of the outbreak strains, ceftriaxone and azithromycin were the only options left for patient therapy. The IncN plasmid is one of the major vehicles for the dissemination of the ESBL CTX-M-1 and plasmid-mediated quinolone resistance (PMQR) genes in *Salmonella* isolates from humans, animals and the environment.[38,39]

The SNPs associated with complete resistance to fluoroquinolone antibiotics were observed in five isolates from Gweru, suggesting ongoing evolution of *S. enterica* ser. Typhi strain in response to selection pressure from the use of antimicrobials of this class. IncN plasmids have been previously reported to be associated in Pakistan with ESBL in *S. enterica* ser. Typhi,[40] carbapenemase and PMQR genes,[40,41] but ESBL-encoding and carbapenemase genes were not present in isolates in this study.

The identified H58 genotype 4.3.1.1 strains from Harare and Gweru were all MDR with a maximum pair-wise distance of 22 SNPs. This indicated that this *S. enterica* ser. Typhi clade is likely to have been circulating in Harare since 2012 and then spread to Gweru during 2018, resulting in the reported outbreak. Strategies to reduce the impact of AMR pathogens have been focusing on reducing antimicrobial use.[42] However Hendriksen *et al.*[11] observed that antimicrobial use only explained a minor part of the occurrence of AMR across the world. Other factors, such as those related to transmission, including infection control, sanitation, access to clean water, access to assured quality antimicrobials and diagnostics, travel and migration, are also important for a holistic approach to control the impact AMR.[43] Hence, improving sanitation infrastructure, disease prevention and hot-spot surveillance for early detection and pathogen surveillance in informal settlements using metagenomics approaches will be of great importance.[11]

The majority of the isolates belonged to H58 genotype 4.3.1.1, which formed a single distinct subclade that showed a closely related common ancestor and close association with strains from Harare and Gweru. One of the limitations of this study was the small sample size, but in future more isolates from Gweru and Harare can be included. The rapid global spread of H58 strains suggests they may exhibit elevated transmissibility or have a long-term carriage stage.[6] On the basis of GenoTyphi analysis of our dataset, genotype *S. enterica* ser. Typhi 4.3.1 lineage 1 was the most prevalent strain circulating in Zimbabwe from 2012 to 2019. The data presented in this study suggests an association between the presence of MDR genes and sequence types in *S. enterica* ser. Typhi in Zimbabwe.

This information will help to understand the evolution and spread of *S. enterica* ser. Typhi worldwide. We propose that *S. enterica* ser. Typhi genotyping and mapping is performed routinely in reference laboratories in Zimbabwe, to monitor the spread

of these MDR strains. The findings of this study add knowledge regarding the global circulation of sequence types of *S. enterica* ser. Typhi.

Conclusions

The phylogenetic relationship of the H58 genotype 4.3.1.1 *S. enterica* ser. Typhi clade from the Harare 2012 to 2019 outbreaks and in Gweru in 2018 is consistent with an expansion of the clonal spread of H58 between these two cities. The WGS analysis suggests that the strain evolved during the outbreak to reach full-blown ciprofloxacin resistance in the Gweru cluster. Our results have important implications for clinical practice and public health policy. The uncertainties about the epidemiology of the disease highlight the urgent need for improvements in screening, antimicrobial policy, water quality and sanitation. Increased surveillance efforts will be needed to capture the true burden of typhoid in Zimbabwe.

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Transparency declarations

None to declare.

Disclaimer

The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of organizations they represent.

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