Antimicrobial susceptibility and genomic profiling of Salmonella enterica from bloodstream infections at a tertiary referral hospital in Lusaka, Zambia, 2018-2019

IJID Regions 3 (2022) 248–255

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

Antimicrobial susceptibility and genomic profiling of *Salmonella enterica* from bloodstream infections at a tertiary referral hospital in Lusaka, Zambia, 2018–2019

Kaunda Yamba[1,2,*], Christine Kapesa[1], Evans Mpabalwani[3,4], Lottie Hachaambwa[5,6], Anthony Marius Smith[7,14], Andrea Liezl Young[7], David Gally[8], Geoffrey Mainda[9], Mercy Mukuma[10], Mulemba Tillika Samutela[11,12], Annie Kalonda[2,11], James Mwansa[13], John Bwalya Muma[2]

[1] *University Teaching Hospitals, Pathology and Microbiology Laboratory, Lusaka, Zambia*
[2] *University of Zambia, School of Veterinary Medicine, Department of Disease Control, Lusaka, Zambia*
[3] *University of Zambia, School of Medicine, Department of Paediatrics and Child Health*
[4] *University Teaching Hospitals, Children's Hospital, Lusaka, Zambia*
[5] *University of Maryland, School of Medicine, Centre for International Health, Education, and Biosecurity, Baltimore, MD, USA*
[6] *University Teaching Hospital, Infectious Diseases Unit, Department of Internal Medicine, Lusaka, Zambia*
[7] *National Institute for Communicable Diseases, Centre for Enteric Diseases, Johannesburg, South Africa*
[8] *University of Edinburgh, Roslin Institute, Division of Infection and Immunity, Edinburgh, Scotland*
[9] *Ministry of Fisheries and Livestock Department of Veterinary Services, Public Health Unit, Lusaka, Zambia*
[10] *University of Zambia, Department of Food Science and Nutrition, School of Agricultural Sciences, Lusaka, Zambia*
[11] *Department of Biomedical Sciences, School of Health Sciences, Lusaka, Zambia*
[12] *University of Zambia, School of Veterinary Medicine, Department of Paraclinical Studies, Lusaka, Zambia*
[13] *Lusaka Apex Medical University, Lusaka, Zambia*
[14] *University of Pretoria, School of Medicine, Faculty of Health Sciences, Department of Medical Microbiology, Pretoria, South Africa*

ARTICLE INFO

Keywords:
Salmonella enterica
antimicrobial resistance
genetic diversity
serotype
Zambia

ABSTRACT

Objectives: This study investigated antimicrobial susceptibility and genomic profiling of *S. enterica* isolated from bloodstream infections at a tertiary referral hospital in Lusaka, Zambia, 2018–2019.
Method: This was a prospective hospital-based study involving routine blood culture samples submitted to the microbiology laboratory at the University Teaching Hospital. Identification of *S. enterica* and determination of antimicrobial susceptibility profiles was achieved through conventional and automated methods. Whole-genome sequencing (WGS) was conducted, and the sequence data outputs were processed for species identification, serotype determination, multilocus sequence typing (MLST) profile determination, identification of antimicrobial resistance determinants, and phylogeny.
Results: Seventy-six *Salmonella enterica* were isolated and 64 isolates underwent WGS. *Salmonella* Typhi (72%) was the most prevalent serotype. Notable was the occurrence of invasive non-typhoidal *Salmonella* Typhimurium ST313 (3%), resistance to cephalosporins (4%) and ciprofloxacin (5%), multidrug resistance (46%), and reduced susceptibility to ciprofloxacin (30%) and imipenem (3%). Phylogenetic cluster analysis showed multiple *Salmonella* serovars with a wide range of genetic diversity.
Conclusion: The genetic diversity of *Salmonella* Typhi, high prevalence of multidrug resistance, and the emergence of ciprofloxacin and cephalosporin resistance warrants improved hygiene and water and sanitation provision, continued surveillance to apprise antibiograms and inform policy, and the introduction of the typhoid conjugate vaccine.

Introduction

Salmonella enterica is one of the leading causes of community-acquired bloodstream infections in low- and middle-income countries (LMIC). Invasive *Salmonella* infections (typhoidal and non-typhoidal) cause a considerable burden of illness worldwide, estimated at 3.4 million cases and over 600 000 deaths annually, especially in resource-limited settings (Kariuki et al., 2015). *Salmonella* Typhi (*S.* Typhi) is the most prevalent in crowded, underprivileged populations with poor sanitation and lack of access to safe, clean water (Crump et al., 2015; Parry et al., 2015). It has contributed significantly to the global pub-

* Corresponding author.
E-mail address: kaundayamba@gmail.com (K. Yamba).

https://doi.org/10.1016/j.ijregi.2022.04.003
Received 9 March 2022; Received in revised form 12 April 2022; Accepted 17 April 2022

lic health problem, increasing the social and economic burden worldwide (Ashton et al., 2016). It is estimated to cause 21.7 to 26.9 million cases and 217 000 deaths annually, with higher case-fatality estimates reported among children and the elderly in LMIC (Buckle et al., 2012; Crump et al., 2004; Stanaway et al., 2019b). These numbers suggest higher incidence in Africa and lower incidence in Asia than previously thought (Crump, 2014). Diagnosis based on clinical and antibody tests is not accurate, and the global burden of typhoid fever is usually underestimated in most endemic areas due to the unavailability of blood cultures and the lack of well-established laboratories and population-based national surveillance systems (Kariuki et al., 2010a; Mogasale et al., 2014).

There has been an alarming increase in invasive NTS (iNTS) that cause diseases such as bacteraemia and meningitis, with higher case-fatality rate estimates among children ≤ 5 years, the elderly ≥ 70 years, people with HIV infection, and those in areas of low sociodemographic development in sub-Saharan Africa (SSA), where iNTS infections are prevalent (Gordon, 2008; Kariuki et al., 2015; Stanaway et al., 2019a). *S.* Typhimurium and *S.* Enteritidis are the most widely reported invasive serovars across SSA. *S.* Typhimurium multilocus sequence type 313 (ST313) has been linked to niche adaptation, with some traits observed in *S.* Typhi and *S.* Paratyphi A (Okoro et al., 2012). More importantly, most *S.* Typhimurium isolates causing invasive disease are multidrug resistant (MDR), thereby compromising the clinical treatment of the disease (Gordon et al., 2008). iNTS might be linked to a diverse host niche, including several animal reservoirs, indicating the need for a 'one-health' approach (Cuypers et al., 2018).

The typhoid conjugate vaccine (TCV) has been advocated for use in the national immunization programmes in Zambia, but vaccination is yet to commence. A recent study using mathematical modelling to estimate the effect of vaccination on antimicrobial-resistant typhoid fever in 73 countries eligible for Gavi support, predicted positive outcomes after the introduction of routine immunization with TCV at age 9 months, with a catch-up campaign up to age 15 years (Birger et al., 2022). Fluoroquinolones and cephalosporins are now the recommended drugs to treat invasive *Salmonella* infection, but the last decade has seen a rise in resistance to fluoroquinolones (Hendriksen et al., 2015; Kariuki et al., 2015). Early initiation of effective antimicrobial therapy shortens the duration of illness and reduces the risk of complications and death (Kariuki et al., 2015). Profiling antimicrobial susceptibility provides information on effective treatment options, thereby reducing morbidity and mortality (Kabwama et al., 2017). This study investigated antimicrobial susceptibility and genomic profiling of *S. enterica* isolated from bloodstream infections at a tertiary referral hospital in Lusaka, Zambia, 2018–2019.

Methodology

Study design and site

This facility-based prospective study was conducted at the University Teaching Hospital (UTH) in Lusaka, Zambia. As part of routine diagnosis and patient care, blood culture samples were received in the microbiology laboratory between January 2018 and December 2019 and were followed up for *Salmonella* isolation. UTH is a national tertiary referral hospital in Lusaka, with a bed capacity of about 1665, offering specialized care to patients from Lusaka and other parts of the country. The approximate proportion of patients from Lusaka was 90% with 10% from other parts of the country.

Specimen collection and processing

Blood was drawn from patients suspected of typhoid fever before antibiotic treatment was commenced. The blood was inoculated in two automated aerobic blood culture bottles (BD) and incubated in a Bactec machine (BD Bactec FX, Wokingham, Berkshire, UK). All blood culture samples that flagged positive were sub-cultured on MacConkey, blood, and chocolate agar plates (Oxoid, Basingstoke, UK) as per the UTH microbiology protocol. After 24 hours' aerobic incubation at 37°C, MacConkey agar plates were examined for non-lactose fermenters suggestive of *Salmonella*. For identification, all the suspected isolates were further subjected to biochemical tests (Oxoid, Basingstoke, UK) or analyzed using a VITEK® 2 Compact instrument (Biomerieux), utilizing GN86 ID cards when available. *Salmonella enterica* and some confirmed *S.* Typhi isolates were then stored in duplicate in glycerol at −80°C.

Antimicrobial susceptibility testing

The presumptive *Salmonella enterica* isolates were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method. The 0.5 McFarland was determined using a DensiCHECK instrument (Biomerieux), after which the suspension was spread on Mueller-Hinton agar plates (Oxoid, Basingstoke, UK). The following antibiotics (Oxoid, Basingstoke, UK) were used: ampicillin-AMP (10 µg), chloramphenicol-CHL (30 µg), cotrimoxazole-SXT (1.25/23.75 µg), tetracycline – TCY (30 µg), ciprofloxacin – CIP (5 µg), nalidixic acid – NAL (30 µg), cefotaxime – CTX (30 µg), cefepime – CPM (30 µg), and imipenem – IMP (10 µg). The Mueller-Hinton agar plates were then incubated aerobically at 37°C for 24 hours. After incubation, the zones of inhibition were measured in millimetres and interpreted as susceptible, intermediate, or resistant, according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). All the antibiotics were quality controlled using the ATCC *Escherichia coli* 25922 control strain.

Analysis of AST data

Data were managed in Excel® spreadsheets and exported to STATA® 14 for analysis. The proportions of resistance (R%), intermediate (I%), susceptible (S%), and multidrug resistance (MDR%) were estimated with corresponding 95% confidence intervals. MDR isolates were defined as isolates with resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (Cuypers et al., 2018). Association between MDR and biodata (age, sex) and the presumptive diagnosis were examined using Fisher's exact test.

Genomic DNA isolation from bacteria and whole-genome sequencing

The isolates were shipped to the National Institute for Communicable Diseases (NICD), South Africa for whole-genome sequencing (WGS). Genomic DNA was isolated from bacteria using an Invitrogen PureLink Microbiome DNA Purification Kit (Invitrogen, Waltham, Massachusetts, USA). WGS was performed using Illumina NextSeq (Illumina, San Diego, California, USA) next-generation sequencing technology. DNA libraries were prepared using a Nextera DNA Flex Library Preparation Kit (Illumina) followed by 2×150 bp paired-end sequencing runs with ~80 times coverage.

Genomic sequence analysis

Raw genomic sequencing data were assembled using SPAdes software (version 3.15). Assembled data were analyzed using online bioinformatics pipelines at the Center for Genomic Epidemiology (CGE) of the Technical University of Denmark (http://www.genomicepidemiology.org/services/). These pipelines included KmerFinder 3.2 for species identification, MLST 2.0 for the determination of MLST profile, and ResFinder 4.1 for identifying antimicrobial resistance determinants. *Salmonella* serovar determination was performed using the online bioinformatics pipeline SeqSero2 version 1.1.0 (http://denglab.info/SeqSero2).

Raw sequencing data (FastQ files for paired-end reads) were uploaded and investigated at the EnteroBase web-based platform

Table 1
Demographics of patients with *Salmonella enterica* infections, serovar/serotype, and seasonal distribution

Characteristics Number of isolates: 76Sequenced isolates: 64	*Salmonella enterica* serovars								
	*S.*Typhi	*S.* Typhimurium	*S.*Enteritidis	*S.*Paratyphi A	*S.*Braenderup	*S.*Heidelberg	*S.*Weltevreden	*S.*Salamae	Total% (*n*)
Total	**58**	**2**	**7**	**1**	**1**	**3**	**1**	**3**	**100% (76)**
Sequence types (ST)	ST1 (44) ST2 (2)	ST313 (2)	ST11 (3) ST366 (3)	ST85 (1)	–	ST15 (3)	ST365 (1)	–	**92% (59/64)**
Gender									
Female: 43% (33/76)	25	1	3	0	0	2	1	1	43% (33/76)
Male: 57% (43/76)	33	1	4	1	1	1	0	2	57% (43/76)
Age									
0–15 years	37 (63%)	1 (50%)	5 (50%)	0	1 (100%)	2 (67%)	1 (100%)	3 (100%)	61% (50/76)
16–35years	11 (23%)	0	1 (25%)	1 (100%)	0	1 (33%)	0	0	23% (14/76)
≥ 36 years	10 (14%)	1 (50%)	1 (25%)	0	0	0	0	0	16% (12/76)
Total (*n*)	**58**	**2**	**7**	**1**	**1**	**3**	**1**	**3**	**100% (76)**
Season									
Rainy	29	0	2	0	1	3	1	1	58% (37/64)
Dry (cold)	10	1	1	0	0	0	0	0	19% (12/64)
Dry (hot)	11	1	2	1	0	0	0	0	23% (15/64)
Total (*n*) of sequenced isolates	**50**	**2**	**5**	**1**	**1**	**3**	**1**	**1**	**64**

Rainy season: November to April, **Dry season (cold)**: May to August, **Dry season (hot)**: September to October

(http://enterobase.warwick.ac.uk/species/index/senterica). EnteroBase analysis included serotype and MLST confirmation using various genomic tools and genomic comparison of isolates based on core-genome multilocus sequence typing (cgMLST) data, using the cgMLST V2 + HierCC V1 scheme. The cgMLST scheme incorporates 3002 genes. Phylogenetic cluster analysis of cgMLST data was depicted using a GrapeTree-generated minimum spanning tree using the MSTree V2 algorithm (https://bitbucket.org/enterobase/enterobase-web/wiki/GrapeTree). Short-read sequence data have been deposited at the NCBI Sequence Read Archive under BioProject identification number **PRJEB43596**.

Results

Patient demographics and clinical symptoms

In total, 7180 blood culture specimens were processed during the study period. The majority of *Salmonella* cases were in males (56.7%; 95% CI 43.6–68.9), compared with 43.3% (95% CI 31.1–56.4) in females. The median age of the patients was 13.5 years (range 2–54 years), with an age group distribution of 0–15 years at 61% (50/76), 16–35 years at 23% (14/76), and ≥ 36 years at 16% (12/74). Most of the cases occurred in the rainy season between November and April (Table 1). The presumptive diagnosis based on symptoms associated with the illness had a wide range of differentials, with enteric fever being the highest at 66.7% (95% CI 51.2–79.2), followed by sepsis at 17.8% (95% CI 8.9–32.4) (Table 2).

Table 2
Distribution of presumptive diagnosis for cases subjected to the microbiology laboratory for *Salmonella enterica* isolation (2018–2019)

Presumptive diagnosis	Proportion	95%CI
Enteric fever	66.7%	51.2–79.2
Hepatitis	4.4%	1–16.9
Sepsis	17.8%	8.9–32.4
Sepsis in leukemia patients	2.2%	0.03–15.1
Peritonitis	2.2%	0.03–15.1
Rheumatic heart disease (RHD)	4.4%	1.1–15.1
Disseminated TB	2.2%	0.03–15.1

Confirmed Salmonella enterica *cases and confirmed serovars*

Seventy-six *Salmonella enterica* were isolated and 64 isolates underwent WGS analysis, while the remaining 12 *S.* Typhi that were identified using the VITEK® GN83 ID (Biomerieux) were not sequenced because they were not stored at the time of isolation. The serovars of the sequenced isolates were as follows: *S.* Typhi 72% (n = 46), *S.* Paratyphi A 2% ($n = 1$), *S.* Weltevreden 2% ($n = 1$), and *S.* Braenderup 2% ($n = 1$). The other serovars were *S.* Enteritidis 11% ($n = 7$), *S.* Typhimurium 3% ($n = 2$), *S.* Heidelberg 5% ($n = 3$), and *S. enterica* subsp. *Salamae* 5% ($n = 3$) (Table 1).

MLSTs of Salmonella enterica

In total, eight *Salmonella enterica* sequence types (STs) (1, 2, 11, 15, 85, 313, 365, and 366) were identified using multilocus sequence typing (MLST). *S.* Typhi and *S.* Enteritidis isolates were assigned to two sequence types each: ST1 ($n = 44$) and ST2 ($n = 2$) for *S.* Typhi, and ST11 ($n = 4$) and ST366 ($n = 3$) for *S.* Enteritidis. The two *S.* Typhimurium isolates were assigned to ST313, a serotype known to cause invasive disease in SSA countries. Other STs identified were: ST85 ($n = 1$); *S.* Paratyphi A, ST15 ($n = 3$); *S.* Heidelberg, ST365 ($n = 1$); and *S.* Weltevreden (Table 1).

Antimicrobial resistance

The *Salmonella enterica* isolates were tested for susceptibility to nine antibiotics. The highest levels of resistance observed were to ampicillin (83%), cotrimoxazole (73%), and chloramphenicol (49%) (Figure 1 and Table 3). Although ciprofloxacin resistance was at 5%, the percentage of isolates showing intermediate resistance to ciprofloxacin (30%) was alarming. Notable was the resistance to nalidixic acid (18%), of which 14% and 2% of these isolates were intermediate and resistant to ciprofloxacin, respectively. Third- and fourth-generation cephalosporin resistance was at 4%. There was no resistance to imipenem recorded – only reduced susceptibility (intermediate) at 3% (Table 3). Of the 76 tested isolates, 46% were classified as MDR (Table 4).

Genes associated with antimicrobial resistance in the different antimicrobial classes were as follows: beta-lactams – *blaTEM-1B* (67%),

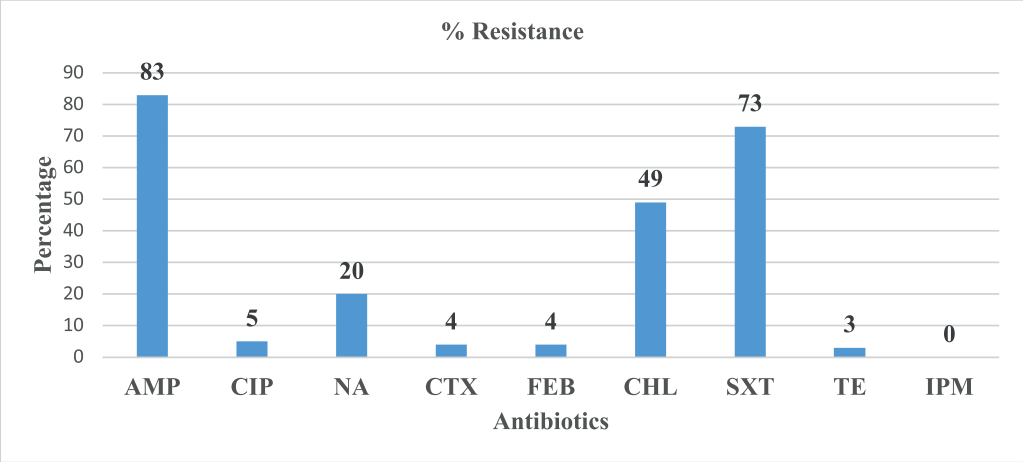

Figure 1. Resistance patterns to all antibiotics tested.
Abbreviations: AMP – ampicillin, CIP – ciprofloxacin, NA – nalidixic acid, CTX – cefotaxime, FEP – cefepime, CHL – chloramphenicol, SXT – trimethoprim-sulfamethoxazole, TE – tetracycline, IPM – imipenem

Table 3
Antimicrobial susceptibility results (RIS proportion and 95% CI) and resistance genes

Antibiotics class	Antibiotics	Susceptibility results Resistance (R)% (95% CI)	Intermediate (I)% (95% CI)	Susceptible (S)% (95% CI)	AMR resistance determinants
Beta-lactams	AMP	83 (72.2–90.3)	1 (0.2–9.7)	16 (8.7–26.1)	*BlaTEM-1B* (43)
Quinolones	CIP	5 (2.1–14.4)	30 (19.8–41.5)	65 (52.8–75.2)	*gyrA* [S83Y, 50% (4/8); D87N, 50% (4/8)], *gyrB*
	NA	20 (9.7–27.8)	–	80 (72.2–90.3)	[S464Y, 100% (1/1)], *parC* [T57S, 100% (8/8)]
Third- and fourth- generation	CTX	4 (1.3–12.6)	–	96 (87.4–98.7)	None
cephalosporins	FEB	4 (1.3–12.6)	–	96 (87.4–98.7)	
Phenols	CHL	49 (37.6–61.1)	–	51 (39–62.4)	catA1 68% (26)
Folate pathway antagonist	SXT	73 (61.5–82.4)	10 (4.7–19.5)	17 (9.7–27.8)	*Sul1* alone (0), *sul2* (18), *sul 1/sul2* combined (24), *dfr A1* (2), *dfr A7* (22), *dfr A14* (18), *dfr A7/dfr 14* combined (2)
Tetracycline	TE	3 (0.7–10.9)	–	97 (89.1–99.3)	None
Carbapenem	IPM	–	3 (0.7–10.9)	97 (89.1–99.3)	–

Abbreviations: AMP – ampicillin, CIP – ciprofloxacin, NA – nalidixic acid, CTX – cefotaxime, FEP – cefepime, CHL – chloramphenicol, SXT – trimethoprim-sulfamethoxazole, TE – tetracycline, IPM – imipenem

Table 4
Distribution of multidrug resistance (MDR) in the different *Salmonella* serovars ($n = 76$)

Serovars	Multidrug resistant (MDR) Number of species (number of MDR isolates)	% MDR
S. Enteritidis	7 (1)	14%
S. Heidelberg	3 (1)	33%
S. Paratyphi A	1 (0)	0
S. Typhi	58 (31)	53%
S. Typhimurium	2 (2)	100%
S. Weltevreden	1 (0)	0
S. Salamae	3 (0)	0
S. Braenderup	1 (0)	0
Total	**76 (35)**	**46% (35/76)**

MDR – resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole

phenicols (68%), folate pathway antagonist (*sul2* 33%, *sul1/sul2* combined 44%, *dfr A1* 4%, *dfr A7* 40%, *dfr A7/dfr 14* combined 4%). Reduced susceptibility and resistance to quinolones were associated with mutations in the quinolone resistance-determining region (QRDR) of DNA gyrase and topoisomerase IV, whose subunits are encoded respectively by *gyrA, gyrB, parC*, and *parE* genes. Mutations were identified in the following codons: *gyrA* (S83Y 50%, D87N 50%), *gyrB* (S464Y 100%), and *parC* (T57S 100%) (Table 3). The 50% isolates with *parC* mutations were all susceptible to ciprofloxacin and nalidixic acid. In comparison, the isolates with *gyrA* 50% and *gyrB* 6% mutations had reduced susceptibility (intermediate) to ciprofloxacin and were resistant to nalidixic acid. There were no CTX and CIP resistance gene determinants found in ResFinder 4.1 pipelines at the time of analysis, but AMR determinants were never investigated in other databases, so the possibility that beta-lactam and quinolone resistant determinants could be found in these other databases cannot be excluded.

Phylogenetic relatedness

A cluster of isolates (regarded as highly related isolates) was defined as two or more isolates that differed by no more than five allele differences following cgMLST analysis. A cluster of isolates defined a distinct genotype. Core genome MLST data analysis showed varied genetic diversity (multiple genotypes) among isolates. In total, 15 genotypes were identified among 46 *S.* Typhi isolates. Other *Salmonella* serovars were genetically very distant (thousands of allele differences) from *S.* Typhi isolates; *S.* Enteritidis showed three genotypes, *S.* Typhimurium showed one genotype, *S.* Heidelberg showed one genotype, and *S. enterica* subsp. *salamae* showed one genotype (Figure 2). In order to determine the phylogenetic relatedness and diversity of our *S.* Typhi strains against others in the existing database, EnteroBase was searched for the nearest strain

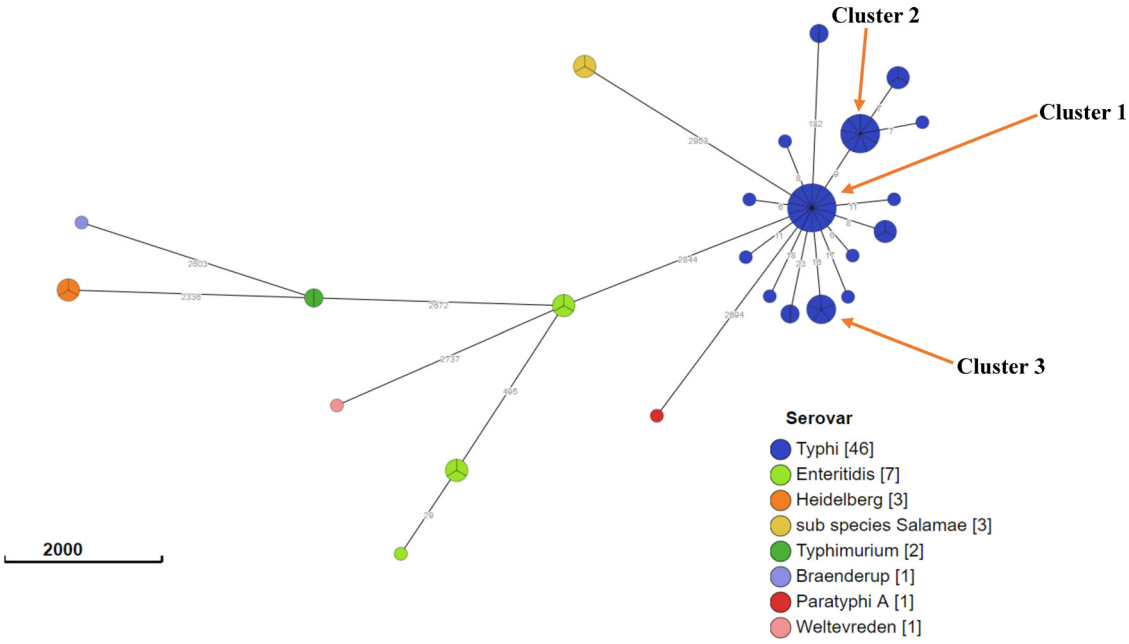

Figure 2. Genetic relatedness of different *Salmonella enterica* serovars
Minimum spanning tree drawn using cgMLST data from *S. enterica* isolated from Lusaka, Zambia, 2018–2019. The circular nodes represent isolate(s) with identical cgMLST profiles; the more significant the node, the more isolates are reflected. The number of values between adjacent nodes indicates the number of allele differences between nodes (isolates). Even with ≤ 5 allele differences, there was a wide range of genetic diversity and varying strains.

matches, using a search criterion (cut-off) of up to 10 allele differences. Comparative analysis was performed for only our largest three *S*. Typhi clusters (clusters represented by ≥ 5 isolates). These three largest clusters were named cluster 1 (14 isolates), cluster 2 (nine isolates), and cluster 3 (five isolates) (Figure 2). For cluster 1, close matches were found to isolates from the Zambian 2010 outbreak, Tanzania, Malawi, South Africa, the UK, and the USA. For clusters 2 and 3, close matches were found to isolates from Zambia (2011), Tanzania, and Malawi. The closest matches dated as far back as 2008, with Tanzania having the highest number of *S*. Typhi isolates with closest matches to our isolates.

Discussion

The highest disease burden was in children (67%), similar to Asia and Malawi (Meiring et al., 2021). A wide range of symptoms associated with this illness was also noted, with attending clinicians placing enteric fever (66.7%) at the top of their presumptive diagnosis list. In comparison, sepsis was second at 17.8%. With this observation, it was assumed that most of our patients received ceftriaxone as empiric treatment, because this is the drug of choice for patients presenting with fever in the Zambian setting (Masich et al., 2020). However, the presumptive diagnosis of some of the known complications of enteric fever, such as hepatitis (4.4%) and peritonitis (2.2%), could be an indication that 6.6% of our patients presented to our health facility late, thereby delaying the commencement of effective antimicrobial treatment (Contini, 2017; María et al., 2019).

Most cases occurred in the rainy season (58%), followed by the hot/dry season (23%). Of particular importance during the hot weather and heavy rainfall season is increased transmission of enteric (typhoid) fever (Saad et al., 2018). Heavy rainfall leads to flooding, a known risk factor for enteric fever spread, as this can cause the mixing of drinking water sources with open sewers that contains fecal matter (Vollaard et al., 2004). This is common in lower- and middle-income countries (LMIC) with poor drainage, waste disposal, and sanitation facilities (Corner et al., 2013). The rapid replication of bacteria such as *Salmonella* in warmer conditions (Akil et al., 2014), coupled with limited supplies of clean water during hot and dry periods, could lead to water scarcity forcing people to consume contaminated water (Singh et al., 2001). This could explain our study's finding of 23% of cases being detected in the hot/dry season.

S. Typhi showed predominance (72%) as a causative agent of enteric fever over the other serovars, which was consistent with studies previously carried out in Zambia (Hendriksen et al., 2015) and other countries – Tanzania (Msemo et al., 2019), China (Qian et al., 2020), and India (Misra et al., 2016). However, the findings differed from those of studies carried out in Malawi (Feasey et al., 2015), Kenya (Muthumbi et al., 2015), Ghana (Dekker et al., 2018; Labi et al., 2014), Burkina Faso (Guiraud et al., 2017), and The Gambia (Kwambana-Adams et al., 2015), which found the incidence of iNTS cases to be higher than that of *S*. Typhi cases. The serovar differences in these countries could be attributed to differences in climate and landscape (Akil et al., 2014; Maurer et al., 2015), and the prevalence of predisposing factors such as HIV associated with iNTS (Guiraud et al., 2017). Zambia attained the 90:90:90 UNAIDS targets for HIV epidemic control in 2020 (UNAIDS, 2015). This has seen the number of new HIV infections reduce and an increase in people living with HIV (PLWH) on antiretroviral therapy, thereby reducing the proportion of immunocompromised population (UNICEF, 2020).

Isolation of *S*. Typhi strains from various endemic regions traversing over a century confirms the predominance of two sequence types (ST1 and ST2) coexisting in the endemic regions (Yap et al., 2016). This could be due to international travel and the uniqueness of ST1/ST2 virulence genes, which support successful dissemination (Yap et al., 2016). Comparable to our findings, *S*. Enteritidis isolates ST11 were also found in Ghana (Aldrich et al., 2019) and The Gambia (Darboe et al., 2022). Although *S*. Typhimurium ST313 is the leading cause of invasive *Salmonella* infections in SSA (Kariuki and Onsare, 2015; Kingsley et al., 2009; Okoro et al., 2012), this finding is contrary to the low prevalence (3%) observed in our study and no presence reported in The Gambia (Darboe et al., 2022). *S*. Typhimurium ST313 is rarely isolated from outside SSA. Compared with the *S*. Typhimurium ST19 that causes diarrhea

in humans, the virulence of ST313 could be attributed to genome degradation and conversion of a more host-restricted existent characteristic of *S*. Typhi infections (Kariuki and Onsare, 2015).

Resistance to ciprofloxacin and ceftriaxone was relatively low in our setting, although there was an alarming rise in reduced susceptibility to ciprofloxacin (30%). These findings are comparable to those in Ghana (Labi et al., 2014), Kenya (Muthumbi et al., 2015), and India (Menezes et al., 2012) but differ from findings in Italy (García-Fernández et al., 2015) and Pakistan (Klemm et al., 2018) that showed high resistance to fluoroquinolones and ceftriaxone, respectively. The percentage of MDR *S*. Typhi isolates (51%) in our study was lower than reported in Tanzania and Kenya – 81% and 77.2%, respectively (Kariuki et al., 2010b; Msemo et al., 2019). Of note was the reduction in MDR *S*. Typhi from 84% in a previous study in Zambia (Kalonda et al., 2015) to 51% in our study. This observation could relate to changes in prescribing patterns and antimicrobial use, such as reduced use of ampicillin, cotrimoxazole, and chloramphenicol as first-line antibiotic treatment for enteric fever (Browne et al., 2020; Menezes et al., 2012). In contrast to our findings, a systematic review observed an increase in MDR *Salmonella* infections in Kenya (60% to 82% between 1990 and 1994), Malawi (0% to 88% between 1994 and 2009), and Nigeria (37% to 100% between 1998 and 2014) (Browne et al., 2020).

The gene *blaTEM-1B*, responsible for resistance to ampicillin, was the most common resistance gene found, which was in agreement with a study in India (Katiyar et al., 2020). Resistance determinants for phenicols, folate pathway antagonist, and fluoroquinolone resistance found in our study were similar to those in other studies (Das et al., 2017; Gaind et al., 2006; García-Fernández et al., 2015; Menezes et al., 2012). From our analysis, none of the strains carried multiple fluoroquinolone substitutions within *gyrA, gyrB* and *parC* – the types of mutation likely to impact fitness. This can explain our observation of nalidixic acid-resistant strains with reduced susceptibility to fluoroquinolones, similar to findings reported in China (Qian et al., 2020; Wu et al., 2010).

The development of fluoroquinolone resistance is understood to often involve a build-up of multiple mutations in a stepwise progression, with mutations that alter *gyrA* at codon 83 being considered the first step to the selection of high-level resistance (Qian et al., 2020). Although the contribution of *parC* T57S substitution to quinolone resistance is still not well understood (Chang et al., 2021; Wang et al., 2017), a 50% prevalence of the *parC* T57S substitution alone was observed. This mutation may carry a small capability cost, which would explain its prevalence in the susceptible strains in our study. However, it may acquire a different impact on fitness when associated with *gyrA* or with multiple other topoisomerase mutations (Chang et al., 2021). This is because the development of fluoroquinolone resistance in *Salmonella* is an endpoint result of the accumulation of several biochemical mechanisms (Giraud et al., 2006). The isolate that was resistant to both ciprofloxacin and nalidixic acid was negative for all the screened quinolone-resistance genes; this finding could be credited to different mechanisms, such as over-activation of multidrug efflux pumps and decreased outer membrane permeability, which contributes to the resistance of *Salmonella* to fluoroquinolone (Poole, 2000).

Phylogenetic cluster analysis using cgMLST data confirmed multiple *Salmonella* serovars. This observation confirmed that NTS infections are sporadic in our community, and most of the outbreaks are driven by the human-specific *S*. Typhi. Assessment of genetic similarities between our *S*. Typhi isolates to strains from other countries, such as Tanzania, Malawi, South Africa, the UK, and the USA, suggested travel-associated spread, with a higher prevalence of geographic association among bordering countries in the region. Similar to our phylogenetic relatedness findings, a study that utilized WGS data for nearly 2000 isolates sourced from over 60 countries found the global *S*. Typhi population to be highly structured, with dozens of subclades displaying geographical restriction (Wong et al., 2016). Analysing WGS data from different regions helps to generate a robust genotyping system that gives an insight into local *S*. Typhi populations, and helps identify recent introductions into new or previously endemic locations, thereby providing information on their likely geographical sources (Wong et al., 2016).

Conclusion

The *Salmonella* infections in our community were driven by *S*. Typhi. The evolution and genetic diversity of *S*. Typhi, coupled with a high prevalence of MDR and emergence of resistance to ciprofloxacin and cephalosporins, warrants improved hygiene and water and sanitation provision, continued surveillance to apprise hospital antibiograms and inform policy, and the introduction of the typhoid conjugate vaccine.

Notes

Authors' contributions

Conceptualisation: KY and JBM. Data collection: KY and CK. Laboratory analysis: KY, CK, MTS, and AK. WGS and analysis: KY, AMS, and ALY. Data analysis: KY and JBM. Writing original draft: KY. Review and editing: JBM, DG, GM, MM, JM, EM, LH, AMS, MTS, and AK. All authors read and approved the final manuscript.

Funding

The study was funded by the African Centre of Excellence for Infectious Diseases of Humans and Animals (ACEIDHA) project, University of Zambia, grant no P151847.

Conflicts of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

The findings and conclusions in this article are those of the authors and do not represent those of their affiliated organizations.

Acknowledgements

The authors acknowledge the Ministry of Health, the University Teaching Hospital, and the Pathology and Microbiology Department's Bacteriology Unit for providing a conducive working environment to enable this work. The authors also acknowledge the Fleming Fund Regional Whole-Genome Sequencing (WGS) in Africa Project (SEQAFRICA) and the National Institute for Communicable Diseases (NICD), South Africa regional sequencing centre for facilitating the WGS. The WGS was funded by the UK Department of Health and Social Care, managed by the Fleming Fund, and performed under the auspices of the SEQAFRICA Project. The Fleming Fund is a £265 million UK aid programme supporting up to 24 low- and middle-income countries (LMICs) in generating, sharing, and using data on antimicrobial resistance, and works in partnership with Mott MacDonald, the Management Agent for the Country and Regional Grants and Fellowship Programme.

Ethical approval

Ethical approval was obtained from the ERES Converge Research Ethics Board. The hospital management granted permission to use the samples received in the microbiology laboratory for this study.

References

Akil L, Ahmad HA, Reddy RS. Effects of climate change on Salmonella infections. Foodborne Pathog Dis 2014;11:974. doi:10.1089/FPD.2014.1802.

Aldrich C, Hartman H, Feasey N, Chattaway MA, Dekker D, Al-Emran HM, et al. Emergence of phylogenetically diverse and fluoroquinolone resistant Salmonella enteritidis as a cause of invasive nontyphoidal Salmonella disease in Ghana. PLoS Negl Trop Dis 2019;13. doi:10.1371/journal.pntd.0007485.

Ashton PM, Nair S, Peters TM, Bale JA, Powell DG, Painset A, et al. Identification of Salmonella for public health surveillance using whole genome sequencing. PeerJ 2016;2016. doi:10.7717/peerj.1752.

Birger R, Antillón M, Bilcke J, Dolecek C, Dougan G, Pollard AJ, et al. Estimating the effect of vaccination on antimicrobial-resistant typhoid fever in 73 countries supported by Gavi: a mathematical modelling study. Lancet Infect Dis 2022;0. doi:10.1016/S1473-3099(21)00627-7.

Browne AJ, Kashef Hamadani BH, Kumaran EAP, Rao P, Longbottom J, Harriss E, et al. Drug-resistant enteric fever worldwide, 1990 to 2018: a systematic review and meta-analysis. BMC Med 2020;18. doi:10.1186/s12916-019-1443-1.

Buckle GC, Walker CLF, Black RE. Typhoid fever and paratyphoid fever: systematic review to estimate global morbidity and mortality for 2010. J Glob Health 2012;2:1. doi:10.7189/jogh.02.010401.

Chang MX, Zhang JF, Sun YH, Li RS, Lin XL, Yang L, et al. Contribution of different mechanisms to ciprofloxacin resistance in Salmonella spp. Front Microbiol 2021;12:1030. doi:10.3389/fmicb.2021.663731.

CLSI. M100 Performance Standards for Antimicrobial Susceptibility Testing: a CLSI supplement for global application. 2018.

Contini S. Typhoid intestinal perforation in developing countries: still unavoidable deaths? World J Gastroenterol 2017;23:1925–31. doi:10.3748/wjg.v23.i11.1925.

Corner RJ, Dewan AM, Hashizume M. Modelling typhoid risk in Dhaka Metropolitan Area of Bangladesh: the role of socio-economic and environmental factors. Int J Heal Geogr 2013;12:1–15. doi:10.1186/1476-072X-12-13.

Crump JA. Updating and refining estimates of typhoid fever burden for public health action. Vol. 2. 2014. https://doi.org/10.1016/S2214-109X(14)70306-7

Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Vol. 82. 2004.

Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. Clin Microbiol Rev 2015;28:901–37. doi:10.1128/CMR.00002-15.

Cuypers WL, Jacobs J, Wong V, Klemm EJ, Deborggraeve S, Van Puyvelde S. Fluoroquinolone resistance in Salmonella: insights by whole-genome sequencing. Microb Genomics 2018;4. doi:10.1099/MGEN.0.000195.

Darboe S, Bradbury RS, Phelan J, Kanteh A, Muhammad A-K, Worwui A, et al. Genomic diversity and antimicrobial resistance among non-typhoidal Salmonella associated with human disease in The Gambia. Microb Genomics 2022;8:785. doi:10.1099/mgen.0.000785.

Das S, Samajpati S, Ray U, Roy I, Dutta S. Antimicrobial resistance and molecular subtypes of Salmonella enterica serovar Typhi isolates from Kolkata, India over a 15 years period 1998–2012. Int J Med Microbiol 2017;307:28–36. doi:10.1016/J.IJMM.2016.11.006.

Dekker D, Krumkamp R, Eibach D, Sarpong N, Boahen KG, Frimpong M, et al. Characterization of Salmonella enterica from invasive bloodstream infections and water sources in rural Ghana. BMC Infect Dis 2018;18. doi:10.1186/s12879-018-2957-4.

Feasey NA, Masesa C, Jassi C, Faragher EB, Mallewa J, Mallewa M, et al. Three epidemics of invasive multidrug-resistant salmonella bloodstream infection in Blantyre, Malawi, 1998–2014. Clin Infect Dis 2015;61:S363–71. doi:10.1093/cid/civ691.

Gaind R, Paglietti B, Murgia M, Dawar R, Uzzau S, Cappuccinelli P, et al. Molecular characterization of ciprofloxacin-resistant Salmonella enterica serovar Typhi and Paratyphi A causing enteric fever in India. J Antimicrob Chemother 2006;58:1139–44. doi:10.1093/jac/dkl391.

García-Fernández A, Gallina S, Owczarek S, Dionisi AM, Benedetti I, Decastelli L, et al. Emergence of ciprofloxacin-resistant Salmonella enterica serovar Typhi in Italy. PLoS One 2015;10. doi:10.1371/JOURNAL.PONE.0132065.

Giraud E, Baucheron S, Cloeckaert A. Resistance to fluoroquinolones in Salmonella: emerging mechanisms and resistance prevention strategies. Microbes Infect 2006;8:1937–44. doi:10.1016/J.MICINF.2005.12.025.

Gordon MA. Salmonella infections in immunocompromised adults. J Infect 2008;56:413–22. doi:10.1016/j.jinf.2008.03.012.

Gordon MA, Graham SM, Walsh AL, Wilson L, Phiri A, Molyneux E, et al. Epidemics of invasive Salmonella enterica serovar Enteritidis and S. enterica serovar Typhimurium infection associated with multidrug resistance among adults and children in Malawi 2008;46. https://doi.org/10.1086/529146

Guiraud I, Post A, Diallo SN, Lompo P, Maltha J, Thriemer K, et al. Population-based incidence, seasonality and serotype distribution of invasive salmonellosis among children in Nanoro, rural Burkina Faso. PLoS One 2017;12. doi:10.1371/journal.pone.0178577.

Hendriksen RS, Leekitcharoenphon P, Lukjancenko O, Lukwesa-Musyani C, Tambatamba B, Mwaba J, et al. Genomic signature of multidrug-resistant Salmonella enterica serovar Typhi isolates related to a massive outbreak in Zambia between 2010 and 2012. J Clin Microbiol 2015;53:262–72. doi:10.1128/JCM.02026-14.

Kabwama SN, Bulage L, Nsubuga F, Pande G, Oguttu DW, Mafigiri R, et al. A large and persistent outbreak of typhoid fever caused by consuming contaminated water and street-vended beverages: Kampala, Uganda, January–June 2015. BMC Public Health 2017;17. doi:10.1186/s12889-016-4002-0.

Kalonda A, Kwenda G, Lukwesa-Musyani C, Samutela MT, Mumbula M, Kaile T, et al. Characterization of antimicrobial resistance in Salmonella enterica serovars Typhi and Paratyphi B in Zambia. Jour Med Sc Tech J Med Sci Tech 2015;4.

Kariuki S, Gordon MA, Feasey N, Parry CM. Antimicrobial resistance and management of invasive Salmonella disease HHS Public Access. Vaccine 2015;33:21–9. doi:10.1016/j.vaccine.2015.03.102.

Kariuki S, Onsare RS. Epidemiology and genomics of invasive nontyphoidal Salmonella infections in Kenya. Clin Infect Dis 2015;61:S317–24. doi:10.1093/cid/civ711.

Kariuki S, Revathi G, Kiiru J, Mengo DM, Mwituria J, Muyodi J, et al. Typhoid in Kenya is associated with a dominant multidrug-resistant Salmonella enterica serovar Typhi haplotype that is also widespread in Southeast Asia. J Clin Microbiol 2010;48:2171–6. doi:10.1128/JCM.01983-09.

Katiyar A, Sharma P, Dahiya S, Singh H, Kapil A, Kaur P. Genomic profiling of antimicrobial resistance genes in clinical isolates of Salmonella Typhi from patients infected with typhoid fever in India. Sci Reports 2020;10:1–15. doi:10.1038/s41598-020-64934-0.

Kingsley RA, Msefula CL, Thomson NR, Kariuki S, Holt KE, Gordon MA, et al. Epidemic multiple drug resistant Salmonella Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. Genome Res 2009;19:2279–87. doi:10.1101/gr.091017.109.

Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, et al. Emergence of an extensively drug-resistant Salmonella enterica serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. MBio 2018;9. doi:10.1128/mBio.00105-18.

Kwambana-Adams B, Darboe S, Nabwera H, Foster-Nyarko E, Ikumapayi UN, Secka O, et al. Salmonella infections in The Gambia, 2005–2015. Clin Infect Dis 2015;61:S354–62. doi:10.1093/cid/civ781.

Labi A-K, Obeng-Nkrumah N, Addison NO, Donkor ES. Salmonella blood stream infections in a tertiary care setting in Ghana. BMC Infect Dis 2014;14:1–10. doi:10.1186/S12879-014-0697-7.

María L, Espinoza C, McCreedy E, Holm M, Im J, Mogeni OD, et al. Impact of duration of illness preceding hospitalization on typhoid fever complications. Clin Infect Dis 2019;69:435–83. doi:10.1093/cid/ciz477.

Masich AM, Vega AD, Callahan P, Herbert A, Fwoloshi S, Zulu PM, et al. Antimicrobial usage at a large teaching hospital in Lusaka, Zambia. PLoS One 2020;15. doi:10.1371/JOURNAL.PONE.0228555.

Maurer JJ, Martin G, Hernandez S, Cheng Y, Gerner-Smidt P, Hise KB, et al. Diversity and persistence of Salmonella enterica strains in rural landscapes in the southeastern United States. PLoS One 2015;10. doi:10.1371/JOURNAL.PONE.0128937.

Meiring JE, Shakya M, Khanam F, Voysey M, Phillips MT, Tonks S, et al. Burden of enteric fever at three urban sites in Africa and Asia: a multicentre population-based study. Lancet Glob Heal 2021;9:e1688–96. doi:10.1016/S2214-109X(21)00370-3.

Menezes GA, Harish BN, Khan MA, Goessens WHF, Hays JP. Antimicrobial resistance trends in blood culture positive Salmonella Typhi isolates from Pondicherry, India, 2005–2009. Clin Microbiol Infect 2012;18:239–45. doi:10.1111/j.1469-0691.2011.03546.x.

Misra R, Thakare R, Amrin N, Prasad KN, Chopra S, Dhole TN. Antimicrobial susceptibility pattern and sequence analysis of DNA gyrase and DNA topoisomerase IV in Salmonella enterica serovars Typhi and Paratyphi A isolates with decreased susceptibility to ciprofloxacin. Trans R Soc Trop Med Hyg 2016;110:472–9. doi:10.1093/TRSTMH/TRW051.

Msemo OA, Mbwana J, Mahende C, Malabeja A, Gesase S, Crump JA, et al. Epidemiology and antimicrobial susceptibility of salmonella enterica bloodstream isolates among febrile children in a rural district in northeastern Tanzania: a cross-sectional study. Clin Infect Dis 2019;68:S177–82. doi:10.1093/cid/ciy1126.

Muthumbi E, Morpeth SC, Ooko M, Mwanzu A, Mwarumba S, Mturi N, et al. Invasive Salmonellosis in Kilifi, Kenya. Clin Infect Dis 2015;61:S290. doi:10.1093/CID/CIV737.

Okoro CK, Kingsley RA, Connor TR, Harris SR, Parry CM, Al-Mashhadani MN, et al. Intracontinental spread of human invasive Salmonella Typhimurium pathovariants in sub-Saharan Africa. Nat Genet 2012;44:1215–21. doi:10.1038/ng.2423.

Parry CM, Thieu NTV, Dolecek C, Karkey A, Gupta R, Turner P, et al. Clinically and microbiologically derived azithromycin susceptibility breakpoints for Salmonella enterica serovars Typhi and Paratyphi A. Antimicrob Agents Chemother 2015;59:2756–64. doi:10.1128/AAC.04729-14.

Poole K. Efflux-mediated resistance to fluoroquinolones in gram-negative bacteria. Antimicrob Agents Chemother 2000;44:2233–41. doi:10.1128/AAC.44.9.2233-2241.2000.

Qian H, Cheng S, Liu G, Tan Z, Dong C, Bao J, et al. Discovery of seven novel mutations of gyrB, parC and parE in Salmonella Typhi and Paratyphi strains from Jiangsu Province of China. Sci Reports 2020;10:1–8. doi:10.1038/s41598-020-64346-0.

Saad NJ, Lynch VD, Antillón M, Yang C, Crump JA, Pitzer VE. Seasonal dynamics of typhoid and paratyphoid fever. Sci Reports 2018;8:1–9. doi:10.1038/s41598-018-25234-w.

Singh RB, Hales S, Wet N de Raj R, Hearnden M, Weinstein P. The influence of climate variation and change on diarrheal disease in the Pacific Islands. Environ Health Perspect 2001;109:155. doi:10.1289/EHP.01109155.

Stanaway JD, Parisi A, Sarkar K, Blacker BF, Reiner RC, Hay SI, et al. The global burden of non-typhoidal Salmonella invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis 2019a;19:1312–24. doi:10.1016/S1473-3099(19)30418-9/ATTACHMENT/5475A56B-6957-4F67-BB90-483D83F1C426/MMC1.PDF.

Stanaway JD, Reiner RC, Blacker BF, Goldberg EM, Khalil IA, Troeger CE, et al. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis 2019b;19:369–81.

UNAIDS. Zambia | UNAIDS. Webpage 2015. https://www.unaids.org/en/regionscountries/countries/zambia (accessed April 4, 2022)

UNICEF. HIV/AIDS | UNICEF Zambia 2020. https://www.unicef.org/zambia/hivaids (accessed April 4, 2022)

Vollaard AM, Ali S, Van Asten HAGH, Widjaja S, Visser LG, Surjadi C, et al. Risk factors for

typhoid and paratyphoid fever in Jakarta, Indonesia. J Am Med Assoc 2004;291:2607–15. doi:10.1001/jama.291.21.2607.

Wang J, Li Y, Xu X, Liang B, Wu F, Yang X, et al. Antimicrobial resistance of Salmonella enterica serovar typhimurium in Shanghai, China. Front Microbiol 2017;8:510. doi:10.3389/fmicb.2017.00510.

Wong VK, Baker S, Connor TR, Pickard D, Page AJ, Dave J, et al. An extended genotyping framework for Salmonella enterica serovar Typhi, the cause of human typhoid. Nat Commun 2016;7:1–11. doi:10.1038/ncomms12827.

Wu W, Wang H, Lu J, Wu J, Chen M, Xu Y, et al. Genetic diversity of Salmonella enteric serovar Typhi and Paratyphi in Shenzhen, China from 2002 through 2007. BMC Microbiol 2010;10:1–7. doi:10.1186/1471-2180-10-32.

Yap KP, Ho WS, Gan HM, Chai LC, Thong KL. Global MLST of Salmonella Typhi revisited in post-genomic era: genetic conservation, population structure, and comparative genomics of rare sequence types. Front Microbiol 2016;7:270. doi:10.3389/fmicb.2016.00270.