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Highlights

- *Salmonella enterica* Typhi most prevalent with genetic diversity
- Low prevalence of invasive Non-typhoidal *Salmonella* infections
- *Salmonella enterica* Typhimurium isolated belong to serotype 313
- High prevalence of multidrug resistant strains
- Emergence of fluoroquinolone and cephalosporin resistance

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

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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%), ciprofloxacin (5%), multidrug-resistance (46%), 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, 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 public 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 invasive diseases such as bacteraemia and meningitis with higher case-fatality rates estimates among children $\leq$ 5 years, the elderly $\geq$ 70 years, people with HIV infection and 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 in the national immunization programmes in Zambia, but vaccination is yet to commence. A recent study using mathematical modelling on estimating 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 the 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 1,665, offering specialized care to patients from Lusaka and other parts of the country. The approximate proportion of patients from Lusaka was 90% and 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 the Bactec machine (BD Bactec FX, Wokingham Berkshire, United Kingdom). All blood culture samples that flagged positive were sub-cultured on MacConkey, blood, and chocolate agar plates (Oxoid, Basingstoke, UK) as per 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 VITEK[®] 2 Compact instrument (Biomerieux) using the 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 disc diffusion method. The 0.5 McFarland was determined

using a Densicheck (Biomérieux), 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), co-trimoxazole-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 was managed in Excel[®] spreadsheets and exported to STATA[®] 14 for analysis. The proportion of Resistance (R %), Intermediate (I %), Susceptible (S %) and (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

They 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 x 150 bp paired-end sequencing runs with ~80 times coverage.

Genomic Sequence Analysis

Raw genomic sequencing data was 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 (<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's Demographics and Clinical Symptoms

A total of 7,180 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 to females 43.3% (95% CI: 31.1-56.4). The median age of the patients was 13.5 years (Range: 2 to 54 years) with an age group distribution of 0-15 years 61% (50/76), 16-35 years at 23% (14/76) and ≥ 36 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).

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 (Biomérieux) were not sequenced as 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*

A total of 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 2 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) *S. Weltevreden* (Table 1).

Antimicrobial Resistance

The *Salmonella enterica* isolates were tested for susceptibility to nine antibiotics. The highest resistance was observed to ampicillin (83%), co-trimoxazole (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 4th generation cephalosporin resistance was at 4%. There was no resistance to imipenem recorded but 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, *bla*TEM-1B (67%), phenicols (68%), folate pathway antagonist [sul2 33%, sul1/sul2 combined 44%, *dfr* A1 4%, *dfr* A7 40 %, *dfr* A7/*dfr* 14 combined 4%]. The 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%], *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 genes determinants found

in 'ResFinder 4.1' pipelines at the time of analysis but we never investigated for AMR determinants at other databases, so we cannot exclude the possibility that beta-lactam and quinolone resistant determinants could be found at these other databases.

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. A total of 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, we searched EnteroBase for the nearest strain matches, using a search criteria (cut-off) of up to 10 allele differences. The comparative analysis was performed for only our largest three *S. Typhi* clusters (clusters represented by ≥ 5 isolates). These three largest clusters were named cluster one (14 isolates), cluster two (nine isolates) and cluster three (five isolates) (Figure 2). For cluster one, we found close matches to isolates from the Zambian 2010 outbreak, Tanzania, Malawi, South Africa, United Kingdom and the United States. For cluster two and three, we found close matches to isolates from Zambia (2011), Tanzania and Malawi. The closest matches dated far back as 2008 and Tanzania had 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). We also noted a wide range of symptoms associated with this illness, with attending clinicians having enteric fever (66.7%) at the top of their presumptive diagnosis list. In comparison, sepsis was second at 17.8%. With this observation, we can assume that most of our patients received ceftriaxone as empiric treatment as 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).

Further, 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 is known to cause flooding, a known risk factor for enteric fever spread, as this can cause the mixing of drinking water sources with open sewer that contains faecal matter (Vollaard et al., 2004). This is common in lower-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 23% cases detected in the hot-dry season.

S. Typhi (72%) showed predominance as a causative agent of Enteric fever over the other serovars, which is consistent with a study previously done 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 are different from 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 Gambia (Kwambana-Adams et al., 2015), where they found the incidence of iNTS cases to be higher than *S. Typhi* cases. The serovar differences in these countries could be attributed to the 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 number of immunocompromised population (UNICEF, 2020).

S. Typhi strains isolated from various endemic regions traversing over a century confirms the predominance of two sequence types (ST1 and ST2) co-existing in the endemic regions (Yap et al., 2016). This could be due to international travel and the uniqueness of ST1/ST2 virulence genes that 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 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 in The Gambia (Darboe et al., 2022). *S. Typhimurium* ST313 is rarely isolated from outside SSA. Compared to the *S. Typhimurium* ST19 that causes diarrhoea 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 the findings in Ghana (Labi et al., 2014), Kenya (Muthumbi et al., 2015) and India (Menezes et al., 2012) but are different to findings in Italy (García-Fernández et al., 2015) and Pakistan (Klemm et al., 2018) that found 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; Msemu et al., 2019). Of note is the reduction of MDR *S. Typhi* from 84% in a previous study in Zambia (Kalonda et al., 2015) to 51% in our study. This observation could result from changes in prescribing patterns and antimicrobial use, such as reduced use of ampicillin, co-trimoxazole 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 1990-1994), Malawi (0 to 88% between 1994 to 2009) and Nigeria (37% to 100% between 1998 to 2014) (Browne et al., 2020).

The resistance gene *blaTEM-1B* responsible for resistance to ampicillin was the most common resistance gene found, similar to 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 other studies (Das et al., 2017; Gaiand 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 type of mutations likely to impact fitness. This can explain our observation of nalidixic acid-resistant strains with reduced susceptibility to fluoroquinolones, similar to what was seen 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 to 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 confirms multiple *Salmonella* serovars. This observation confirms that NTS infections are sporadic in our community and most of the outbreaks are driven by the human-specific *S. Typhi*. The results of genetic similarities of our *S. Typhi* isolates to strains from other countries such as Tanzania, Malawi, South Africa, the UK and the United States, suggests 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 from nearly 2,000 isolates sourced from over 60 countries found the global *S. Typhi* population to be highly structured with dozens of subclades that display geographical restriction (Wong et al., 2016). Analysing WGS data

from different regions helps 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 source (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 high prevalence of MDR and emergence of resistance to ciprofloxacin and cephalosporins warrants improved hygiene, 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 have read and approved the final manuscript.

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Conflict 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.

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Ethical Approval

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

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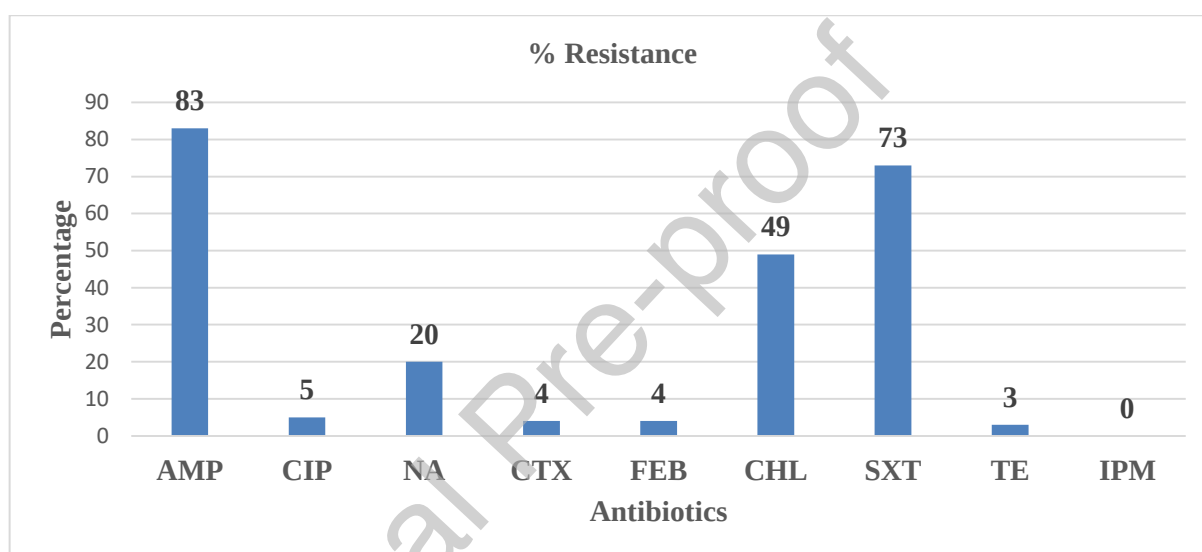


Figure 1: Resistance patterns to all antibiotics tested

Abbreviations: AMP – Ampicillin, CIP – Ciprofloxacin, NA – Nalidixic acid, CTX – Cefotaxime, FEP – Cefepime, CHL – Chloramphenicol, SXT – Trimethoprim-sulphamethoxazole, TE – Tetracycline, IPM – Imipenem

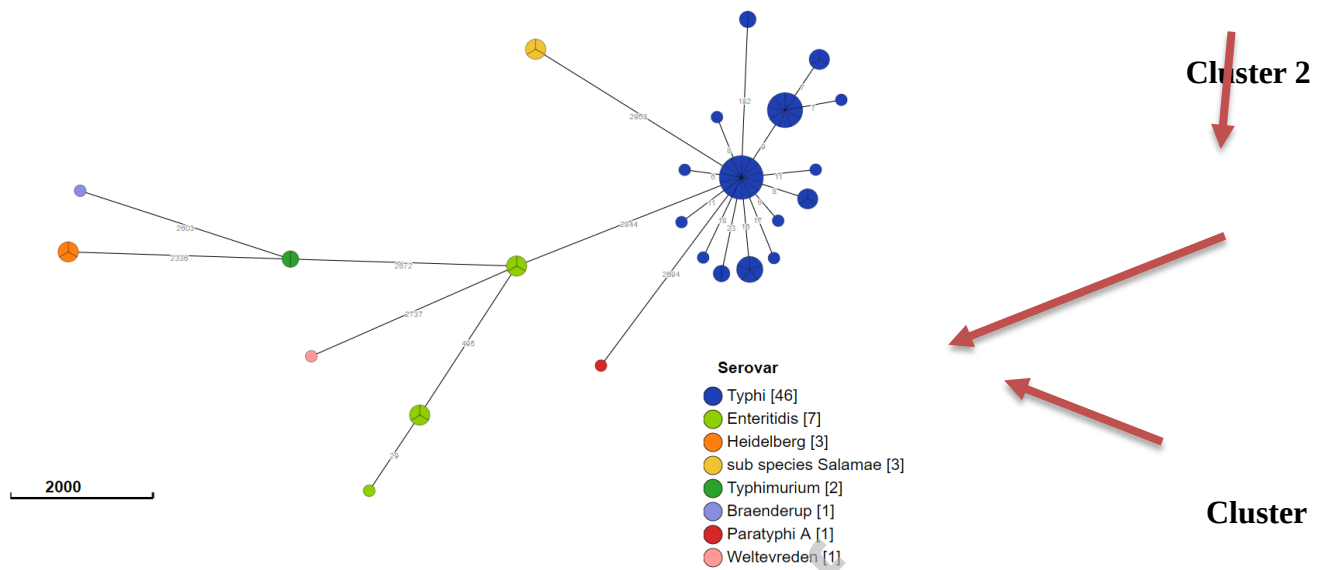


Figure 2: Genetic relatedness of different *Salmonella enterica* serovars

Minimum spanning tree drawn using cgMLST data from *S. enterica* isolated from Lusaka, Zambia, from 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 the ≤ 5 allele differences, there was a wide range of genetic diversity and varying strains.

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

Characteristics	<i>Salmonella enterica</i> serovars								
Number of isolates: 76	S. Typhi	S. Typhimurium	S. Enteritidis	S. Paratyphi A	S. Braenderup	S. Heidelberg	S. Weltevreden	S. Salamae	Total % (n)
Sequence types (ST)	ST1 (44)	ST313 (2)	ST11 (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:	33	1	4	1	1	1	0	2	57%
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	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 sequence d 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

Table 2 Distribution of Presumptive diagnosis of 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 Leukaemia 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

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

Antibiotics class	Antibiotics	Susceptibility results			AMR resistance determinants
		Resistance (R) % (95% CI)	Intermediate (I) % (95% CI)	Susceptible (S) % (95% CI)	
Beta-lactams	AMP	83 (72.2-90.3)	1 (0.2-9.7)	16 (8.7-26.1)	<i>BlaTEM-1B</i> (43)
Quinolones	CIP	5 (2.1-14.4)	30 (19.8-41.5)	65 (52.8-75.2)	<i>gyrA</i> [S83Y, 50% (4/8), D87N, 50% (4/8)], <i>gyrB</i> [S464Y, 100% (1/1)], <i>parC</i> [T57S 100% (8/8)]
	NA	20 (9.7-27.8)	-	80 (72.2-90.3)	
3 rd & 4 th generation cephalosporins	CTX	4 (1.3-12.6)	-	96 (87.4-98.7)	None
	FEB	4 (1.3-12.6)	-	96 (87.4-98.7)	
Phenols	CHL	49 (37.6-61.1)	-	51 (39-62.4)	<i>catA1</i> 68% (26)
Folate pathway antagonist	SXT	73 (61.5-82.4)	10 (4.7-19.5)	17 (9.7-27.8)	<i>Sul1</i> alone (0), <i>sul2</i> (18), <i>sul1/sul2</i> combined (24), <i>dfr A1</i> (2), <i>dfr A7</i> (22), <i>dfr A14</i> (18), <i>dfr A7/dfr 14</i> 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-sulphamethoxazole, TE – Tetracycline, IPM – Imipenem

Table 4: Distribution Multidrug resistance (MDR) in the different *Salmonella* serovars (n=76)

Serovars	Multi-drug 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