The molecular basis of extensively drug-resistant *Salmonella* Typhi isolates from pediatric septicemia patients

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Abstract

Sepsis is a syndromic response to infections and is becoming an emerging threat to the public health sector, particularly in developing countries. *Salmonella* Typhi (*S.* Typhi), the cause of typhoid fever, is one primary cause of pediatric sepsis in typhoid endemic areas. Extensively drug-resistant (XDR) *S.* Typhi is more common among pediatric patients, which is responsible for over 90% of the reported XDR typhoid cases, but the majority of antibiotic resistance studies available have been carried out using *S.* Typhi isolates from adult patients. Here, we characterized XDR *S.* Typhi isolates from a medium size cohort of pediatric typhoid patients to determine their antibiotic-resistance-related gene signatures associated with common treatment options to typhoid fever patients. This study informs the molecular basis of antibiotic-resistance among recent *S.* Typhi isolates from pediatric septicemia patients, therefore providing insights into the development of molecular detection methods and control strategies for XDR *S.* Typhi.

Introduction

Sepsis is a syndromic response to infections and is becoming an emerging threat to the public health sector. The World Health Organization (WHO) estimated that 48.9 million cases of sepsis have been reported and that one person dies every 2.58 seconds around the world (1). Furthermore, 20 million cases were detected among children, with 2.9 million deaths worldwide and 85% of these deaths in developing countries (1). *Salmonella enterica* serovar Typhi (*S.* Typhi), the causative agent of typhoid fever, is one primary cause of pediatric sepsis in typhoid endemic areas including Pakistan (1-3). Antibiotics are the primary treatment options for typhoid fever, but *Salmonella* are continuously evolving to acquire plasmid, prophage, transposon, or chromosomal gene mutation to attain resistance against antibiotics. A myriad of reports indicated the global spread of *S.* Typhi that is resistant to all of the first-line antibiotics, ampicillin, chloramphenicol, and co-trimoxazole, collectively known as multidrug-resistant (MDR) (4-8). All of the identified MDR *S.* Typhi carry the IncHI1 region located on either the plasmid or chromosome, which encodes several antibiotic-resistance genes, including *catA1* (conferring resistance to chloramphenicol), *blaTEM-1* (resistance to ampicillin), *dhfR7*, and *sul1* (resistance to trimethoprim-sulfamethoxazole), among other antibiotics-resistance-related genes found in MDR *S.* Typhi (4, 9-11).

Fluoroquinolones were used to treat MDR cases but became largely ineffective in some endemic regions. Fluoroquinolone-resistant *S.* Typhi encodes the quinolone resistance gene *qnrS* and point mutations in the quinolone resistance determining region (QRDR) harboring the genes for gyrase/topoisomerase II *gyrA* and *gyrB* and topoisomerase IV *parC* and *parE*. For instance, several point mutations occurred in *gyrA* have been correlated to resistance to fluoroquinolones,

55 including M52L, G81C, D82G, S83F/Y/L, D87N/G/A/Y/H, and A119E (10, 12-16). Point
56 mutations in *gyrB, parC,* and *parE* have also been reported, while some variants have been
57 reported only from certain geographical locations (13, 15-20). Some of those mutation sites are
58 near the quinolone binding site, which in many cases results in the inhibition of the binding of
59 antibiotics to topoisomerases (21). *S.* Typhi strains resistant to chloramphenicol, ampicillin, co-
60 trimoxazole, fluoroquinolones, and third-generation cephalosporins were first reported in
61 Hyderabad, Sindh, Pakistan, affecting over 300 cases in 2016 (9), collectively known as
62 extensively drug-resistant (XDR) *S.* Typhi (8, 22). XDR *S.* Typhi isolates commonly harbor an
63 IncY plasmid carrying the extended-spectrum β-lactamase resistance gene *blaCTX-M-15* and
64 quinolone resistance gene *qnrS*, among others (9).
65
66 Drug efflux pump systems also play a significant role in resistance to a wide range of
67 antibiotics. *Salmonella spp.* possess five efflux pump families, including the ATP-binding
68 cassette (ABC) MacAB-TolC system and resistance-nodulation-cell division (RND) AcrAB-
69 TolC system (23). Members of the other families of drug transporters, major facilitator
70 superfamily (MFS), multidrug and toxin extrusion (MATE), and small multidrug resistance
71 (SMR), are located in the inner membrane (IM) of gram-negative bacteria (24). They usually
72 function as independent units in the IM to translocate antibiotics across the membrane bilayer,
73 followed by their cooperation with RND-type efflux pumps to pump out antibiotics across the
74 entire cell envelope (24). In typhoidal *Salmonella*, point mutations at amino acid position 717
75 (R717Q or R717L) on AcrB, the antibiotic-binding subunit of the RND-type AcrAB-TolC efflux
76 pump, have been correlated with resistance to azithromycin in *S.* Typhi and *S.* Paratyphi A,
77 respectively (19, 25, 26).

Antibiotic-resistant *S.* Typhi infection is more common among children; more than 90% of the XDR typhoid cases are currently from children younger than 15 years old of age (9, 27, 28). The typhoid mortality in the pre-antibiotic era was approximately 25% (29). Typhoid fever is only partly preventable by vaccines, and two types of typhoid fever vaccines, the live-attenuated Ty21a and Vi and its conjugate subunit vaccines, are currently available (30). These vaccines exhibit the efficacy of approximately 55-85%, with the Vi-protein conjugate subunit vaccine being the most efficacious (30-32). The recent Vi-protein conjugate subunit vaccine has been demonstrated efficacious among children who are older than six months of age. There are no vaccines for early-life populations younger than six months available (31). Furthermore, *S.* Typhi strains that lack Vi have emerged in some endemic areas (33), which is most likely to make current subunit vaccines ineffective against those variants.

Macrolides (e.g., azithromycin) and carbapenems (e.g., imipenem, meropenem) remain to be "last resort" oral and injectable antibiotics for treating *S.* Typhi infection, respectively. *S.* Typhi strains resistant to macrolide azithromycin have been emerged (34). *S.* Typhi strains resistant to carbapenem antibiotic meropenem, have also been reported, and many cases of invasive nontyphoidal Salmonellae (NTS) resistant to carbapenems have been reported (25, 35-37). Given that typhoid fever vaccines and treatment options have limitations, there is an urgent need for closely monitoring drug-resistance profiles of *S.* Typhi strains at the point-of-care to provide valuable insights into the development of control strategies against drug-resistant *S.* Typhi.

Results

XDR *S.* Typhi strains isolated from children at various developmental stages

We isolated *S.* Typhi strains from 45 typhoid fever-suspected pediatric septicemia patients with 1-13 years of age (68.89% male and 31.11% female) who have visited the Fatima Memorial Hospital, Lahore, Punjab, Pakistan, between October 2019 to January 2020. Punjab is the most populous province approximately 1,044 km away from Hyderabad, Sindh, Pakistan, where the first XDR *S.* Typhi was reported (Figure 1A). Before samples were transferred to researchers, all *S.* Typhi samples were de-identified, number-based identification codes were assigned to samples (Table 1). The data were analyzed anonymously throughout the study. Positive blood culture bottles from the initial step using a fully automated culture and test system for patient blood specimens were sub-cultured on blood and MacConkey agar plates and incubated overnight at 37ºC. Preliminary identification of the isolates was conducted according to colony morphology and culture characteristics, followed by polymerase chain reaction (PCR) and Sanger sequencing-based molecular determination (Figure 1B and C). These results indicate that all suspected patient specimens carried *S.* Typhi and all isolates are XDR since they are resistant to both first- and second-line antibiotics (Figure 1 and Table 1). XDR *S.* Typhi isolates exhibit similar minimum inhibitory concentration (MIC) values across 45 isolates with some variations, indicating that antibiotic resistance phenotypes do not correlate with age, sex, or hospital wards (Table 1).

Molecular basis of resistance to first-line antibiotics among XDR *S.* Typhi isolates

We hypothesized that resistance to first- and second-line antibiotics among XDR *S.* Typhi isolates is primarily due to the acquisition and/mutation of antibiotic-resistance-related

124 genes. To carry out a series of molecular characterization via PCR and/or PCR amplicon
125 sequencing, we selected 18 *S.* Typhi samples based on sex, age, hospital wards, antibiotic-
126 resistance profile, and MIC (Table 2). According to child development milestones defined by the
127 Centers for Disease Control and Prevention (CDC), age groups were split into toddlers (ages 1 to
128 2), preschoolers (ages 3 to 5), school-age children (ages 6 to 12), and adolescents (ages 13 to 18)
129 (Figure 2A). To understand the molecular basis of the MDR phenotype resistant to all the first-
130 line antibiotics with clinical relevance, we have designed primers specific to *catA1*, *blaTEM1*,
131 *dhfR7*, and *sul1*, MDR-related genes encoded in the IncHI1 region (Table 3). Using these
132 primers, we evaluated the presence of these MDR-related genes in 18 selected *S.* Typhi isolates
133 via PCR analysis. All PCR reactions resulted in amplicons with expected size for specific genes
134 except for two controls, antibiotic-susceptible *S.* Typhi ISP2825 (ISP) and a control PCR
135 reaction mixture that did not contain any *S.* Typhi genomic DNA (N) (Figure 2B-D). Drug-
136 susceptible *S.* Typhi ISP clinical isolate available in the laboratory was used as a control since all
137 *S.* Typhi isolates from this cohort were XDR (Table 1). These results indicate that the MDR
138 phenotype exhibited by these XDR *S.* Typhi isolates is most likely due to MDR-related genes
139 encoded in the IncHI1 region.
140
141 **Molecular basis of fluoroquinolone-resistance among XDR *S.* Typhi isolates**
142 The acquisition of an IncY region harboring *qnrS* and one or more point mutations on the
143 genes *gyrA*, *gyrB*, *parC*, and/or *parE*, referred to as the quinolone resistance determining region
144 (QRDR), have been correlated to fluoroquinolone-resistance among typhoidal *Salmonella*
145 strains. A PCR primer set specific for *qnrS* was designed and used to investigate whether XDR *S.*
146 Typhi isolates encode this fluoroquinolone-resistance-related gene. We found that, unlike

147 antibiotic-susceptible *S.* Typhi ISP2825, all XDR *S.* Typhi isolates tested encode *qnrS* (Figure
148 3A). Fluoroquinolone-resistant *Salmonella* strains have been reported to carry point mutations in
149 *gyrA*, *gyrB*, *parC*, and/or *parE*, which exhibits variations depending on geographical locations
150 (10, 12-20) (Table 4). Specific primer sets for the known mutations on these four genes were
151 designed for PCR and PCR amplicon sequencing (Table 3). Consistent with their essential roles
152 in bacterial cell replication, both antibiotic-susceptible *S.* Typhi ISP2825 and all XDR *S.* Typhi
153 isolates resulted in PCR products with expected size for the four topoisomerase genes (Figure
154 3B-E). To determine whether XDR *S.* Typhi isolates resistant to fluoroquinolones have point
155 mutations in these topoisomerase genes, we carried out Sanger sequencing of PCR amplicons.
156 Consistent with the antibiotic-susceptible phenotype, *S.* Typhi ISP2825 carries wild-type
157 topoisomerases. In contrast, we found that all the XDR *S.* Typhi isolates carry a mutant form of
158 *gyrA* encoding for $GyrA^{Ser83Phe}$ (Figure 3F-G). $GyrA^{Ser83Phe}$ has been found most commonly
159 among XDR *S.* Typhi identified from other endemic regions. We also found that these XDR *S.*
160 Typhi isolates from pediatric septicemia patients encode wild-type *gyrB*, *parC*, and *parE* (Figure
161 3G and Table 4).
162
163 **Molecular basis of third-generation cephalosporin-resistance among XDR *S.* Typhi isolates**
164 In addition to $GyrA^{Ser83Phe}$, resistance to second-line antibiotics among XDR *S.* Typhi in
165 Pakistan has been associated with an IncY region carrying the quinolone resistance gene *qnrS*
166 (Figure 3A) and extended-spectrum β-lactamase resistance gene *blaCTX-M-15* (9, 38).
167 Consistent with resistance to second-line antibiotics, fluoroquinolones and cephalosporins,
168 among these XDR *S.* Typhi isolates from pediatric septicemia patients, we found the acquisition
169 of *blaCTX-M-15* among all XDR *S.* Typhi tested (Figure 4). In contrast, antibiotic-susceptible *S.*

Typhi ISP2825 did not result in PCR amplicon for *blaCTX-M-15*, indicating the specificity of the primers used.

Effects of efflux pumps on antibiotic resistance among XDR *S.* Typhi isolates

XDR *S.* Typhi isolates from pediatric typhoid patients exhibit some variations in their antibiotic-resistance/susceptibility profiles across antibiotics tested in Table 1. These results led us to investigate drug efflux pumps in XDR *S.* Typhi isolates since drug efflux pump systems are associated with resistance/susceptibility to a wide range of antibiotics. The recent worrisome trend among some XDR *S.* Typhi includes a correlation between efflux pump mutations and azithromycin resistance. For instance, a point mutation(s) on the antibiotic-binding subunit AcrB of the tripartite AcrAB-TolC efflux pumps (e.g., R717Q or R717L) has recently been correlated to azithromycin-resistance among some *S.* Typhi and *S.* Paratyphi A clinical isolates (19, 25, 26). In addition, *Salmonella* encodes another tripartite efflux pump, ABC-type MacAB-TolC, and three other small efflux pumps, major facilitator superfamily (MFS), multidrug and toxin extrusion (MATE), and small multidrug resistance (SMR), spanning in the inner membrane of the bacteria and therefore need to cooperate with another tripartite efflux pump such as RND-type AcrAB-TolC in exporting antibiotics (24). In *Neisseria gonorrhoeae* that, like *S.* Typhi, is also a human-adapted gram-negative bacterial pathogen, point mutations on the promoter of *macA* of the tripartite MacAB-TolC efflux pump have been correlated to azithromycin-resistance (39).

To assess whether point mutations on tripartite efflux pumps have occurred among XDR *S.* Typhi isolates from pediatric patients, we have determined *macA* promoter and *acrB*

sequences via PCR and PCR amplicon sequencing using specific primer sets summarized in Table 3. We found that all XDR *S.* Typhi isolates tested carry wild-type -10 promoter sequence in the *macA* promoter and wild-type Arg at position 717 on the AcrB protein (Figure 5A-C). These results are consistent with azithromycin-susceptibility (2-8 µg/ml) among XDR *S.* Typhi isolates from our pediatric septicemia patient cohort (Figure 5C and Table 1).

The expression of drug-efflux pumps is tightly regulated. For instance, AcrR represses *acrAB* gene expression by binding to the operator and inhibiting the transcription of *acrAB*. In 3-dimensional protein structure, wild-type AcrR protein monomer forms nine α-helices crucial for homodimer assembly, DNA-binding, and ligand-binding for its function in repressing *acrAB* expression (40, 41). Through whole-genome sequencing analysis of the latest NCBI RefSeq dataset of the fully assembled complete genome of *S.* Typhi (107 in total; Table 5), we found that two *S.* Typhi strains (RefSeq assembly accession IDs GCF_001121865.2 and GCF_900205275.1) carry a variant form of AcrR with 48 amino acid difference at the C-terminus (Figure 6A). Unlike the repressor AcrR variant, these two *S.* Typhi strains still carry wild-type RobA (NP_463442.1) and MarA (WP_000091194.1), activators for the *acrAB* gene expression, and wild-type AcrA (NP_459471.1), AcrB (NP_459470.1), and MacA (NP_459918.1) (data not shown). These results led us to investigate whether our XDR *S.* Typhi isolates carry a variant form of AcrR. Consistent with our azithromycin-susceptibility data, we found that all the XDR *S.* Typhi isolates from our pediatric septicemia patient cohort carry wild-type AcrR (Figure 6B-C).

Discussion

XDR *S.* Typhi is more common among pediatric patients but the majority of antibiotic resistance studies available have been carried out using *S.* Typhi isolates from adult patients. Here, we characterized *S.* Typhi isolates from a medium size cohort of pediatric typhoid patients to determine antibiotic-resistance-related gene signatures associated with their drug-resistant profiles. This study provides a valuable overview of the recent (2019-2020) populations in the setting of Lahore, Pakistan, among a septic pediatric cohort and provides insights into the development of simple, cost-effective molecular detection methods with point-of-care testing potential.

Biochemical method-mediated identification assisted by the streamlined automated system was further validated via molecular typing for typhoidal *Salmonella* specific gene sequences (42-44). Antibiotic resistance profiles were obtained through the automated test system that followed the CLSI 2018 guidelines. To determine the molecular basis of the antibiotic resistance profiles among these XDR *S.* Typhi, we have designed primer sets and optimized PCR reaction conditions for antibiotic resistance genes harbored in the IncHI1 (*catA1*, *blaTEM1*, *dhfR7*, and *sul1*) and IncY (*qnrS and blaCTX-M-15*) regions, contributing to resistance to front-line and second-line antibiotics, respectively. Overall findings across the XDR *S.* Typhi isolates for these antibiotic resistance genes are in agreement with our MIC results and other reports correlated to molecular determinants of MDR and XDR phenotypes among recent XDR *S.* Typhi isolates from adult typhoid patients (9).

Besides *qnrS*, mutations on *gyrA*, *gyrB*, *parC*, and *parE* also contribute to fluoroquinolone-resistance, which exhibits more diverse patterns depending on geographical

239 locations. Our XDR *S.* Typhi isolates from pediatric patients in Northern Pakistan carry GyrA^{S83F}
240 across all samples tested, while the remaining genes were found to be wild-type (Figure 3 and
241 Table 4). This result indicates that XDR *S.* Typhi circulating in this geographical location is
242 different from the ones prevalent in other locations that exhibit different QRDR mutation
243 signatures. Future investigations on XDR *S.* Typhi isolates from adult patients in the same
244 geographical location would inform us about whether a divergent host adaptation process has
245 occurred in pediatric and adult patients.
246
247 Macrolides such as azithromycin are considered the only remaining oral antibiotic option
248 in treating XDR *S.* Typhi resistant to both front-line and second-line antibiotics. An additional
249 treatment option against XDR *S.* Typhi, although requiring injection, are carbapenems such as
250 imipenem and meropenem. Our XDR *S.* Typhi isolates from pediatric patients are susceptible to
251 azithromycin, imipenem, and meropenem (Table 1). In contrast, 5% and 48% of recent *S.* Typhi
252 isolates (n=81) from a mid-size adult cohort in Northern Punjab were resistant to azithromycin
253 and meropenem, respectively (36), supporting the concept of a divergent host adaptation and/or
254 transmission process in child and adult groups. These results also support a possibility that, with
255 time, molecular determinants for azithromycin-resistance and meropenem-resistance would
256 likely be adopted in nearly all *S.* Typhi circulating locally and globally.
257
258 Current analysis indicates that our XDR *S.* Typhi isolates from pediatric septicemia
259 patients in Punjab do not carry molecular determinants that have been correlated to
260 azithromycin-resistance in typhoidal *Salmonella*, *S.* Typhi and *S.* Paratyphi A, and another
261 human-adapted Gram-negative pathogen *N. gonorrhoeae* (Figure 5). Besides wild-type AcrB and

wild-type -10 promoter sequence in the *macA* promoter across our XDR *S.* Typhi isolates, WGS analysis of the latest NCBI RefSeq dataset of the fully assembled complete genome of *S.* Typhi (107 in total), conducted as part of this study, indicates the emergence of *S.* Typhi strains carrying the frameshifted AcrR variant, the repressor of the *acrAB* efflux pump components. Although further investigations are required to understand the consequence of having the AcrR frameshifted variant in antibiotic-resistance, it is intriguing to hypothesize that the AcrR variant is less effective in repressing the expression of *acrAB*, therefore contributing to antibiotic-resistance such as azithromycin. We also found that those *S.* Typhi strains carrying the AcrR variant carry wild-type RobA and MarA, activators for the *acrAB* gene expression, and wild-type AcrAB, collectively supporting the hypothesis that possession of the AcrR frameshifted variant is an adaptation/evolution outcome, rather than a stochastic event outcome.

The emergence and spread of *S.* Typhi resistant to macrolides and carbapenems are a serious global health concern, deserving close surveillance for local and global spread. We envision that some of the methods detecting key molecular determinants for *S.* Typhi antibiotic resistance used in the current study could be developed as a surveillance strategy and point-of-care testing strategy. The detection and analysis methods for resistance to front-line and second-line antibiotics described in the study are straightforward. Besides efflux pump related molecular determinants described in the study, the future surveillance strategy could include additional molecular traits predicted to be associated with resistance to macrolides and carbapenems in *S.* Typhi. For instance, in *Enterobacteriaceae*, *erm* genes encoding for methylases to modify target sites, *ere* genes for esterase transferases, and *mph* genes for phosphor transferases are known to confer macrolide-resistance by altering the structure of antibiotics (45). Similarly, carbapenem

resistance in *S.* Typhi can be acquired by mutational events or gene acquisition via horizontal gene transfer, leading to the overexpression of efflux pumps that expel carbapenems and the acquisition of carbapenemases. The most effective carbapenemases known that hydrolyze carbapenem and spread across many bacterial pathogens are KPC, VIM, IMP, NDM and OXA-48 types (46).

In summary, this study informs the molecular basis of antibiotic-resistance among recent *S.* Typhi isolates from pediatric septicemia patients and provides insights into the development of molecular detection and control strategies for XDR *S.* Typhi.

Materials & Methods

Ethics Statement

Before initiating this research, ethical approval was obtained following the Declaration of Helsinki from the Institutional Review Board (IRB# FMH-03-2020-IRB-774-F), the Fatima Memorial Hospital Lahore. In addition, informed consent was obtained from a legal guardian of each study participant. Informed consent was read to the person in the language they understood and signed appropriately. They were willing to provide a sample and utilize the isolates for research. They were assured that the samples would be used solely for research purposes and that personal information would be kept confidential. Before samples were transferred to researchers, all XDR *S.* Typhi samples were de-identified, number-based identification codes were assigned to samples (Tables 1 and 2). The data were analyzed anonymously throughout the study.

S. Typhi isolation from patient specimens and MIC determination

The BACT/ALERT® 3D Microbial Detection System with PF/PF plus culture bottles (bioMérieux, France), an automated bacterial culture and antibiotic-resistance test system capable of incubating, agitating, and continuously monitoring aerobic and anaerobic media inoculated with patient specimens was used in this study. The samples were collected between October 2019 to January 2020. In brief, 1-4 mL blood samples of each septicemia suspected child based on their age and bodyweight were taken and placed in BacT/ALERT PF/PF plus bottles for up to 5 days. The bottles contained BacT/Alert FAN Plus media with Adsorbent Polymeric Beads (APB) that neutralized antimicrobials (47). Positive blood culture bottles were sub-cultured on blood and MacConkey agar plates and incubated overnight at 37ºC aerobically. Preliminary identification of the isolates was conducted according to colony morphology and culture characteristics. MIC determinations were made using the automatic VITEK 2 compact system (bioMérieux, France) and antibiotics interpretation was carried out as per the clinical laboratory standards institute (CLSI) 2018 guidelines (https://clsi.org).

XDR *S.* Typhi samples selection for detailed molecular characterization

Of 45 XDR *S.* Typhi isolates, 18 isolates were selected for detailed molecular characterization based on their MIC results, gender, age, and hospital wards, representing all 45 XDR isolates (Tables 1 and 2).

PCR-based detection of antibiotic-resistance-related genes among XDR *S.* Typhi isolates

Bacterial genomic DNA was prepared using a DNeasy bacterial DNA extraction kit (QIAGEN) following the vendor's recommendation. The PCR primer sequences and reaction conditions used are summarized in Table 3. Green Taq DNA polymerase with provided buffers (GenScript)

was used for *pltB, blaTEM1, dhfR7, sul1, catA1, parC, parE, blaCTXM15, macA, acrB*, and *acrR*. Phusion high fidelity DNA polymerase with the provided GC buffer (New England BioLabs) or Herculase II fusion DNA polymerase (Agilent) was used for *gyrA, gyrB,* and *qnrS*. PCR reaction steps were: pre-denaturation at 95℃ for 3min, 34 cycles of denaturation at 95℃ for 30 sec, annealing (see Table 3), and extension at 72℃ for 1 kb/min (see Table 3 for amplicon size), and final extension at 72℃ for 7 min using a C1000 Touch Thermal Cycle (BIO-RAD). PCR results were run on 1% agarose gels and imaged using an iBright CL1500 Imaging system (ThermoFisher Scientific).

Sanger sequencing of PCR amplicons

When indicated, PCR amplicons were extracted from agarose gels for sequencing analysis by using the QIAEX II gel extraction system (QIAGEN, cat # 20051), followed by standard Sanger sequencing (The Cornell Institute of Biotechnology or Eton Bioscience Inc). The primer sequences used for Sanger sequencing are summarized in Table 3.

Whole-genome sequencing analysis for efflux pump-related genes

The latest NCBI RefSeq dataset of the fully assembled complete genome of *S.* Typhi (107 in total; Table 5**)** was collected on Feb 26, 2021. The 107 complete whole-genome sequences were utilized to analyze the sequence variations for *acrR* (NP_459472.1) using 'General Feature Formats (gff)' files with a bash script (grep acrR *.gff | grep pseudo=true). Two whole genome sequences (GCF_001121865.2 and GCF_900205275.1) that have an *acrR* variant were further analyzed with CLC Main Workbench 8.1.3 (QIAGEN) for multidrug efflux pump-related genes:

353 *macA* (NP_459918.1), *acrA* (NP_459471.1), *acrB* (NP_459470.1), *marA* (WP_000091194.1),
354 and *robA* (NP_463442.1).
355
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360
361 **Author contributions**
362 C.K. conducted experiments shown in Figs. 1-6 using genomic DNA received from M.U.Q., and
363 prepared the manuscript draft. D.P.N. contributed to experiments shown in Figs. 1-6. G.Y.L.
364 conducted WGS analysis for efflux pump-related genes shown in Fig. 6. R.S.K. contributed to
365 antibiotic resistance-related gene sequence analysis. I.L. A.B. and Q.A. isolated XDR *S.* Typhi
366 strains, conducted MIC determination experiments, and genomic DNA preparations. M.U.Q
367 supervised the isolation and antibiotic resistance phenotype characterization study. J.S.
368 supervised the molecular characterization study using genomic DNA received, and prepared the
369 manuscript with input from all authors.
370
371 **Declaration of interests**
372 The authors declare no competing interests.
373
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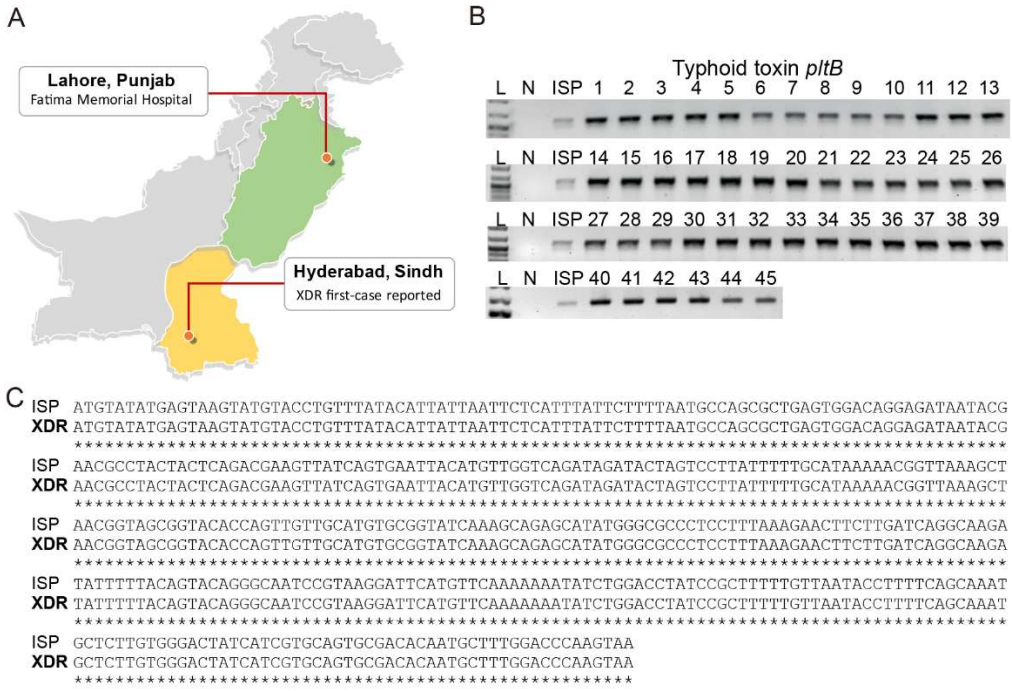


Figure 1. XDR *S.* Typhi strains isolated from children ages 1-13 years old. A, Cartoon showing the geographical location that XDR *S.* Typhi characterized in this study was isolated compared to the location that the first case of XDR *S.* Typhi was reported. **B**, PCR reactions of 45 XDR isolates for typhoid toxin *pltB*. **C**, *pltB* sequences from all 45 XDR isolates (XDR, one representative is shown) were identical to typhoid toxin *pltB* sequence of *S.* Typhi ISP2825 (ISP). ISP, antibiotic-susceptible *S.* Typhi ISP2825. N, negative control containing all PCR components except for *S.* Typhi genomic DNA. See Table 1 for sample information.

A

#	Age	Sex	AMP	SXT
Toddlers (1-2 yrs)				
16	2	F	R	R
2	2	M	R	R
20	2	M	R	R
23	2	M	R	R
27	2	M	R	R
Pre-schoolers (3-5 yrs)				
33	3	F	R	R
9	3	M	R	R
17	3	M	R	R
31	3	M	R	R
40	4	F	R	R
12	4	F	R	R
1	5	M	R	R
School-age children (6-12 yrs)				
15	9	F	R	R
6	11	M	R	R
8	11	M	R	R
36	11	M	R	R
Adolescents (13-18 yrs)				
38	13	F	R	R
7	13	M	R	R

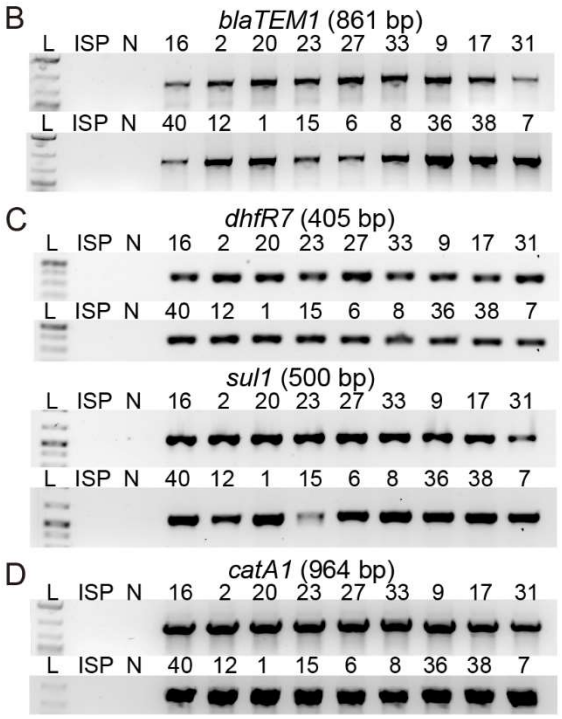
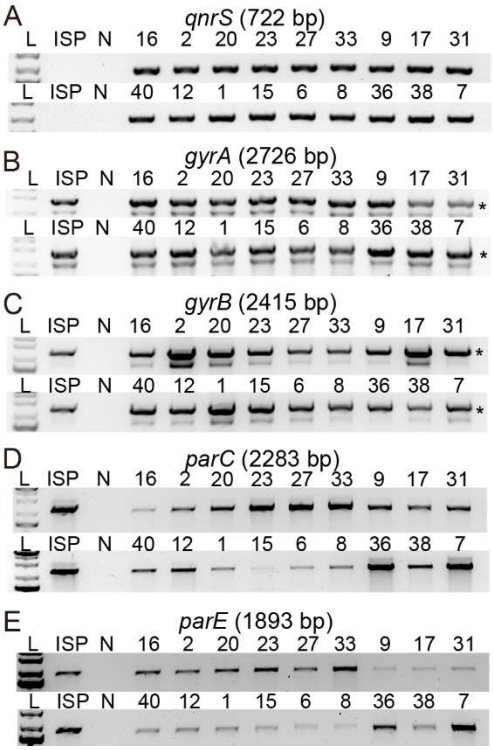
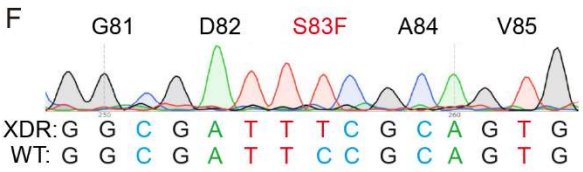


Figure 2. Molecular basis of resistance to first-line antibiotics among XDR *S.* Typhi isolates. A, Sample information and antibiotic resistance profiles of select of XDR *S.* Typhi isolates for molecular characterization. See Table 2 for details. **B**, PCR reactions for *blaTEM1*. **C**, PCR reactions for *dhfR7* and *sul1*. **D**, PCR reactions for *catA1*. ISP, antibiotic-susceptible *S.* Typhi ISP2825. N, negative control containing all PCR components except for *S.* Typhi genomic DNA. See Table 3 for details.





G

ID	Age	Sex	CIP	*gyrA*	*gyrB*	*parC*	*parE*
Toddlers (1-2 yrs old)							
16	2	F	R	S83F	WT	WT	WT
2	2	M	R	S83F	WT	WT	WT
20	2	M	R	S83F	WT	WT	WT
23	2	M	R	S83F	WT	WT	WT
27	2	M	R	S83F	WT	WT	WT
Pre-schoolers (3-5 yrs old)							
33	3	F	R	S83F	WT	WT	WT
9	3	M	R	S83F	WT	WT	WT
17	3	M	R	S83F	WT	WT	WT
31	3	M	R	S83F	WT	WT	WT
40	4	F	R	S83F	WT	WT	WT
12	4	F	R	S83F	WT	WT	WT
1	5	M	R	S83F	WT	WT	WT
School-age children (6-12 yrs old)							
15	9	F	R	S83F	WT	WT	WT
6	11	M	R	S83F	WT	WT	WT
8	11	M	R	S83F	WT	WT	WT
36	11	M	R	S83F	WT	WT	WT
Adolescents (13-18 yrs old)							
38	13	F	R	S83F	WT	WT	WT
7	13	M	R	S83F	WT	WT	WT

Figure 3. Molecular basis of fluoroquinolone-resistance among XDR *S.* Typhi isolates. A-E, PCR reactions for *qnrS* (**A**), *gyrA* (**B**), *gyrB* (**C**), *parC* (**D**), and *parE* (**E**). See Table 3 for details. ISP, antibiotic-susceptible *S.* Typhi ISP2825. N, negative control containing all PCR components except for *S.* Typhi genomic DNA. *, PCR amplicons specific to *gyrA* in B and *gyrB* in C. **F**, Representative sequencing chromatogram showing GyrA S83F mutation. **G**, Summary of PCR amplicon sequencing analysis for *gyrA, gyrB, parC,* and *parE*. See Table 4 for additional information. WT, wild type for the known mutations.

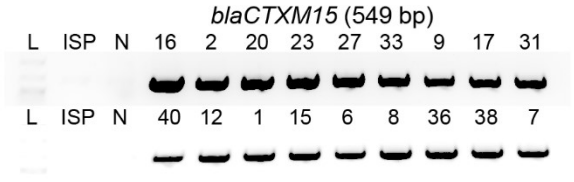
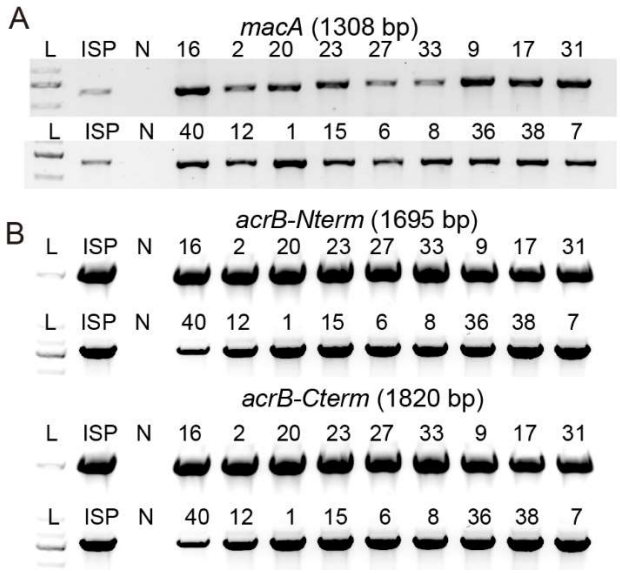


Figure 4. Molecular basis of third-generation cephalosporin-resistance among XDR *S.* Typhi isolates. PCR reactions for *blaCTXM15*. ISP, antibiotic-susceptible *S.* Typhi ISP2825. N, negative control containing all PCR components except for *S.* Typhi genomic DNA.



C

ID	Age	Sex	AZM	*macA*	*acrB*
Toddlers (1-2 yrs)					
16	2	F	8	WT	WT
2	2	M	2	WT	WT
20	2	M	8	WT	WT
23	2	M	4	WT	WT
27	2	M	8	WT	WT
Pre-schoolers (3-5 yrs)					
33	3	F	4	WT	WT
9	3	M	4	WT	WT
17	3	M	2	WT	WT
31	3	M	8	WT	WT
40	4	F	4	WT	WT
12	4	F	2	WT	WT
1	5	M	8	WT	WT
School-age children (6-12 yrs)					
15	9	F	2	WT	WT
6	11	M	4	WT	WT
8	11	M	4	WT	WT
36	11	M	8	WT	WT
Adolescents (13-18 yrs)					
38	13	F	4	WT	WT
7	13	M	4	WT	WT

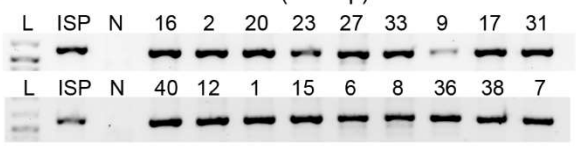
Figure 5. *macA* and *acrB* sequence analysis among XDR *S.* Typhi isolates. A-B, PCR reactions for *macA* (**A**) and *acrB* (**B**). *acrB* was split into two pieces for more productive PCR reaction outcomes (*acrB*-Nterm and *acrB*-Cterm). ISP, antibiotic-susceptible *S.* Typhi ISP2825. N, negative control containing all PCR components except for *S.* Typhi genomic DNA. See Table 3 for details. **C**, Summary of PCR amplicon sequencing analysis for *macA* and *acrB*. WT, wild type for the known mutations.

A

```
            181       190                 200
Ty2_AcrR    APQSFDLKKEARAYVTILLEMYQLCPTLRA
CT18_AcrR   APQSFDLKKEARAYVTILLEMYQLCPTLRA
SGB92_AcrR  APQSFDLKKRSSRLRHDPAGDVSIVSDAAR
403Ty_AcrR  APQSFDLKKRSSRLRHDPAGDVSIVSDAAR
            *********.:           .: .

            211   217                 238
Ty2_AcrR    STVNGSP--------------------
CT18_AcrR   STVNGSP--------------------
SGB92_AcrR  VDGQRLPLIIFQENSWTFSVSLFCLLQA
403Ty_AcrR  VDGQRLPLIIFQENSWTFSVSLFCLLQA
               :  *
```

B



C

ID	Age	Sex	AZM	*acrR*
Toddlers (1-2 yrs)				
16	2	F	8	WT
2	2	M	2	WT
20	2	M	8	WT
23	2	M	4	WT
27	2	M	8	WT
Pre-schoolers (3-5 yrs)				
33	3	F	4	WT
9	3	M	4	WT
17	3	M	2	WT
31	3	M	8	WT
40	4	F	4	WT
12	4	F	2	WT
1	5	M	8	WT
School-age children (6-12 yrs)				
15	9	F	2	WT
6	11	M	4	WT
8	11	M	4	WT
36	11	M	8	WT
Adolescents (13-18 yrs)				
38	13	F	4	WT
7	13	M	4	WT

Figure 6. AcrR sequence analysis among XDR *S.* Typhi isolates. A, AcrR amino acid sequence comparison analysis of the latest RefSeq dataset of the fully assembled complete genome of *S.* Typhi (107 in total) collected from NCBI as of Feb 26, 2021. Ty2, *S.* Typhi Ty2 (RefSeq assembly accession: GCF_000007545.1, assembly name: ASM754v1, strain: Ty2, submitter: University of Wisconsin). CT18, *S.* Typhi CT18 (RefSeq assembly accession: GCF_000195995.1, assembly name: ASM19599v1, strain: CT18, submitter: Sanger Institute). SGB92, *S.* Typhi SGB92 (RefSeq assembly accession: GCF_001121865.2, assembly name: 404Ty, strain name: SGB92, submitter: Wellcome Sanger Institute). 403Ty, *S.* Typhi 403Ty-sc-1979084 (RefSeq assembly accession: GCF_900205275.1, assembly name: 403Ty, isolate: 403Ty-sc-1979084, submitter: Wellcome Sanger Institute). See Table 5 for details. **B**, PCR reactions for *acrR*. ISP, antibiotic-susceptible *S.* Typhi ISP2825. N, negative control containing all PCR components except for *S.* Typhi genomic DNA. **C**, Summary of PCR amplicon sequencing analysis for *acrR*. WT, wild type for the known mutations.

Table 1. The minimum inhibitory concentration (MIC) results (μg/ml) of all samples used in this study, related to Fig. 1.

#	Age *	Sex	Wards **	AMP ***	SXT	CIP	CTX	CRO	PIP/TZB	AMC	AZM	IPM	MEM
1	5	M	E	≥32	≥4/76	≥64	8	32	32/4 - 64/4	≥32/16	8	0.25	0.25
2	2	M	I	≥32	≥4/76	32	8	≥64	4/4-128/4	8/4	2	0.5	0.25
3	4	F	E	≥32	≥4/76	≥64	≥64	32	32/4 - 64/4	16/8	8	0.5	0.25
4	10	M	I	≥32	≥4/76	32	32	8	32/4 - 64/4	16/8	4	0.5	0.25
5	11	M	M	≥32	≥4/76	8	≥64	≥64	32/4 - 64/4	≥32/16	4	0.25	0.5
6	11	M	C	≥32	≥4/76	≥64	32	≥64	4/4-128/4	8/4	4	0.25	0.5
7	13	M	E	≥32	≥4/76	≥64	≥64	32	4/4-128/4	4/2	4	0.25	0.5
8	11	M	E	≥32	≥4/76	32	≥64	≥64	≥128/4	≥32/16	4	0.5	0.5
9	3	M	C	≥32	≥4/76	8	≥64	≥64	4/4-128/4	4/2	4	0.25	0.5
10	4	M	E	≥32	≥4/76	≥64	32	≥64	≥128/4	≥32/16	4	0.5	0.5
11	4	F	G	≥32	≥4/76	≥64	32	32	≥128/4	≥32/16	2	0.5	0.5
12	4	F	E	≥32	≥4/76	≥64	8	≥64	32/4 - 64/4	16/8	2	0.5	0.25
13	5	M	O	≥32	≥4/76	32	≥64	32	≥128/4	16/8	2	0.5	0.5
14	4	M	I	≥32	≥4/76	≥64	≥64	≥64	≥128/4	16/8	8	0.5	0.5
15	9	F	O	≥32	≥4/76	32	32	≥64	4/4-128/4	8/4	2	0.5	0.5
16	2	F	O	≥32	≥4/76	≥64	≥64	≥64	≥128/4	16/8	8	0.5	0.25
17	3	M	E	≥32	≥4/76	≥64	≥64	32	32/4 - 64/4	≥32/16	2	0.25	0.25
18	6	M	C	≥32	≥4/76	≥64	≥64	32	32/4 - 64/4	16/8	8	0.25	0.25
19	8	M	C	≥32	≥4/76	≥64	≥64	≥64	≥128/4	16/8	8	0.25	0.25
20	2	M	W	≥32	≥4/76	32	≥64	32	32/4 - 64/4	16/8	8	0.25	0.25
21	6	M	W	≥32	≥4/76	≥64	32	≥64	≥128/4	≥32/16	2	0.25	0.25
22	5	M	E	≥32	≥4/76	≥64	≥64	32	32/4 - 64/4	≥32/16	8	0.5	0.25
23	2	M	I	≥32	≥4/76	≥64	≥64	≥64	32/4 - 64/4	16/8	4	0.5	0.5
24	7	M	MI	≥32	≥4/76	≥64	8	32	32/4 - 64/4	≥32/16	8	0.5	0.5
25	5	M	E	≥32	≥4/76	8	≥64	≥64	≥128/4	16/8	4	0.25	0.25
26	11	M	E	≥32	≥4/76	≥64	≥64	32	≥128/4	16/8	8	0.25	0.25
27	2	M	E	≥32	≥4/76	≥64	≥64	32	≥128/4	≥32/16	8	0.25	0.5
28	7	M	W	≥32	≥4/76	≥64	32	≥64	≥128/4	16/8	4	0.5	0.5
29	2	F	W	≥32	≥4/76	≥64	32	8	4/4-128/4	8/4	4	0.5	0.25
30	6	M	E	≥32	≥4/76	≥64	≥64	≥64	32/4 - 64/4	16/8	4	0.5	0.25
31	3	M	C	≥32	≥4/76	≥64	≥64	8	32/4 - 64/4	≥32/16	8	0.25	0.25
32	5	M	E	≥32	≥4/76	≥64	32	32	32/4 - 64/4	16/8	4	0.25	0.25
33	3	F	E	≥32	≥4/76	≥64	≥64	≥64	32/4 - 64/4	16/8	4	0.25	0.25
34	6	M	E	≥32	≥4/76	32	≥64	≥64	32/4 - 64/4	≥32/16	4	0.5	0.5
35	6	F	O	≥32	≥4/76	≥64	≥64	≥64	32/4 - 64/4	8/4	4	0.25	0.5
36	11	M	C	≥32	≥4/76	≥64	≥64	8	≥128/4	8/4	8	0.25	0.5
37	2	F	W	≥32	≥4/76	32	32	≥64	4/4-128/4	8/4	4	0.5	0.5
38	13	F	F	≥32	≥4/76	32	≥64	≥64	32/4 - 64/4	8/4	4	0.5	0.5
39	10	F	E	≥32	≥4/76	≥64	≥64	8	32/4 - 64/4	8/4	8	0.5	0.25

40	4	F	O	≥32	≥4/76	8	≥64	≥64	32/4 - 64/4	8/4	4	0.25	0.25
41	5	M	O	≥32	≥4/76	≥64	≥64	32	≥128/4	≥32/16	8	0.25	0.5
42	5	M	E	≥32	≥4/76	≥64	32	≥64	32/4 - 64/4	16/8	4	0.25	0.25
43	8	F	E	≥32	≥4/76	32	32	≥64	≥128/4	8/4	4	0.25	0.5
44	2	F	O	≥32	≥4/76	≥64	≥64	≥64	32/4 - 64/4	≥32/16	4	0.5	0.25
45	1	M	E	≥32	≥4/76	≥64	8	32	32/4 - 64/4	≥32/16	4	0.25	0.25

*, Age in years.

**, E, Peads Emergency; I, PAED-ICU; M, Male Medical; C, Clinical Laboratory; G, General OPD; O, Peads Medical OPD; W, Pead Medical Ward; MI, Pead Medical ICU; F, FMH-executive clinic.

***, Antibiotic acronym: AMP, ampicillin, SXT, trimethoprim-sulfamethoxazole, CIP, ciprofloxacin, CTX, cefotaxime, CRO, ceftriaxone, AZM, azithromycin, PIP, piperacillin, TZB, tazobactam, AMC, amoxicillin/ clavulanic acid, IPM, imipenem, MEM, meropenem.

54 **Table 2. Select of samples for molecular characterization, related to Figs 2-6.**

Lab No	Age (Yr)	Sex	AMP	SXT	CIP	CTX	CRO	PIP/TZB	AMC/CLA	AZM	IPM	MEM
			MDR		XDR							
Toddlers (1-2 yrs)												
16	2	F	R	R	R	R	R	I	I	S	S	S
2	2	M	R	R	R	R	R	S	S	S	S	S
20	2	M	R	R	R	R	R	I	I	S	S	S
23	2	M	R	R	R	R	R	I	I	S	S	S
27	2	M	R	R	R	R	R	I	I	S	S	S
Pre-schoolers (3-5 yrs)												
33	3	F	R	R	R	R	R	I	I	S	S	S
9	3	M	R	R	R	R	R	S	S	S	S	S
17	3	M	R	R	R	R	R	I	I	S	S	S
31	3	M	R	R	R	R	R	I	I	S	S	S
40	4	F	R	R	R	R	R	I	S	S	S	S
12	4	F	R	R	R	R	R	I	I	S	S	S
1	5	M	R	R	R	R	R	I	I	S	S	S
School-age children (6-12 yrs)												
15	9	F	R	R	R	R	R	S	S	S	S	S
6	11	M	R	R	R	R	R	S	S	S	S	S
8	11	M	R	R	R	R	R	I	I	S	S	S
36	11	M	R	R	R	R	R	I	S	S	S	S
Adolescents (13-18 yrs)												
38	13	F	R	R	R	R	R	I	S	S	S	S
7	13	M	R	R	R	R	R	S	S	S	S	S

55

56 **Table 3. PCR and sequencing primers and PCR conditions used in this study, related to Methods.**

Genes	Primers	Primer sequences (5'-3')	Annealing temp.	Extension time (min)	Amplicon size (bp)
pltB	Forward	TAAACCATGATAGACTGG	55℃	0.5	657
	Reverse	GAAAGTTACGGTTATACC			
	Sequencing	TAAACCATGATAGACTGG			
blaTEM1	Forward	AACCCTGGTAAATGCTTC	55℃	1	861
	Reverse	GTATATATGAGTAAACTTGG			
catA1	Forward	GAAGATCACTTCGCAGAATAA	45℃	1	964
	Reverse	CAGCAATAGACATAAGCG			
dhfR7	Forward	GCAACGTCAGAAAATGGC	60℃	0.5	405
	Reverse	AAACTGCTCAAAAAGGAAATTGA			
sul1	Forward	GTATTGCGCCGCTCTTAGAC	60℃	0.5	500
	Reverse	AGGGTTTCCGAGAAGGTGAT			
qnrS	Forward	TATAATGGTAGTCTAGCCC	52℃	1	722
	Reverse	GATGTGTGATTTTAAACG			
gyrA	Forward	CTTTGAATCCGGGATACAG	55℃	2	2726
	Reverse	CCTTTTTCTTGTCTATGGAA			
	Sequencing	CTTTGAATCCGGGATACAG			
gyrB	Forward	GAAAAGGGTAAAATAACGG	55℃	2	2415
	Reverse	CATCATGATGCCCTGGCCAG			
	Sequencing	GAATAAAACGCCGATCCAC			
parC	Forward	ATAGGGTATTATCTGCGGC	55℃	2.5	2283
	Reverse	GAATAAACAACGGTTTTACG			
	Sequencing	ATAGGGTATTATCTGCGGC			
parE	Forward	TGCACAGTTGCTGACAATC	55℃	2	1893
	Reverse	TCGGATTCTCTTATCCGGCCTG			
	Sequencing	CTGTGGCTGAACCAGAAC			
blaCTXM15	Forward	GATGTGCAGCACCAGTAAAG	52℃	0.5	549
	Reverse	AACGATATCGCGGTGATCTG			
macA	Forward	CTGTAAGCTGTGTCATGATCG	52℃	1	1308
	Reverse	CTCACATTGCACAGTTCAAGC			
	Sequencing	CTGTAAGCTGTGTCATGATCG			
acrB(1)	Forward	GGTTAAAGTGCAGGAAATTACCG	50℃	1.5	1695
	Reverse	CTACGCTATCGGTGTAGTGAT			
acrB(2)	Forward	GACGATGCTCAAACCCGT	50℃	1.5	1820
	Reverse	GCCAACTTTCCTAAGAAAAAGCC			
	Sequencing	GACTTCGAGTTGATTGACCA			
acrR	Forward	CACCGACATATGGCACGAA	52℃	1	633
	Reverse	CAGCGTCGGACACAATTGATA			
	Sequencing	GAAAGTTACGATCGGATTGA			

57

58 **Table 4. Sequencing results associated with fluoroquinolone resistance, related to Fig. 3.**

Genes	Mutations	References	**Findings**
gyrA	M52L	(14)	**Wild type**
	G81C	(16)	**Wild type**
	D82G	(16)	**Wild type**
	S83F/Y/L	(13, 20)	**S83F**
	D87Y/H/N/G/A	(13, 20, 48)	**Wild type**
	A119E	(16)	**Wild type**
gyrB	S464Y/F/T	(17)	**Wild type**
	Q465L		**Wild type**
	E466D		**Wild type**
	A468E		**Wild type**
parC	T57S	(18, 19)	**Wild type**
	G72S	(13)	**Wild type**
	G78D	(16)	**Wild type**
	D79G/R	(16, 18)	**Wild type**
	S80R/I	(16, 20)	**Wild type**
	G84G/K	(18, 20)	**Wild type**
	W106G	(15)	**Wild type**
parE	D420N	(20)	**Wild type**
	Y434S	(18)	**Wild type**
	S458P	(16)	**Wild type**

59

60 **Table 5. Details of the 107 completed *S.* Typhi genomes used in the study, related to Fig. 6.**

Accession number	Assembly number	BioProject	BioSample	BioSample ID	Serovar
GCF_000007545.1	ASM754v1	PRJNA371	SAMN02604095	2604095	Typhi
GCF_000195995.1	ASM19599v1	PRJNA236	SAMEA1705914	25445	Typhi
GCF_000245535.1	ASM24553v1	PRJNA80939	SAMN02603101	2603101	Typhi
GCF_000385905.1	ASM38590v1	PRJNA34855	SAMN02603210	2603210	Typhi
GCF_001048035.2	ERL103914	PRJEB3215	SAMEA2072815	2363281	Typhi
GCF_001048375.2	M223	PRJEB3215	SAMEA2156512	2372886	Typhi
GCF_001095585.2	ERL114000	PRJEB3215	SAMEA2072817	2363290	Typhi
GCF_001104165.2	E00-7866	PRJEB3215	SAMEA2072798	2363309	Typhi
GCF_001104885.2	10349_1#89_2	PRJEB3215	SAMEA2072799	2363310	Typhi
GCF_001118185.2	H12ESR00394-001A	PRJEB3215	SAMEA2150110	2379332	Typhi
GCF_001119245.2	76_1292	PRJEB3215	SAMEA1930246	2387556	Typhi
GCF_001121865.2	404Ty	PRJEB3215	SAMEA2072936	2363311	Typhi
GCF_001127485.2	ERL041834	PRJEB3215	SAMEA2072498	2363237	Typhi
GCF_001135805.2	ERL072973	PRJEB3215	SAMEA2072503	2363252	Typhi
GCF_001148125.2	ERL024120	PRJEB3215	SAMEA2072494	2363225	Typhi
GCF_001148305.2	ERL082356	PRJEB3215	SAMEA2072647	2363255	Typhi
GCF_001163025.2	H12ESR04734-001A	PRJEB3215	SAMEA2072794	2363303	Typhi
GCF_001165785.2	ERL024919	PRJEB3215	SAMEA2072495	2363228	Typhi
GCF_001302605.1	ASM130260v1	PRJNA286155	SAMN03765654	3765654	Typhi
GCF_001357935.2	80_2002	PRJEB3215	SAMEA1930249	2387559	Typhi
GCF_001360555.2	2010_7898	PRJEB3215	SAMEA2058419	2301303	Typhi
GCF_001362095.2	034151_4	PRJEB3215	SAMEA2072676	2363233	Typhi
GCF_001362135.2	11909_3	PRJEB3215	SAMEA2072788	2363294	Typhi
GCF_001362195.2	H12ESR00755-001A	PRJEB3215	SAMEA2072793	2363301	Typhi
GCF_001362335.2	Ty2	PRJEB3215	SAMEA2072934	2363305	Typhi
GCF_003429465.1	ASM342946v1	PRJNA398278	SAMN07507058	7507058	Typhi
GCF_003716995.1	ASM371699v1	PRJNA474465	SAMN09320528	9320528	Typhi
GCF_003717015.1	ASM371701v1	PRJNA474465	SAMN09320527	9320527	Typhi
GCF_003717035.1	ASM371703v1	PRJNA474465	SAMN09320526	9320526	Typhi
GCF_003717055.1	ASM371705v1	PRJNA474465	SAMN09320523	9320523	Typhi
GCF_003717075.1	ASM371707v1	PRJNA474465	SAMN09320522	9320522	Typhi
GCF_003717095.1	ASM371709v1	PRJNA474465	SAMN09320521	9320521	Typhi
GCF_003717115.1	ASM371711v1	PRJNA474465	SAMN09320520	9320520	Typhi
GCF_003717135.1	ASM371713v1	PRJNA474465	SAMN09320519	9320519	Typhi
GCF_003717215.1	ASM371721v1	PRJNA474465	SAMN09320518	9320518	Typhi
GCF_003717285.1	ASM371728v1	PRJNA474465	SAMN09320517	9320517	Typhi
GCF_003717355.1	ASM371735v1	PRJNA474465	SAMN09320516	9320516	Typhi
GCF_003717395.1	ASM371739v1	PRJNA474465	SAMN09320514	9320514	Typhi
GCF_003717435.1	ASM371743v1	PRJNA474465	SAMN09320513	9320513	Typhi
GCF_003717455.1	ASM371745v1	PRJNA474465	SAMN09320512	9320512	Typhi
GCF_003717475.1	ASM371747v1	PRJNA474465	SAMN09320511	9320511	Typhi
GCF_003717515.1	ASM371751v1	PRJNA474465	SAMN09320510	9320510	Typhi
GCF_003717535.1	ASM371753v1	PRJNA474465	SAMN09320509	9320509	Typhi

GCF_003717575.1	ASM371757v1	PRJNA474465	SAMN09320507	9320507	Typhi
GCF_003717615.1	ASM371761v1	PRJNA474465	SAMN09320564	9320564	Typhi
GCF_003717635.1	ASM371763v1	PRJNA474465	SAMN09320563	9320563	Typhi
GCF_003717655.1	ASM371765v1	PRJNA474465	SAMN09320562	9320562	Typhi
GCF_003717675.1	ASM371767v1	PRJNA474465	SAMN09320561	9320561	Typhi
GCF_003717695.1	ASM371769v1	PRJNA474465	SAMN09320560	9320560	Typhi
GCF_003717715.1	ASM371771v1	PRJNA474465	SAMN09320559	9320559	Typhi
GCF_003717735.1	ASM371773v1	PRJNA474465	SAMN09320558	9320558	Typhi
GCF_003717755.1	ASM371775v1	PRJNA474465	SAMN09320557	9320557	Typhi
GCF_003717775.1	ASM371777v1	PRJNA474465	SAMN09320556	9320556	Typhi
GCF_003717795.1	ASM371779v1	PRJNA474465	SAMN09320555	9320555	Typhi
GCF_003717815.1	ASM371781v1	PRJNA474465	SAMN09320554	9320554	Typhi
GCF_003717835.1	ASM371783v1	PRJNA474465	SAMN09320553	9320553	Typhi
GCF_003717855.1	ASM371785v1	PRJNA474465	SAMN09320552	9320552	Typhi
GCF_003717875.1	ASM371787v1	PRJNA474465	SAMN09320551	9320551	Typhi
GCF_003717895.1	ASM371789v1	PRJNA474465	SAMN09320550	9320550	Typhi
GCF_003717915.1	ASM371791v1	PRJNA474465	SAMN09320549	9320549	Typhi
GCF_003717935.1	ASM371793v1	PRJNA474465	SAMN09320548	9320548	Typhi
GCF_003717955.1	ASM371795v1	PRJNA474465	SAMN09320547	9320547	Typhi
GCF_003717975.1	ASM371797v1	PRJNA474465	SAMN09320546	9320546	Typhi
GCF_003717995.1	ASM371799v1	PRJNA474465	SAMN09320545	9320545	Typhi
GCF_003718015.1	ASM371801v1	PRJNA474465	SAMN09320544	9320544	Typhi
GCF_003718035.1	ASM371803v1	PRJNA474465	SAMN09320543	9320543	Typhi
GCF_003718055.1	ASM371805v1	PRJNA474465	SAMN09320542	9320542	Typhi
GCF_003718075.1	ASM371807v1	PRJNA474465	SAMN09320541	9320541	Typhi
GCF_003718095.1	ASM371809v1	PRJNA474465	SAMN09320540	9320540	Typhi
GCF_003718115.1	ASM371811v1	PRJNA474465	SAMN09320539	9320539	Typhi
GCF_003718135.1	ASM371813v1	PRJNA474465	SAMN09320538	9320538	Typhi
GCF_003718155.1	ASM371815v1	PRJNA474465	SAMN09320537	9320537	Typhi
GCF_003718175.1	ASM371817v1	PRJNA474465	SAMN09320536	9320536	Typhi
GCF_003718195.1	ASM371819v1	PRJNA474465	SAMN09320535	9320535	Typhi
GCF_003718235.1	ASM371823v1	PRJNA474465	SAMN09320533	9320533	Typhi
GCF_003718255.1	ASM371825v1	PRJNA474465	SAMN09320532	9320532	Typhi
GCF_003718275.1	ASM371827v1	PRJNA474465	SAMN09320531	9320531	Typhi
GCF_003718295.1	ASM371829v1	PRJNA474465	SAMN09320530	9320530	Typhi
GCF_003718315.1	ASM371831v1	PRJNA474465	SAMN09320529	9320529	Typhi
GCF_003718355.1	ASM371835v1	PRJNA474465	SAMN09320578	9320578	Typhi
GCF_003718375.1	ASM371837v1	PRJNA474465	SAMN09320577	9320577	Typhi
GCF_003718395.1	ASM371839v1	PRJNA474465	SAMN09320576	9320576	Typhi
GCF_003718415.1	ASM371841v1	PRJNA474465	SAMN09320575	9320575	Typhi
GCF_003718435.1	ASM371843v1	PRJNA474465	SAMN09320574	9320574	Typhi
GCF_003718455.1	ASM371845v1	PRJNA474465	SAMN09320573	9320573	Typhi
GCF_003718475.1	ASM371847v1	PRJNA474465	SAMN09320572	9320572	Typhi
GCF_003718495.1	ASM371849v1	PRJNA474465	SAMN09320571	9320571	Typhi
GCF_003718515.1	ASM371851v1	PRJNA474465	SAMN09320570	9320570	Typhi
GCF_003718535.1	ASM371853v1	PRJNA474465	SAMN09320569	9320569	Typhi
GCF_003718555.1	ASM371855v1	PRJNA474465	SAMN09320568	9320568	Typhi
GCF_003718575.1	ASM371857v1	PRJNA474465	SAMN09320567	9320567	Typhi
GCF_003718595.1	ASM371859v1	PRJNA474465	SAMN09320566	9320566	Typhi

GCF_003718615.1	ASM371861v1	PRJNA474465	SAMN09320565	9320565	Typhi
GCF_003718635.1	ASM371863v1	PRJNA474465	SAMN09320525	9320525	Typhi
GCF_003718655.1	ASM371865v1	PRJNA474465	SAMN09320506	9320506	Typhi
GCF_003719215.1	ASM371921v1	PRJNA474465	SAMN09320534	9320534	Typhi
GCF_003719235.1	ASM371923v1	PRJNA474465	SAMN09320508	9320508	Typhi
GCF_003719255.1	ASM371925v1	PRJNA474465	SAMN09320515	9320515	Typhi
GCF_003719555.1	ASM371955v1	PRJNA471337	SAMN09208111	9208111	Typhi
GCF_004136335.1	ASM413633v1	PRJNA480202	SAMN09630442	9630442	Typhi
GCF_005885835.1	ASM588583v1	PRJNA543969	SAMN11792777	11792777	Typhi
GCF_900185485.1	BL60006	PRJEB21155	SAMEA104109193	7190870	Typhi
GCF_900205255.1	1554	PRJEB5919	SAMEA3109638	3338098	Typhi
GCF_900205265.1	lupe_GEN0059_5	PRJEB5919	SAMEA2564024	3071773	Typhi
GCF_900205275.1	403Ty	PRJEB5919	SAMEA2564027	3071775	Typhi
GCF_900205295.1	E98-3139	PRJEB5919	SAMEA2467787	3000319	Typhi
GCF_901457615.1	ERS3381924	PRJEB32272	SAMEA5577690	11516613	Typhi

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