Typhoid in Kenya is associated with a dominant multidrug resistant *Salmonella Typhi* haplotype that is also widespread in South East Asia.

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**Running title:** Multidrug-resistant haplotype H58 *S. Typhi* in Kenya

**Key words:** Multidrug-resistant haplotype H58, typhoid, decreased ciprofloxacin susceptibility, Kenya

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Abstract

In sub-Saharan Africa the burden of typhoid fever, caused by *Salmonella enterica* serovar Typhi (*S. Typhi*) remains largely unknown, in part because of a lack of blood or bone marrow culture facilities. We characterised a total of 323 *S. Typhi* isolated from outbreaks in Kenya over the period 1988-2008 for antimicrobial susceptibility, and phylogenetic relationship using single nucleotide polymorphism (SNP) analysis. There was a dramatic increase in the number and percentage of multidrug-resistant (MDR) *S. Typhi* over the study period. Overall, only 54 (16.7%) of *S. Typhi* were fully sensitive while the majority 195 (60.4%) were multiply resistant to most commonly available drugs - ampicillin, chloramphenicol, tetracycline and co-trimoxazole 74 (22.9%) isolates were resistant to a single antimicrobial, usually ampicillin, cotrimoxazole or tetracycline.

Resistance to these antibiotics was encoded on self-transferrable IncHI1 plasmids of the ST6 sequence type. Of the 94 representative *S. Typhi* selected for genome-wide haplotype analysis sensitive isolates fell into several phylogenetically different groups whereas MDR isolates all belonged to a single haplotype, H58, associated with MDR and decreased ciprofloxacin susceptibility which is also dominant in many parts of SE Asia. Derivatives of the same *S. Typhi* lineage, H58, are responsible for multidrug resistance in Kenya and parts of SE Asia, suggesting intercontinental spread of a single MDR clone. Given the emergence of this aggressive MDR haplotype, careful selection and monitoring of antibiotic usage will be required in Kenya and potentially other regions of sub-Saharan Africa.
Introduction

Typhoid fever, caused by *Salmonella enterica* serovar Typhi (*S*. *Typhi*) is an important disease in many developing countries. It is estimated that there are approximately 22 million typhoid cases and ~200,000 deaths per year world-wide (10). However, the true global distribution of typhoid fever is not well documented. For example, in Africa the overall burden of typhoid fever remains largely unknown, mainly because facilities capable of performing the blood culture tests essential for diagnosis are absent from many regions. Some local estimates on typhoid incidence in different African regions have been made. Typhoid incidence rates of 39/100,000 and 59/100,000 have been reported for Kenya/East Africa and Egypt, respectively (10, 28), but these figures may be an under-estimate due to under reporting as only severely ill patients seek treatment in hospital. In other studies, Weeramanthri *et al*. (30) observed that over a 5-year period typhoid remained a common cause of septicemic illness in The Gambia, while in Nigeria (2) and Ghana (6) cases of ileal perforation due to typhoid were documented.

Problems are also emerging with the clinical treatment of typhoid in resource poor settings. For many years, the antibiotics chloramphenicol, ampicillin, and co-trimoxazole formed the mainstay of typhoid treatment. However, outbreaks of MDR *S*. *Typhi* (20, 24, 25) prompted the widespread use of fluoroquinolones, such as ciprofloxacin and ofloxacin. Fluoroquinolone usage was followed by the emergence of nalidixic acid resistant *S*. *Typhi* exhibiting reduced susceptibility to fluoroquinolones in the early 1990s (18, 22) and these have since become widespread (1, 12, 16, 19, 25). Thus, the spread of MDR and fluoroquinolone resistance in *S*. *Typhi* is presenting significant clinical challenges.

Better methods for monitoring the emergence and spread of MDR *S*. *Typhi* would facilitate disease control and treatment. However, this monophyletic (clonal) pathogen presents particular challenges.
in this regard. Studies on the population structure of *S. Typhi* have shown that this human adapted pathogen exhibits extremely limited genetic variation, challenging our ability to develop discriminatory tools of value in the field (4, 11, 25, 27). However, the application of novel deep sequencing and bioinformatic approaches have succeeded in stratifying the *S. Typhi* population into distinct phylogenetic lineages based on over 1,000 single nucleotide polymorphisms (SNPs) distributed throughout the chromosome. Typing of these chromosomal SNPs allows isolates from typhoid patients to be mapped to specific points on the phylogenetic tree of *S. Typhi* (11, 27). This provides an unequivocal test of the genetic relatedness of multiple *S. Typhi* isolates, which can be inferred from their relative positions in the phylogenetic tree. In particular, isolates sharing identical haplotypes, mapping to the leaf nodes of the *S. Typhi* phylogenetic tree, are deemed to be very closely related even if they are isolated in widely different geographical locations.

In Kenya, MDR *S. Typhi* isolated from adults and school-age children associated with sporadic outbreaks in resource-poor settings especially in slum areas have been reported (13, 15). Here, we analysed a collection of 323 *S. Typhi* isolated at three hospitals in Nairobi, Kenya between 1988 and 2008 for their population structure. We used a novel SNP typing method capable of simultaneously interrogating ~1500 points of potential variation on the *S. Typhi* genome in a single DNA sample. Using this powerful high through-put approach we show that a particular MDR-associated haplotype H58, previously shown to be widespread in several countries in Asia, has become dominant, replacing more divergent, antimicrobial-susceptible *S. Typhi*.

**Materials and Methods**

**Patients.** Adults and children reporting to various hospitals in Nairobi with fever and referred for blood culture investigations were included in this study. These were routine self-reported endemic typhoid cases from around Nairobi province detected through routine outpatient care and treatment
procedure. The Centre for Microbiology Research, KEMRI, conducted this hospital–based periodic surveillance for blood-culture confirmed typhoid cases in the National Referral Hospital and two other private hospitals that together have a bed-capacity of 3,240. During the study period a total of 6,750 patients presenting with fever were seen by clinician on duty and recommended for blood culture for confirmation of diagnosis. Blood cultures were performed at each hospital’s Microbiology Laboratory and isolates identified as *S*. Typhi were confirmed at the KEMRI laboratory and archived for further analysis. For ease of analysis, surveillance periods were divided into three phases: 1988-1993, 1994-2000 and 2001-2008 during which periods we conducted funded surveillance studies. The study was approved by the National Ethical Review Board (Kenya).

**Bacterial isolation.** At the respective hospital laboratory blood for culture was obtained from patients with suspected typhoid and before antibiotic treatment was commenced. Blood was cultured in broth media containing brain heart infusion and para-aminobenzoic acid, incubated at 37°C and sub-cultured when turbid onto sheep blood agar and MacConkey plates. Bacterial isolates were identified by biochemical tests using API 20E strips (bioMerieux, Basingstoke, UK) and serotyped using agglutinating antisera (Murex Diagnostics, Dartford, UK). *S*. Typhi isolates were stored at -80°C on protect beads (Technical Service Consultants Ltd, Heywood, UK) until analysed.

**Antimicrobial susceptibility testing.** *S*. Typhi isolates were tested for susceptibility to antimicrobials initially by a controlled disk diffusion technique on Diagnostic Sensitivity Testing (DST) agar (Oxoid Ltd., Basingstoke, UK) plates containing 5% lysed horse blood. The antibiotic disks (all from Oxoid) contained ampicillin (10µg), tetracycline (30µg), co-trimoxazole (25µg), chloramphenicol (30µg), gentamicin (10µg), co-amoxyclov (20:10µg), ciprofloxacin (5µg), ceftriaxone (30µg) and nalidixic acid (30µg). Minimum inhibitory concentrations (MICs) of these
antibiotics were determined using the E-test strips (AB Biodisk, Solna, Sweden) according to the manufacturer’s instructions. *Escherichia coli* ATCC 25922 (with known MICs) was used as a control for potency of antibiotics. Disk sensitivity tests and MICs were interpreted according to the guidelines provided by the Clinical and Laboratory Standards Institute (3). **Isolates resistant to two or more of the antimicrobials tested were categorised as multidrug resistant while an MIC of 0.1-1 µg/mL was defined as decreased ciprofloxacin susceptibility (DCS)** (9).

**Determination of chromosomal haplotype.** A total of 98 isolates representative of both sensitive and MDR (resistant to 2 or more antimicrobials tested) phenotypes from across the three study periods were selected (for additional typing see Supplementary Figure 1). The chromosomal haplotype of *S. Typhi* isolates was determined based on the SNPs present at 1,485 chromosomal loci identified previously (11, 27) [see also Supplementary Methods and Supplementary Table]. These and eight IncHI1 plasmid loci identified previously (25) were investigated using a GoldenGate custom panel according to the manufacturer’s standard protocols (Illumina), [see Supplementary Methods]. A maximum-likelihood phylogenetic tree based on chromosomal SNPs was constructed using the RAxML software (29).

**Mating experiments and incompatibility grouping.** Mating experiments were performed as described previously (15). Plasmid DNA extraction was performed using a Plasmid Mini Prep Kit (Qiagen Ltd., West Sussex, UK) according to manufacturer’s instructions. Plasmid molecular sizes were determined by co-electrophoresis with plasmids of known molecular sizes from *E. coli* strains V517 (35.8, 4.8, 3.7, 2.6, 2.0, 1.8, 1.4 MDa) and 39R861 (98, 42, 24, 4.6 MDa). PCR-based replicon typing was used to identify the FIA, FIB, FIC, HI1, HI2 and FIIA replicons, which represent the major plasmid incompatibility groups circulating in *S. Typhi*, using primers developed by (5). Reaction conditions consisted of 50 ng of plasmid DNA and 100 nM concentrations of each primer.
in a buffer composed of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, a 200 µM concentration of dNTP mixture and 1 U of Taq polymerase in a final volume of 50µl.

Amplification conditions were 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension step of 72°C for 10 min. PCR products were resolved by electrophoresis on 1.2% gels at 120 V run for 1 h. Plasmid DNA preparations from MDR S. Typhi and total DNA from five fully sensitive isolates (negative controls) were used as a template for PCR.

**PCR and sequencing to detect mutations leading to reduced susceptibility.** All nalidixic acid-resistant S. Typhi strains were selected for amplification of the quinolone resistance determining region (QRDR) in the chromosome with four primer pairs as described previously (15). In addition to detect plasmid-encoded quinolone resistance, we applied qnr-gene primers, QP1 (5’-ATAAAGTTTTTCAGCAAGAGG; starting at the 12th qnr nucleotide) and QP2 (5’-ATCCAGATCGGCAAAGGTATA), to detect qnrA gene on plasmids (length, 657 bp). For qnrB, primers FQ1 (5’-ATGACGCCATTACTGTATAA) and FQ2 (5’GATCGCAATGTGTGAAGTTA) were used (13). Two sets of primers, aac(6')-1b-cr 1 (aac(6')-1b-cr1 F (5’ATATGCGGATCCAATGAGCAACGACAAAACAAAGTTAG3’) aac(6')-1b-cr1 R (5’ATATGCGAATTCTTTACATC3’), aac(6')-1b-cr2 F (5’TTGCAATGCTGAATGGAGAG3’) and aac(6')-1b-cr 2 (5’TTGCAATGCTGAATGGAGAG3’), aac(6')-1b-cr2 R (5’TGTTTGGATCCTTACCT3’) were used to detect a ciprofloxacin acetylation enzyme designated aac(6')-1b-cr (confers low level ciprofloxacin resistance, MIC=0.25 µg/ml) and located on a mobile transposon. Amplification conditions consisted of 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension step of 72°C for 10 min.

Sequence determination was performed using the PCR primers for both strands of the amplicons with a dideoxy-chain termination method using an automated DNA sequencer ABI PRISM 377 (Perkin-Elmer, Warrington, United Kingdom) and was analyzed using commercial software.
1 (Lasergene; DNASTar Inc., Madison, Wis.).
Results

Trends in antimicrobial susceptibility of S. Typhi isolated in Nairobi between 1988-2008

A total of 323 S. Typhi isolates from three hospitals covering the Nairobi region of Kenya during the period 1988-2008 were included in this study. Of these, 54 (16.7%) isolates were fully susceptible to all eight antibiotics tested (see Methods) and over half of these sensitive isolates were obtained during the first part of the surveillance period (1988-1993) (see Table 1 and Supplementary Figure 1). A total of 74 (22.9%) isolates were resistant to only one antimicrobial (usually ampicillin, cotrimoxazole or tetracycline). In contrast, 195 (60.4%) S. Typhi were MDR, but only 5 (2.6%) of these were isolated during this first phase (1988-1993). Indeed, there was a significant peak of MDR S. Typhi isolated between 2001 and 2006 and, although sensitive S. Typhi were isolated throughout the whole study period, they were dominated by MDR isolates after 2001.

The majority of MDR isolates (78%) were uniformly resistant to ampicillin, chloramphenicol and tetracycline (MICs of >256 µg/ml), streptomycin (MIC of >1024 µg/ml) and co-trimoxazole (MIC of >32 µg/ml), which are among the most readily available antibiotics in Kenya. Thus, overall there was a significant trend towards increasing levels of resistance to antibiotics over the period of study (Table 1 and Supplementary Figure 1). In addition, the proportion of S. Typhi isolates resistant to nalidixic acid (MICs > 16 µg/ml) also increased significantly from 8 (5.6%) during the period 1994-2000 to 25 (18.4%) during the period 2001-2008 (p-value <0.01). Of these nalidixic acid resistant S. Typhi a total of 16 (64.0%) isolates from the 2001-2008 surveillance period showed decreased ciprofloxacin susceptibility (DCS) (MIC= 0.1-1 µg/ml) (Table 2).

Antimicrobial resistance in S. Typhi isolates

Previous studies have shown that MDR in S. Typhi is predominantly associated with the acquisition of large transmissible IncHI1 plasmids (25). All MDR S. Typhi isolates examined in this study
contained plasmids ~100-110 MDa in size regardless of the period of study and 72 had one or two additional plasmids of 4 to 10 kb. Fully sensitive S. Typhi did not contain any detectable plasmids.

Of the 269 antibiotic-resistant S. Typhi isolates (resistant to a single agent and MDR combined), 121 (45%) that were resistant to a combination of ampicillin, chloramphenicol, tetracycline and co-trimoxazole co-transferred these resistance phenotypes to *E. coli* K12 by *in-vitro* conjugation.

Additionally, all the MDR S. Typhi isolates were positive for the predicted 365 bp PCR product (data not shown) expected for IncHI1 plasmids (15). The 35 S. Typhi that exhibited nalidixic acid resistance (MICs > 16 µg/ml, compared to fully sensitive strains, MICs = 1-4 µg/ml) which included 19 isolates with DCS (MIC=0.1-1.0 µg/ml) were investigated for the presence of SNPs associated with resistance in the *gyrA*, *gyrB*, *parC*, and *parE* genes and for presence of plasmid-mediated resistance to fluoroquinolones. All isolates that were resistant to nalidixic acid (MICs > 16 µg/ml) or exhibited DCS harboured mutations in *gyrA* resulting in amino acid substitution, 83 Serine (TCC) to Leucine (TTG). A total of 16 of the 35 nalidixic acid resistant strains (MIC = 16-20 µg/ml) had MICs of ciprofloxacin below DCS range (0.02-0.1 µg/ml). None of the nalidixic acid-resistant S. Typhi isolates showed any significant point mutations in *gyrB*, *parC* or *parE*.

Further, none of the quinolone- or fluoroquinolone-resistant strains harboured plasmid-mediated quinolone resistance genes detectable in our PCR assays.

**Phylogenetic structure of the S. Typhi population**

In order to determine the phylogenetic relationships between S. Typhi isolates from Kenya and elsewhere a selection of 94 Kenyan isolates (representing both susceptible and antibiotic resistant isolates from the study periods – see Supplementary Figure 1) were subjected to SNP typing at 1,485 different chromosomal loci. These SNPs were discovered within a global S. Typhi collection (11, 27) and are distributed throughout the S. Typhi chromosome, providing unequivocal mapping features. The SNP typing was performed using a novel approach based on small aliquots of DNA
purified from individual isolates, analysed by GoldenGate assay (Illumina). The assay determines SNP alleles at hundreds of sites within a single sample and has been successfully used for genotyping human DNA (11, 27). We adapted the GoldenGate platform to investigate SNPs in S. Typhi DNA, requiring some modifications to standard data analysis methods (see Methods and Supplementary Methods). SNP typing assigned the S. Typhi isolates to eight different haplotypes, with H58 being dominant. Figure 1a shows the distribution of Kenyan isolates on the S. Typhi phylogenetic tree defined by sequenced control strains (27). The majority of isolates (71; 75.5%) were H58, which is known to be the dominant haplotype associated with MDR in Asia (4, 11, 25). The proportion of H58 S. Typhi isolates increased from 11/23 (47.8%) in the period 1988-1998, to 62/71 (87.3%) in 2001-2008 (Figure 1b). The other haplotypes were represented by fewer than eight isolates in each case, which were predominantly drug sensitive isolates (Figures 1a and 1b). Of the 94 S. Typhi isolates that were subjected to SNP analysis, 63 (67%) were MDR and all of these were H58. Each of the MDR S. Typhi carried an IncHI1 plasmid as outlined above. Since IncHI1 plasmid SNPs (25) were included in the SNP typing assays we determined that all of the MDR S. Typhi isolates harboured IncHI1 plasmids of the same subtype, ST6 (Figure 2b). We have recently identified a number of SNPs that allow us to discriminate further within the H58 lineage (11). Using these SNPs H58 isolates could be further subdivided at eight variable SNP loci; four different subtypes were identified, including two distinct lineages I and II (Figure 2a). Lineage II was detected throughout the study period. However, lineage I was only detected between 2004-2008, during which time it comprised 45.8% of H58 isolates (Figure 2b). Interestingly, all the nalidixic acid resistant isolates were H58, and resistance was significantly more common among lineage II H58 isolates than lineage I H58 isolates (90% versus 23%, respectively (p-value < 0.01). DSC isolates (MIC=0.1-1.0 µg/ml) were also more common among H58 lineage II (24%) compared to H58 lineage I (9.1%) or non-H58 isolates (8%).
Discussion

In this report we document the first DNA sequence-based analysis of *S. Typhi* associated with typhoid in an endemic region within the African continent. Our data unequivocally proves that a rise in the incidence of MDR *S. Typhi* throughout the study period is associated with a single bacterial haplotype, H58, which has also undergone expansion in South East Asia (4, 25). Thus, a single *S. Typhi* haplotype has now been linked to the spread of the MDR genotype on two continents. H58 is currently detected to be the phylogenetic lineage of *S. Typhi* responsible for MDR in Kenya and possibly globally, suggesting that the current wave of MDR *S. Typhi* may be spreading as a single clone to different parts of the world driven by antibiotic usage. Intriguingly, H58 isolates appear to be capable of acquiring an MDR genotype, including the rise in DCS phenotype, while remaining fit and competitive. At present we do not know why this is but it is an area worthy of further investigation, potentially serving as a study model for other aggressive MDR lineages in other pathogens. Interestingly, H58 is the dominant MDR *S. Typhi* haplotype currently circulating in Pakistan and India (our unpublished data), which have strong cultural links with Kenya and it is tempting to speculate that these isolates may have arrived in Africa as a consequence of such links. All the H58 MDR *S. Typhi* examined in this study contained large self-transmissible IncHI1 plasmids. Similar MDR IncHI1 plasmids carrying resistance to nearly all commonly available antibiotics have been characterized in *S. Typhi* from various countries in Asia including Vietnam (7), India (12), and Pakistan (21). It is significant that all the Kenyan IncHI1 plasmids belonged to the ST6 lineage, again supporting their highly clonal nature. This data is in agreement with previous studies (4, 11, 27).

Our SNP typing approach is based on simultaneously measuring nucleotide changes at 1,485 sites distributed throughout the chromosome and IncHI1 plasmids of *S. Typhi*. Using this technology we previously identified novel H58-associated SNPs that allow discrimination within this haplotype.
(Figure 2a and (11)). Analysis using this subset of SNPs identified two distinct H58 lineages causing disease in Kenya that are acquiring DCS independently. The identification of these ‘local’ SNPs will greatly facilitate studies in the field and could potentially be exploited to identify transmission routes within the region since they provide an unequivocal identity tag for particular isolates.

Although in most endemic parts of Asia and the Indian subcontinent typhoid outbreaks caused by MDR S. Typhi have been well characterized (1, 12) outbreaks in sub-Saharan Africa are rarely documented and data on incidence and antimicrobial susceptibility patterns are scarce. The observed rise in MDR S. Typhi in Kenya is particularly alarming. For example, during the period of our study most of the S. Typhi (73.3%) isolated from blood cultures of patients prior to 1993 were fully sensitive to all antimicrobials. Data from the last 7 years shows that 77.2% of the S. Typhi are MDR, much higher than the reported 52% and 29% prevalence for MDR S. Typhi in Ghana (20) and Egypt (28), respectively. It is, however, close to the high prevalence of MDR S. Typhi noted in South Africa (8) and Nigeria (2) where 65% and 61% isolates were MDR, respectively. In addition in our studies in Nairobi the proportion of S. Typhi that are MDR and also resistant to nalidixic acid has risen from 5.6% in 2000 (13) to 18.4% in 2008. The increased prevalence of MDR S. Typhi strains also resistant to nalidixic acid and with DCS should serve as a warning that soon full fluoroquinolone resistance may emerge that renders these drugs ineffective, as has happened in South East Asia (1, 7, 24, 26) and in parts of West Africa (20, 23) where even moderate rises in MICs has led to clinical treatment failure. Even in non-endemic settings in the USA, patients infected with S. Typhi isolates with similar antibiotic resistance phenotypes show evidence of a longer time to fever clearance and more frequent treatment failure (9). A total of 16 of 35 isolates that were nalidixic acid resistant (MIC = 16-20µg/ml) did not fit into the DCS category but none-the-less had the Ser83Leu gyrA region. The reason for continued susceptibility to ciprofloxacin
resistance in the presence of mutations that are linked to reduced susceptibility is not currently known, but may be associated with other compensating mutations elsewhere in the genome.

The major concern in Kenya has been the increasing rate of over-the-counter sale without a prescription of fluoroquinolones and indeed all other first-line antibiotics for typhoid fever, particularly as a result of poor diagnosis of suspected cases that report to privately owned clinics that abound in the poor informal settlements (slums). Our sentinel surveillance programmes involved patients treated at the main referral hospital and two other private facilities in Nairobi, Kenya, since 1988. The majority of these typhoid cases came from slum areas near the capital city, Nairobi. During our study fewer isolates were obtained during phase 1 (1988-1993) compared to the subsequent two phases of surveillance (35 isolates versus 152 and 136, respectively). It is likely that due to the rapid growth of the slums, as more people moved into the city looking for job opportunities, the limited resources such as water and sanitary services became overwhelmed which may have contributed to increased rates of typhoid fever. Currently these slums are overcrowded, with a population rise from approximately 300,000 in 1988 to nearly 600,000 in 2007 (14, 17), virtually non-existent sewage disposal. Thus, the possibility of hygiene-related disease outbreaks including typhoid remains high.

In conclusion, it appears that a rapidly expanding multidrug and nalidixic acid resistant S. Typhi clone of the H58 haplotype, commonly associated with outbreaks in South East Asia (18, 27), is now commonly isolated in Kenyan typhoid cases. As humans are the only source of infection, and transmission of S. Typhi is by the faecal-oral route through contaminated water or food, prevention measures need to include provision of clean water and sanitation improvements, as well as health education. However, given the very real potential for MDR fluoroquinolone-resistant typhoid outbreaks in Kenya, we propose a more comprehensive approach to prevention of this disease
needs to be considered. A combination of targeted vaccination of high risk populations as a short- to medium-term measure, with the longer term solutions of water and sanitation improvements and elevated living standards, may be the best strategy.

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Table 1. Distribution of drug resistance phenotypes among *S. Typhi* isolated from three surveillance periods.

<table>
<thead>
<tr>
<th>Period of isolation</th>
<th>No. (%) fully susceptible</th>
<th>No. (%) single resistance</th>
<th>No. (%) MDR</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988-1993</td>
<td>33 (73.3)</td>
<td>7 (15.6)</td>
<td>5 (11.1)</td>
<td>45 (13.9)</td>
</tr>
<tr>
<td>1994-2000</td>
<td>11 (7.7)</td>
<td>46 (32.3)</td>
<td>85 (60)</td>
<td>142 (44.0)</td>
</tr>
<tr>
<td>2001-2008</td>
<td>10 (7.4)</td>
<td>21 (15.4)</td>
<td>105 (77.2)</td>
<td>136 (42.1)</td>
</tr>
</tbody>
</table>

Table 2. Trends in resistance to quinolones during the study period

<table>
<thead>
<tr>
<th>Period of isolation</th>
<th>No. Nal(^R) (%)</th>
<th>No. (%) DCS of Nal(^R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988-1993 (N=45)</td>
<td>2 (4.4)</td>
<td>0</td>
</tr>
<tr>
<td>1994-2000 (N=142)</td>
<td>8 (5.6)</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>2001-2008 (N=136)</td>
<td>25 (18.4)</td>
<td>16 (64)</td>
</tr>
</tbody>
</table>

Decreased Ciprofloxacin Susceptibility (DCS): MIC = 0.1-1.0 µg/mL

Nal\(^R\) (nalidixic acid resistant): MIC > 16 µg/mL
Figure Legends

Figure 1. Distribution of S. Typhi haplotypes identified in Kenya. (a) Radial phylogram based on 1,485 SNPs originally detected among 19 whole genome sequences of S. Typhi (11). Black nodes: sequenced control isolates. Coloured nodes: 94 Kenyan isolates (colours randomly assigned to nodes), scaled to reflect the number of isolates found, precise number is given in brackets. (b) Distribution of haplotypes of Kenyan S. Typhi by year of isolation. Colours indicate S. Typhi haplotype as defined in (a).

Figure 2. Subtypes of Kenyan S. Typhi isolates of haplotype H58. (a) Radial phylogram of the H58 cluster of S. Typhi; dashed line indicates where this phylogram links back to the broader S. Typhi phylogram shown in Figure 1. Black nodes: genotyped control isolates including seven sequenced genomes (labeled with strain names, see ref 11) or previously defined (labeled with H groups, see ref 27). Coloured nodes: nodes to which Kenyan isolates were mapped by SNP typing (colours randomly assigned to nodes), labeled with letters. Pale circles highlight two distinct H58 lineages, I and II. (b) Distribution of H58 subtypes of Kenyan S. Typhi by year of isolation, split by presence of MDR IncHI1 plasmids (note than no non-H58 isolates contained plasmids). Legend labels and colours correspond to those defined in (a).
References


