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Typhoid in Kenya Is Associated with a Dominant Multidrug-Resistant *Salmonella enterica* Serovar Typhi Haplotype That Is Also Widespread in Southeast Asia▽†

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In sub-Saharan Africa, the burden of typhoid fever, caused by *Salmonella enterica* serovar Typhi, remains largely unknown, in part because of a lack of blood or bone marrow culture facilities. We characterized a total of 323 *S.* Typhi isolates from outbreaks in Kenya over the period 1988 to 2008 for antimicrobial susceptibilities and phylogenetic relationships using single-nucleotide polymorphism (SNP) analysis. There was a dramatic increase in the number and percentage of multidrug-resistant (MDR) *S.* Typhi isolates over the study period. Overall, only 54 (16.7%) *S.* Typhi isolates were fully sensitive, while the majority, 195 (60.4%), were multiply resistant to most commonly available drugs—ampicillin, chloramphenicol, tetracycline, and cotrimoxazole; 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 isolates 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 Southeast Asia. Derivatives of the same *S.* Typhi lineage, H58, are responsible for multidrug resistance in Kenya and parts of Southeast 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.

Typhoid fever, caused by *Salmonella enterica* serovar 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 worldwide (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 of typhoid incidences 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 underestimates due to underreporting, as only severely ill patients seek treatment in hospitals. 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 (5), 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 cotrimoxazole formed the mainstays of typhoid treatment. However, outbreaks of multidrug-resistant (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 it has since become widespread (1, 12, 16, 19, 25). Thus, the spread of MDR and fluoroquinolone resistance in *S.* Typhi presents 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 (3, 11, 25, 27). However, the application of novel deep-sequencing and bioinformatics approaches has 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, iso-

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lates 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 isolates 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 analyzed a collection of 323 *S*. Typhi isolates from three hospitals in Nairobi, Kenya, between 1988 and 2008 for their population structure. We used a novel SNP-typing method capable of simultaneously interrogating ~1,500 points of potential variation on the *S*. Typhi genome in a single DNA sample. Using this powerful high-throughput approach, we show that a particular MDR-associated haplotype, H58, previously shown to be widespread in several countries in Asia, has become dominant in Kenya, replacing more divergent antimicrobial-susceptible *S*. Typhi strains.

MATERIALS AND METHODS

Patients. Adults and children who reported to various hospitals in Nairobi with fever and who were 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 procedures. 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 who presented with fever were seen by clinicians 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 to 1993, 1994 to 2000, and 2001 to 2008—during which we conducted funded surveillance studies. The study was approved by the National Ethical Review Board (Kenya).

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

Antimicrobial susceptibility testing. *S.* Typhi isolates were initially tested for susceptibility to antimicrobials by a controlled disk diffusion technique on Diagnostic Sensitivity Testing (DST) agar (Oxoid Ltd., Basingstoke, United Kingdom) plates containing 5% lysed horse blood. The antibiotic disks (all from Oxoid) contained ampicillin (10 μg), tetracycline (30 μg), cotrimoxazole (25 μg), chloramphenicol (30 μg), gentamicin (10 μg), amoxicillin-clavulanic acid (20:10 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), and nalidixic acid (30 μg). The MICs of these antibiotics were determined using Etest strips (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. *Escherichia coli* ATCC 25922 (with known MICs) was used as a control for the potencies of antibiotics. Disk sensitivity tests and MICs were interpreted according to the guidelines provided by the Clinical and Laboratory Standards Institute (6). Isolates resistant to two or more of the antimicrobials tested were categorized as multidrug resistant, while an MIC of 0.1 to 1 μg/ml was defined as decreased ciprofloxacin susceptibility (DCS) (9).

Determination of the 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 Fig. S1 in the supplemental material). The chromosomal haplotypes of *S*. Typhi isolates were determined based on the SNPs present at 1,485 chromosomal loci identified previously (11, 27; see also Table S1 and the methods in the supplemental material). 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 the methods in the supplemental material). A maximum-likelihood phylogenetic tree based on chromosomal SNPs was constructed using RAxML software (29).

TABLE 1. Distribution of drug resistance phenotypes among *S*. Typhi isolates from three surveillance periods

Period of isolation	No. (%) fully susceptible	No. (%) with single resistance	No. (%) MDR	Total no. (%)
1988–1993	33 (73.3)	7 (15.6)	5 (11.1)	45 (13.9)
1994–2000	11 (7.7)	46 (32.3)	85 (60)	142 (44.0)
2001–2008	10 (7.4)	21 (15.4)	105 (77.2)	136 (42.1)

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, United Kingdom) according to the manufacturer's instructions. Plasmid molecular sizes were determined by coelectrophoresis with plasmids of known molecular sizes from *E. coli* strains V517 (35.8, 4.8, 3.7, 2.6, 2.0, 1.8, and 1.4 MDa) and 39R861 (98, 42, 24, and 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 (4). The 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 $MgCl_2$, a 200 μM concentration of a deoxynucleoside triphosphate (dNTP) mixture, and 1 U of *Taq* polymerase in a final volume of 50 μl. The 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. The 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. Individual nalidixic acid-resistant *S*. Typhi strains were selected for amplification of the quinolone resistance-determining region (QRDR) in the chromosome with primer pairs, as described previously (15). In addition, to detect plasmid-encoded quinolone resistance, we applied *qnr* gene primers, QP1 (5′-ATAAAG TTTTTCAGCAAGAGG; starting at the 12th *qnr* nucleotide) and QP2 (5′-AT CCAGATCGGCAAAGGTTA), to detect the *qnrA* gene on plasmids (length, 657 bp). For *qnrB*, primers FQ1 (5′-ATGACGCCATTACTGTATAA) and FQ2 (5′GATCGCAATGTGTGAAGTTT) were used (13). Two sets of primers, aac(6′)-1b-cr1 F (5′ATATGCGGATCCAATGAGCAACGCAAAAACAAAG TTAG3′) plus aac(6′)-1b-cr1 R (5′ATATGCGAATTCTTAGGCATCACTGC GTGTTCGCTC3′) and aac(6′)-1b-cr2 F (5′TTGCAATGCTGAATGGAGAG 3′) plus aac(6′)-1b-cr2 R (5′CGTTTGGATCTTGGTGACCT3′) were used to detect a ciprofloxacin-acetylating enzyme, designated aac(6′)-1b-cr (confers low-level ciprofloxacin resistance; MIC = 0.25 μg/ml), located on a mobile transposon. The 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 by a dideoxy chain termination method with an automated DNA sequencer, ABI Prism 377 (Perkin Elmer, Warrington, United Kingdom), and analyzed using commercial software (Lasergene; DNAStar Inc., Madison, WI).

RESULTS

Trends in antimicrobial susceptibility of *S*. Typhi isolated in Nairobi between 1988 and 2008. A total of 323 *S*. Typhi isolates from three hospitals covering the Nairobi region of Kenya during the period 1988 to 2008 were included in this study. Of these, 54 (16.7%) isolates were fully susceptible to all eight antibiotics tested (see Materials and Methods), and over half of these sensitive isolates were obtained during the first part of the surveillance period (1988 to 1993) (Table 1; see also Fig. S1 in the supplemental material). 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 isolates were MDR, but only 5 (2.6%) of these were

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

Period of isolation (*n*)	No. (%) Nal^{ra}	No. (%) DCS^{b} and Nal^{r}
1988–1993 (45)	2 (4.4)	0
1994–2000 (142)	8 (5.6)	3 (37.5)
2001–2008 (136)	25 (18.4)	16 (64)

[a] Nal^{r}, nalidixic acid resistant; MIC > 16 μg/ml.
[b] MIC = 0.1 to 1.0 μg/ml.

isolated during the first phase (1988 to 1993). Indeed, there was a significant peak of MDR *S.* Typhi strains isolated between 2001 and 2006, and although sensitive *S.* Typhi strains were isolated throughout the 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, >256 μg/ml), streptomycin (MIC, >1,024 μg/ml), and cotrimoxazole (MIC, >32 μg/ml), which are among the most readily available antibiotics in Kenya. Thus, overall, there was a significant trend toward increasing levels of resistance to antibiotics over the study period (Table 1; see also Fig. S1 in the supplemental material). 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 to 2000 to 25 (18.4%) during the period 2001 to 2008 ($P < 0.01$). Of these nalidixic acid-resistant *S.* Typhi isolates, a total of 16 (64.0%) isolates from the 2001-to-2008 surveillance period showed DCS (MIC = 0.1 to 1 μg/ml) (Table 2).

Antimicrobial resistance in *S.* Typhi isolates. Previous studies had 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 to 110 MDa in size regardless of the period of the study, and 72 had one or two additional plasmids of 4 to 10 kb. Fully sensitive *S.* Typhi isolates 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 cotrimoxazole cotransferred these resistance phenotypes to *E. coli* K-12 by *in vitro* conjugation. Additionally, all the MDR *S.* Typhi isolates were positive for the 365-bp PCR product (data not shown) predicted for IncHI1 plasmids (15). The 35 *S.* Typhi isolates that exhibited nalidixic acid resistance (MICs, >16 μg/ml, compared to fully sensitive strains with MICs of 1 to 4 μg/ml), which included 19 isolates with DCS (MIC = 0.1 to 1.0 μg/ml), were investigated for the presence of SNPs associated with resistance in the *gyrA*, *parC*, and *parE* genes and for the presence of plasmid-mediated resistance to fluoroquinolones. Sequenced isolates that were resistant to nalidixic acid (MICs > 16 μg/ml) or exhibited DCS harbored mutations in *gyrA* resulting in amino acid substitutions, serine 83 (TCC) to leucine (TTG). A total of 16 of the 35 nalidixic acid-resistant strains (MIC = 16 to 20 μg/ml) had MICs of ciprofloxacin below the DCS range (0.02 to 0.1 μg/ml). None of the nalidixic acid-resistant *S.* Typhi isolates showed any significant point mutations in *parC* or *parE*. Furthermore, none of the quinolone- or fluoroquinolone-resistant strains harbored 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 Fig. S1 in the supplemental material]) 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 and analyzed 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, which required some modifications to standard data analysis methods (see Materials and Methods and the methods in the supplemental material). 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 (3, 11, 25). The proportion of H58 *S.* Typhi isolates increased from 11/23 (47.8%) in the period 1988 to 1998 to 62/71 (87.3%) in 2001 to 2008 (Fig. 1b). The other haplotypes were represented by fewer than eight isolates in each case, which were predominantly drug-sensitive isolates (Fig. 1a and b). Of the 94 *S.* Typhi isolates that were subjected to SNP analysis, 63 (67%) were MDR, and all of those were H58. Each of the MDR *S.* Typhi isolates 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 harbored IncHI1 plasmids of the same subtype, ST6 (Fig. 2b).

We 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 (Fig. 2a). Lineage II was detected throughout the study period. However, lineage I was detected only between 2004 and 2008, during which time it comprised 45.8% of the H58 isolates (Fig. 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; $P < 0.01$). DSC isolates (MIC = 0.1 to 1.0 μg/ml) were also more common among H58 lineage II (24%) than 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 a region of endemicity within the African continent. Our data unequivocally prove that a rise in the incidence of MDR *S.* Typhi throughout the study period is associated with a single bacte-

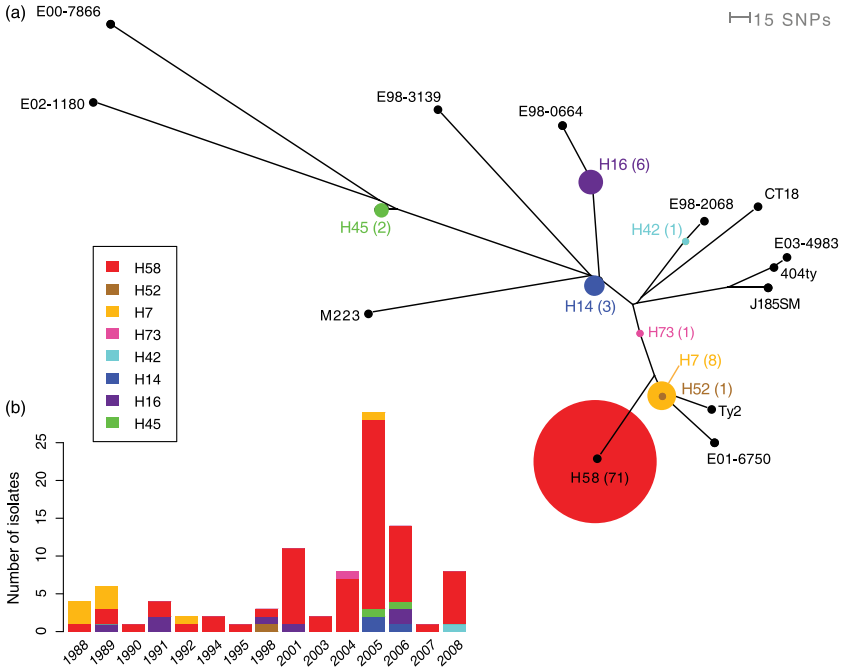

FIG. 1. Distribution of *S*. Typhi haplotypes identified in Kenya. (a) Radial phylogram based on 1,485 SNPs originally detected among 19 whole-genome sequences of *S*. Typhi (11). Black nodes, sequenced control isolates; colored nodes, 94 Kenyan isolates (colors were randomly assigned to the nodes) scaled to reflect the numbers of isolates found; precise numbers are given in parentheses. (b) Distribution of haplotypes of Kenyan *S*. Typhi by year of isolation. The colors indicate *S*. Typhi haplotypes as defined in panel a.

rial haplotype, H58, which has also undergone expansion in Southeast Asia (3, 25). Thus, a single *S*. Typhi haplotype has now been linked to the spread of the MDR genotype on two continents. H58 has been detected as 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 the 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 and will potentially serve 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 been exchanged as a consequence of such links. All the H58 MDR *S*. Typhi isolates 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 isolates 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. These data are in agreement with previous studies (3, 11, 27).

Our SNP-typing approach is based on simultaneously measuring nucleotide changes at 1,485 sites distributed throughout

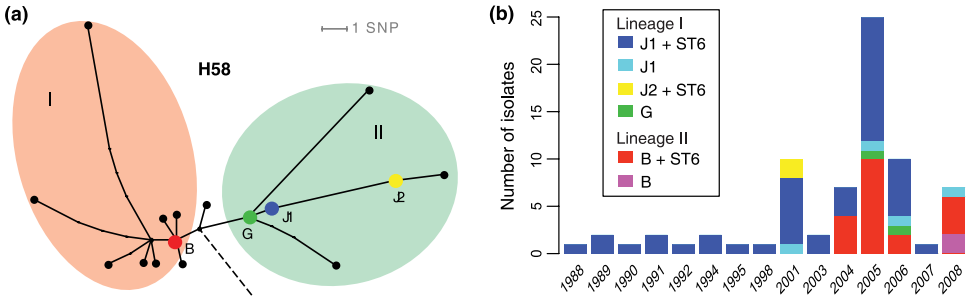

FIG. 2. Subtypes of Kenyan *S*. Typhi isolates of haplotype H58. (a) Radial phylogram of the H58 cluster of *S*. Typhi; the dashed line indicates where this phylogram links back to the broader *S*. Typhi phylogram shown in Fig. 1. Black nodes, genotyped control isolates, including seven genomes sequenced (labeled with strain names [11]) or previously defined (labeled with H groups [27]); colored nodes, nodes to which Kenyan isolates were mapped by SNP typing (colors were randomly assigned to the nodes), labeled with letters. The oval shading highlights two distinct H58 lineages, I and II. (b) Distribution of H58 subtypes of Kenyan *S*. Typhi by year of isolation, split by the presence of MDR IncHI1 plasmids (note than no non-H58 isolates contained plasmids). The labels and colors correspond to those defined in panel a.

the chromosome and IncHI1 plasmids of *S*. Typhi. Using high-throughput sequencing, we previously identified novel H58-associated SNPs that allow discrimination within this haplotype (Fig. 2a) (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 many areas of endemicity in 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 isolates (73.3%) from blood cultures of patients prior to 1993 were fully sensitive to all antimicrobials. Data from the last 7 years show that 77.2% of the *S*. Typhi isolates are MDR, much higher than the reported 52% and 29% prevalences for MDR *S*. Typhi in Ghana (20) and Egypt (28), respectively. It is, however, close to the high prevalences of MDR *S*. Typhi noted in South Africa (8) and Nigeria (2), where 65% and 61% of isolates, respectively, were MDR. In addition, in our studies in Nairobi, the proportion of *S*. Typhi isolates 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 that renders these drugs ineffective may emerge, as has happened in Southeast Asia (1, 7, 24, 26) and in parts of West Africa (20, 23), where even moderate rises in MICs have led to clinical treatment failure. Even in settings in the United States, where *S*. Typhi is not endemic, 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 to 20 μg/ml) did not fit into the DCS category, but nonetheless, sequenced isolates had the Ser83Leu *gyrA* mutation. 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 prescription of fluoroquinolones, and indeed all other first-line antibiotics for typhoid fever, particularly as a result of poor diagnosis of suspected cases in patients who report to the privately owned clinics that abound in the poor informal settlements (slums). Our sentinel surveillance programs 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 to 1993) than in 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) and virtually nonexistent sewage disposal. Thus, the possibility of hygiene-related disease outbreaks, including typhoid, remains high.

In conclusion, it appears that a rapidly expanding multidrug- and partially nalidixic acid-resistant *S*. Typhi clone of the H58 haplotype, commonly associated with outbreaks in Southeast 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 fecal-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 typhoid outbreaks in Kenya caused by MDR fluoroquinolone-resistant *S.* Typhi, we propose that a more comprehensive approach to the 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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