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RESEARCH ARTICLE

Molecular Surveillance Identifies Multiple Transmissions of Typhoid in West Africa

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Abstract

Background
The burden of typhoid in sub-Saharan African (SSA) countries has been difficult to estimate, in part, due to suboptimal laboratory diagnostics. However, surveillance blood cultures at two sites in Nigeria have identified typhoid associated with *Salmonella enterica* serovar *Typhi* (S. Typhi) as an important cause of bacteremia in children.

Methods
A total of 128 S. Typhi isolates from these studies in Nigeria were whole-genome sequenced, and the resulting data was used to place these Nigerian isolates into a worldwide context based on their phylogeny and carriage of molecular determinants of antibiotic resistance.

Results
Several distinct S. Typhi genotypes were identified in Nigeria that were related to other clusters of S. Typhi isolates from north, west and central regions of Africa. The rapidly expanding S. Typhi clade 4.3.1 (H58) previously associated with multiple antimicrobial resistances in Asia and in east, central and southern Africa, was not detected in this study. However, antimicrobial resistance was common amongst the Nigerian isolates and was associated with several plasmids, including the IncHI1 plasmid commonly associated with S. Typhi.

Conclusions
These data indicate that typhoid in Nigeria was established through multiple independent introductions into the country, with evidence of regional spread. MDR typhoid appears to be evolving independently of the haplotype H58 found in other typhoid endemic countries. This study highlights an urgent need for routine surveillance to monitor the epidemiology of typhoid and evolution of antimicrobial resistance within the bacterial population as a means to facilitate public health interventions to reduce the substantial morbidity and mortality of typhoid.

Author Summary
Typhoid fever, a serious bloodstream infection caused by the bacterium *Salmonella* Typhi, is a major cause of disease and death around the world. There have been limited data on the epidemiology of typhoid in many countries in sub-Saharan African, including Nigeria. Recent evidence, however, showed that typhoid was an important cause of bacteremia in children residing in two regions of Nigeria. Here, we analyzed the whole genome sequences of 128 S. Typhi isolates from two studies in order to elucidate the population structure and characterize the genetic components of antimicrobial resistance. We found that the multiple S. Typhi genotypes identified were closely related to other S. Typhi from neighboring regions of Africa and that multidrug resistance (MDR) was common among these isolates, and in many cases was associated with the IncHI1 plasmid known to cause
MDR typhoid. These results provide evidence that typhoid was established in Nigeria as a result of several independent introductions into the country and that there has been extensive exchange of S. Typhi in and around the region of West Africa. This study emphasizes the importance of surveillance to improve our understanding of the epidemiology of typhoid, which is needed to underpin public health measures to reduce the spread of disease and facilitate patient management.

Introduction

Typhoid fever is a systemic infection caused by the Gram-negative bacterium Salmonella enterica serovar Typhi (S. Typhi) that continues to be a serious global health problem and a major cause of morbidity and mortality in low-middle income countries [1]. It is estimated that the yearly incidence of typhoid fever exceeds 20 million cases, with over 200,000 deaths [2, 3]. Defining the burden of typhoid fever is a challenge in settings where there are few diagnostic microbiology facilities, with diagnosis often based on clinical history of fever, malaise, and abdominal pain. Unfortunately, these symptoms have considerable overlap with several other febrile illnesses and clinical diagnosis is therefore inaccurate [4].

Nigeria is one of the most densely populated countries in Africa with large areas of urban development. Thus, it is perhaps surprising that little reliable data are available on microbial culture of the etiologic agents of bacteremia in children or adults. This poses a challenge for data comparison with other regions, including other sub-Saharan African countries where such data are available [5–7]. In general, febrile illnesses among children in Nigeria are presumed by clinicians to be caused by malaria, which is still very common in many parts of the country. Only if fever persists following an empiric course of anti-malarials, is typhoid then considered as a potential cause of infection [8]. In studies from central and northwest Nigeria [9], we found that S. Typhi was the commonest cause of bloodstream infections in children, particularly in those living in the proximity of Abuja city located in central Nigeria.

Until recently, molecular epidemiological studies on S. Typhi were compromised by a lack of genetic resolution, limiting the ability to define the population structure of the bacteria and identify transmission patterns. This is because S. Typhi is a relatively monomorphic pathogen with limited genome variation [10]. However, sequencing-based approaches have facilitated the stratification of S. Typhi into multiple genotypes [11] (see Wong et al. 2016, under review in Nature Communications, NCOMMS-15-25823, manuscript included). Whole genome sequencing in particular can unequivocally identify phylogenetic relationships with important genetic traits such as antimicrobial resistance [12]. Here we report whole genome-based analysis of 128 bloodstream isolates of S. Typhi from children residing in two regions of Nigeria, and compared these with data from other countries in Africa, including the West African subregion.

Methods

Settings

Nigeria has a population of approximately 177 million people making it the most populous country in sub-Saharan Africa [13]. The two study sites in Nigeria were the Federal Capital Territory (FCT) and Kano. The FCT is a federal territory in central Nigeria and covers a land area of 8,000 square kilometers. It is the home of the capital city Abuja, a “planned” city, built in the 1980s. It was officially made Nigeria’s capital in 1991 replacing the previous capital in...
Lagos. In 2006, the population was estimated at 1.7 million [14]. The FCT continue to experience rapid population growth; it has been reported that some areas around Abuja have been growing at an annual rate of 20–30%, and the current population may be as high as 5.7 million [14]. The rapid spread of squatter settlements and shantytowns in and around the city limits contribute to this rapid growth. The rainy season begins in April and ends in October. Within this period there is a brief interlude of Harmattan, occasioned by the Northeast Trade Wind, with the main features of dust haze, intensified coldness and dryness. The annual total rainfall for the FCT is in the range of 1,100 to 1,600 mm. The population is diverse, with increasing representation from the major ethnic groups of Hausa, Yoruba, and Igbos following the development of the FCT and relocation of the federal capital [15]. Of note, there is also perennial malaria transmission, mostly due to Plasmodium falciparum, and the HIV prevalence is 7.5% amongst pregnant women attending antenatal clinics [16].

Kano is the capital of Kano state in northwest Nigeria. According to the 2006 census, Kano state has a population of 9.38 million, which is comprised predominantly of Hausa and Fulani ethnic groups [17]. It is recognized as one of the fastest growing cities in Nigeria with a population density of about 1,000 inhabitants per km². It lies within the Sahel savannah region with daily mean temperature of about 30–33°C during the dry months of March to May and 10°C during the autumn months of September to February. Rainy season varies from year to year, but typically commences in May and ends in October, with an average annual rainfall of 600mm. The dry season starts from November to April [18]. The entire state is within the meningococcal disease belt and malarial transmission is seasonal [17]. HIV prevalence among women attending antenatal clinic is 1.3% [16].

Enrolment sites
The enrolment sites at FCT are as previously described [9, 15]. Briefly, children aged less than 5 years were enrolled from primary, secondary and tertiary healthcare facilities on presentation with an acute febrile illness and symptoms suggestive of sepsis. In Kano, we enrolled children from Aminu Kano Teaching Hospital (AKTH), Hasiya Bayero Pediatric Hospital and Murtala Specialist Hospital. While AKTH serves as a tertiary referral center, the other two facilities provide primary and secondary healthcare services. The combined outpatient attendance for children at these three facilities is about 1,000 daily. Both study sites included patients from the newer settlements on the outskirts of Abuja and around Kano where the level of sanitation is poor and access to potable water limited.

Data collection
A structured questionnaire was used to collate the clinical information. Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Nebraska Medical Center [19]. IBM SPSS for statistics was used for data analysis. Dichotomous variables were analyzed using $\chi^2$ or $\chi^2$ for trend tests [20].

Ethics statement
Clinical information was collected using a structured questionnaire after obtaining a signed informed consent from the child’s parent or legal guardian. This study was approved by the ethics committees of the FCT, National Hospital Abuja, Zankli Medical Center, Federal Medical Center Keffi, Aminu Kano Teaching Hospital, and UNMC, Omaha Institutional Review Board.
Blood culture processing

Blood sampling and processing were as previously described [9, 15]. Briefly, we utilized only aerobic blood culture bottles and held cultures in the Bactec 9050 incubator for a maximum of 5 days. Bacteria were identified by a combination of colony morphology and biochemical assays. For example, the API 20E system (bioMérieux, France) was used to identify Enterobacteriaceae. Antimicrobial susceptibility profiles of the bacteria were determined by the Kirby-Bauer disk diffusion test using standard interpretative criteria [21] for locally available antimicrobials (amoxicillin, co-amoxiclav, ceftazidime, ceftriaxone, nalidixic acid, ciprofloxacin, ofloxacin, sulfamethoxazole, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, streptomycin, gentamicin, kanamycin, azithromycin, imipenem) in order to provide immediate management of patients. Bacterial isolates were stored in skimmed milk at -70°C and further characterized at the Clinical Microbiology Laboratory of the University of Nebraska Medical Center (UNMC).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed at the UNMC Microbiology laboratory using the Epsilometer test (Etest; bioMérieux, France) according to standard methods. Minimum inhibitory concentration (MIC) values were interpreted according to Clinical Laboratory Standards Institute (CLSI) standards [21]. Due to the lack of CLSI standards, a streptomycin MIC of ≥16 mg/L was considered resistant in these studies.

Salmonella serotyping

All Salmonella isolates were identified to the serotype level using the Bioplex 200 (Bio-Rad) as previously described using the CDC standard Salmonella molecular serotyping protocol [22–24]. A total of 128 S. Typhi isolates were identified in these studies for whole genome sequencing.

DNA sequencing

S. Typhi DNA was prepared using the Wizard Genomic DNA Kit (Promega, Madison, WI, USA) as per manufacturer's instructions. Index-tagged paired end Illumina sequencing libraries were prepared as previously described [25]. These were combined into pools each containing 96 uniquely tagged libraries and sequenced on the Illumina Hiseq2000 or Miseq platforms (Illumina, San Diego, CA, USA) according to manufacturer's protocols to generate tagged 100 or 150 base pair (bp) paired-end reads with an insert size of 300–400 bp. Sequence reads were deposited in the European Nucleotide Archive under accession ERP005877 and a full list of accession numbers for each sample is available in S1 Table. Sequence data from 1,831 additional S. Typhi isolates from 63 countries, generated previously in the same manner (Wong et al. 2015) [12], were also included in the study (reads are available in the European Read Archive under accession ERP001718).

Read alignment and SNP detection

For analysis of single nucleotide polymorphisms (SNPs), the paired-end reads were mapped to the reference genome of S. Typhi CT18 (accession number AL513382), including the chromosome and plasmids pHCM1 and pHCM2 [26], using SMALT (version 0.7.4) (http://www.sanger.ac.uk/resources/software/smalt/). SNPs were identified as previously described, using samtools mpileup [27] and filtering with a minimum mapping quality of 30 and a quality ratio cut-off of 0.75 [25]. The allele at each locus in each isolate was determined by reference to the
consensus base in that genome, using samtools mpileup [27] and removing low confidence alleles with consensus base quality ≤20, read depth ≤5 or a heterozygous base call. SNPs called in phage regions, repetitive sequences (354 kbp; ~7.4% of bases in the S. Typhi CT18 reference chromosome, as defined previously [10]) or recombinant regions (~180 kbp; <4% of CT18 reference chromosome, identified using an approach described previously [25, 28]) were excluded, resulting in a final set of 23,300 chromosomal SNPs.

**Phylogenetic analysis**

The maximum likelihood (ML) phylogenetic tree was built from 23,300 SNP alignment of 1,961 isolates, including one S. Paratyphi A (accession number ERR326600) to provide an out-group for tree rooting. We used RAxML (version 7.0.4) [29] with the generalized time-reversible model and a Gamma distribution to model site-specific rate variation (the GTR + substitution model; GTRGAMMA in RAxML). Support for the ML phylogeny was assessed via 100 bootstrap pseudo-replicate analyzes of the alignment data. The ML trees were displayed and annotated using iTOL [30, 31].

**In silico resistance plasmid and resistance gene analysis**

Plasmids and acquired antimicrobial resistance genes were detected, and their precise alleles determined, using the mapping-based allele typer SRST2 [32] together with the ARG-Annot database of antimicrobial resistance genes [33] and the PlasmidFinder database of plasmid replicons [34]. SRST2 was also used to identify mutations in the gyrA, gyrB, parC and parE genes that have been associated with resistance to quinolones in Salmonella and other Gram-negative bacteria [35–38].

**Results**

**Typhoid surveillance**

Blood cultures were performed for the evaluation of 10,133 acutely ill children, aged 0–60 months, from September 2008 until April 2015, in the FCT (including Abuja) and Kano located in central and northwest Nigeria, respectively [9]. At FCT 6,082 children were enrolled between June 2012 and March 2015, of whom 457 (8%) had clinically significant bacteremia. Of these 110 (24%) had invasive salmonellosis, consisting of S. Typhi in 84 cases and non-typhoidal salmonellae (NTS) in 26 cases. In Kano from January 2014 until April 2015 clinically significant bacteremia was detected in 609 (15%) of 4,051 children: salmonellae accounted for 364 (60%) of 609 cases, of which 296 were S. Typhi and 68 were NTS. Across both regions Salmonella species accounted for 24–60% of bacteremia with S. Typhi being the most common serovar isolated with a total of 380 isolates (76–79%) [9].

**Phylogenetic analysis of Nigerian S. Typhi**

A selection of one hundred and twenty-two S. Typhi from the FCT and six from Kano, all isolated between 2008–2013, were randomly selected and sequenced via Illumina HiSeq and MiSeq (see Methods). The genomes of the Nigerian isolates were compared to that of the S. Typhi CT18 reference strain and a previously published global collection of approximately 2,000 S. Typhi isolates [12]. A phylogeny was built by extracting single nucleotide polymorphisms (SNPs) from the whole genome sequences, excluding likely recombination events and repetitive sequences that could confound phylogenetic analysis as described in Methods. The SNP data were also used to assign each isolate to one of 62 previously defined genotypes; details of the source and genotype of all Nigerian isolates is given in Table 1 and S1 Table.
Table 1. Summary of genotypes of Nigerian S. Typhi.

| Laboratory name | Year of isolation | Location | Roumagnac haplotype* | Primary clade | Clade | Subclade |
|-----------------|-------------------|----------|----------------------|---------------|-------|----------|
| PO_30           | 2008              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_601          | 2009              | Abuja    | Untypeable           | 1             | 1     | 0.0.3    |
| PO_132          | 2009              | Abuja    | Untypeable           | 2             | 2.3   | 2.3.1    |
| PO_107          | 2009              | Abuja    | Untypeable           | 2             | 2.3   | 2.3.1    |
| PO_1057         | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_1060         | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_1063         | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_187          | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_227          | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_293          | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_351          | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_355          | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_771          | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_812          | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_919          | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_575          | 2009              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_260          | 2009              | Abuja    | H52                  | 4             | 4.1   | 4.1.0    |
| PO_1102         | 2010              | Abuja    | Untypeable           | 1             | 1     | 0.0.3    |
| PO_1131         | 2010              | Abuja    | Untypeable           | 1             | 1     | 0.0.3    |
| 3135STDYS6861198| 2010              | Abuja    | Untypeable           | 1             | 1     | 0.0.3    |
| 3135STDYS6861206| 2010              | Abuja    | Untypeable           | 1             | 1     | 0.0.3    |
| 3135STDYS6861239| 2010              | Abuja    | Untypeable           | 1             | 1     | 0.0.3    |
| 3135STDYS6861254| 2010              | Abuja    | Untypeable           | 2             | 2.1   | 2.1.0    |
| PO_1255         | 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_1098         | 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_1101         | 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_1210         | 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_1242         | 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| PO_1265         | 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS686190 | 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS6861230| 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS6861262| 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS6861271| 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS6861184| 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS6861208| 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS6861216| 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS6861224| 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS6861232| 2010              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS6861183| 2010              | Abuja    | H42                  | 3             | 3.3   | 3.3.0    |
| PO_1232         | 2010              | Abuja    | H52                  | 4             | 4.1   | 4.1.0    |
| 3135STDYS6861222| 2010              | Abuja    | H52                  | 4             | 4.1   | 4.1.0    |
| 3135STDYS6861246| 2010              | Abuja    | H52                  | 4             | 4.1   | 4.1.0    |
| 3135STDYS6861270| 2010              | Abuja    | H52                  | 4             | 4.1   | 4.1.0    |
| 3135STDYS6861199| 2010              | Abuja    | H52                  | 4             | 4.1   | 4.1.0    |
| 3135STDYS6861247| 2010              | Abuja    | H52                  | 4             | 4.1   | 4.1.0    |
| PO_1110         | 2010              | Abuja    | H52                  | 4             | 4.1   | 4.1.1    |
| PO_1256         | 2011              | Abuja    | Untypeable           | 1             | 1     | 0.0.3    |

(Continued)
| Laboratory name  | Year of isolation | Location | Roumagnac haplotype* | Primary clade | Clade | Subclade |
|-----------------|-------------------|----------|----------------------|---------------|-------|----------|
| 3135STDYS861272 | 2011              | Abuja    | Untypeable           | 2             | 2.1   | 2.1.0    |
| 3135STDYS861209 | 2011              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861248 | 2011              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861256 | 2011              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861264 | 2011              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861193 | 2011              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861201 | 2011              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861233 | 2012              | Abuja    | Untypeable           | 2             | 2.3   | 2.3.1    |
| 3135STDYS861241 | 2012              | Abuja    | Untypeable           | 2             | 2.3   | 2.3.1    |
| 3135STDYS861273 | 2012              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861217 | 2012              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861225 | 2012              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861195 | 2012              | Abuja    | Untypeable           | 2             | 2.3   | 2.3.1    |
| 3135STDYS861211 | 2012              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861235 | 2012              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861243 | 2012              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861210 | 2013              | Kano     | Untypeable           | 2             | 2.3   | 2.3.1    |
| 3135STDYS861226 | 2013              | Kano     | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861202 | 2013              | Kano     | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861234 | 2013              | Kano     | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861242 | 2013              | Kano     | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861218 | 2013              | Kano     | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861196 | 2013              | Abuja    | Untypeable           | 1             | 1     | 0.0.1    |
| 3135STDYS861244 | 2013              | Abuja    | Untypeable           | 1             | 1     | 0.0.1    |
| 3135STDYS861359 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861290 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861298 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861350 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861287 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861280 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861289 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861337 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861353 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861361 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861369 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861253 | 2013              | Abuja    | Untypeable           | 2             | 2.2   | 2.2.0    |
| 3135STDYS861342 | 2013              | Abuja    | Untypeable           | 2             | 2.3   | 2.3.1    |
| 3135STDYS861334 | 2013              | Abuja    | Untypeable           | 2             | 2.3   | 2.3.1    |
| 3135STDYS861294 | 2013              | Abuja    | H56                  | 3             | 3.1   | 3.1.0    |
| 3135STDYS861312 | 2013              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861320 | 2013              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861268 | 2013              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861276 | 2013              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861229 | 2013              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861237 | 2013              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861245 | 2013              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |
| 3135STDYS861314 | 2013              | Abuja    | H56                  | 3             | 3.1   | 3.1.1    |

(Continued)
distribution of the 128 Nigerian S. Typhi within the global phylogenetic tree is shown in S1 Fig. This global phylogeny includes 238 isolates from other countries in Africa, and the Nigerian isolates all cluster with other African isolates. Detailed phylogenetic relationships amongst the 366 African isolates are shown in Fig 1, and an interactive version of the phylogeny and map are available for exploration online at http://microreact.org/project/styphi_nigeria.

The majority of Nigerian S. Typhi (84/128, 66%) belonged to genotype 3.1.1 (these isolates were assigned to H56 under the old typing scheme of Roumagnac et al (2006) [11]). This dominant genotype is relatively common across Africa, predominantly western and central countries (Fig 1). The Nigerian isolates formed a tight phylogenetically clustered subgroup within the 3.1.1 subclade (Fig 1), suggesting recent local expansion, and included isolates from both
Abuja and Kano, suggesting intra-country transmission. Interestingly, in the wider African collection genotype 3.1.1 was represented by isolates from neighboring Cameroon and across West Africa (Benin, Togo, Ivory Coast, Burkina Faso, Mali, Guinea and Mauritania) suggesting long-term inter-country exchange within the region (Fig 1). Most of the remaining isolates belonged to four other genotypes, indicating that these are also established genotypes in circulation at the study sites in Nigeria. These genotypes, highlighted in Fig 1, are 2.2.0 (n = 13), 2.3.1 (n = 8), 4.1.0 (n = 8, H52 under the old scheme) and 0.0.3 (n = 7, H12). Nigerian isolates of genotypes 2.2.0 and 2.3.1 were closely related to isolates from neighboring Cameroon and West African countries and not found elsewhere, supporting regional transmission similar to the dominant genotype 3.1.1 (see map in Fig 1), while genotype 4.1.0 was more widespread across Africa. Interestingly genotype 0.0.3 (previously identified in India and Malaysia), which accounted for >5% of Nigerian isolates, maps very close to the root of the global S. Typhi tree, suggestive of older circulating isolates. A further six other genotypes were also detected amongst the Nigerian isolates, represented by 1–2 isolates each (Table 1). Of note, genotype 4.3.1 (H58), which has become dominant elsewhere in sub-Saharan Africa and accounts for the majority of antimicrobial resistant typhoid globally, was not detected in the Nigerian studies.

Fig 1. Distribution of Nigerian S. Typhi isolates in Africa in this study. A maximum likelihood tree of 366 S. Typhi isolates constructed using 9,352 SNPs from whole genome sequence from 128 Nigerian isolates and 238 isolates from other regions of Africa is shown on the left. The geographical location of isolation is highlighted on the maps of Africa displayed on the right (http://microreact.org/showcase/). S. Typhi isolates from Abuja (122 isolates) and Kano (6) are denoted using red and orange squares, respectively. Colored circles on both the tree and maps represent isolates from other regions of Africa. The common genotypes of the Nigerian isolate are highlighted by a grey ring surrounding the tree with the corresponding geographical location marked on the map. Branch lengths are indicative of the estimated substitution rate per variable site.

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Antimicrobial resistant S. Typhi in Nigeria

Fig 2 shows the proportion of S. Typhi isolates that were resistant to one or more antimicrobials, and the proportion that were multidrug-resistant (MDR; defined as resistance to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole), each year from 2008–2013. The majority of isolates were MDR throughout this period (Fig 2).

Fig 3 and Table 2 show the distribution of antimicrobial resistance determinants in the Nigerian isolates. Most of the 3.1.1 (H56) isolates carried genes encoding resistance to ampicillin, chloramphenicol, tetracycline and sulfamethoxazole (blaTEM-1, catA1, tetB, dfrA15, sul1). These were located on an IncHI1 plasmid, similar to that commonly found in MDR S. Typhi 4.3.1 (H58). The same profile was identified in a single isolate of 0.0.3, indicative of local plasmid transfer between the co-circulating genotypes. Genotype 2.3.1 isolates were found to carry IncHI1 plasmids encoding these resistance genes, as well as resistance determinants sul2 and strAB. An IncHI1 plasmid carrying blaTEM and tetB was also identified in one 2.2.0 isolate. Interestingly, nine genotype 3.1.1 isolates lacked the IncHI1 plasmid. However, four of these carried plasmids of other incompatibility groups. Three isolates (3135STDY5861338; 3135STDY5861351; 3135STDY5861282) harbored a novel IncY plasmid (blaTEM-198, catA1, tetB, dfrA14, sul1) and one (3135STDY5861242) harbored a plasmid-related to the Kpn3 plasmid (blaTEM-198, tetAR, dfrA14, sul1, sul2, strAB and also qnr-S, which mediates fluoroquinolone resistance). Thus, plasmid-mediated MDR is common in Nigerian S. Typhi from the regions under study.

We identified only six S. Typhi isolates with quinolone resistance-associated mutations in gyrA (one with S83F; five with S83Y). The affected isolates were all of the dominant genotype 3.1.1, including the three that carried IncY plasmids and three that carried IncHI1 plasmids. No other polymorphisms were detected in the quinolone resistance determining regions of the gyrA or parC genes of Nigerian S. Typhi isolates.

Discussion

Here, S. Typhi is shown to be a common cause of bacteremia and fever among children living in two geographically distinct regions of Nigeria. Studies on typhoid within Nigeria have been
relatively rare, even though it is a country with a large population and extensive urbanization. Indeed, S. Typhi is the most common bacterial cause of bloodstream infections. Phylogenetic analysis identified distinct clusters of S. Typhi, with isolates of genotype 3.1.1 representing 66% of all isolates. Other common genotypes included 2.2.0 and 2.3.1, which have been previously reported in Africa, and genotypes 4.1.0 and 0.0.3, which were previously reported in Asia. The presence of multiple genotypes in these comparatively small regions suggests typhoid has been established for some time and that different waves of disease have entered the regions at different times. It is also interesting that the different clades of Nigerian isolates distributed across the phylogeny frequently map adjacent to other S. Typhi isolates from other African countries. For example, genotype 3.1.1 maps adjacent to S. Typhi isolates from both west and north...
Table 2. Summary of drug resistance of Nigerian S. Typhi.

| Laboratory name | Subclade | Plasmids | Resistance genes | gyrA mutations |
|-----------------|----------|----------|------------------|----------------|
| 3135STDY5861196 | 0.0.1    | -        | -                | -              |
| 3135STDY5861244 | 0.0.1    | -        | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_601          | 0.0.3    | -        | -                | -              |
| PO_1102         | 0.0.3    | -        | -                | -              |
| PO_1131         | 0.0.3    | -        | -                | -              |
| 3135STDY5861198 | 0.0.3    | -        | -                | -              |
| 3135STDY5861206 | 0.0.3    | -        | -                | -              |
| 3135STDY5861239 | 0.0.3    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_1256         | 0.0.3    | -        | -                | -              |
| 3135STDY5861254 | 2.1.0    | -        | -                | -              |
| 3135STDY5861272 | 2.1.0    | -        | -                | -              |
| 3135STDY5861209 | 2.2.0    | -        | -                | -              |
| 3135STDY5861359 | 2.2.0    | IncHI1   | bla-TEM, tetB    | -              |
| 3135STDY5861290 | 2.2.0    | -        | -                | -              |
| 3135STDY5861298 | 2.2.0    | -        | -                | -              |
| 3135STDY5861350 | 2.2.0    | -        | -                | -              |
| 3135STDY5861287 | 2.2.0    | -        | -                | -              |
| 3135STDY5861280 | 2.2.0    | -        | -                | -              |
| 3135STDY5861289 | 2.2.0    | -        | -                | -              |
| 3135STDY5861337 | 2.2.0    | -        | -                | -              |
| 3135STDY5861353 | 2.2.0    | -        | -                | -              |
| 3135STDY5861361 | 2.2.0    | -        | -                | -              |
| 3135STDY5861369 | 2.2.0    | -        | -                | -              |
| 3135STDY5861253 | 2.2.0    | -        | -                | -              |
| PO_132          | 2.3.1    | IncHI1   | dfrA, sul1, tetB, strA, strB, sul2, aad | -              |
| PO_107          | 2.3.1    | IncHI1   | dfrA, tetB, strA, strB, sul2, aad | -              |
| 3135STDY5861233 | 2.3.1    | IncHI1   | dfrA, sul1, bla-TEM, tetB, strA, strB, sul2, aad | -              |
| 3135STDY5861241 | 2.3.1    | IncHI1   | dfrA, sul1, bla-TEM, tetB, strA, strB, sul2, aad | -              |
| 3135STDY5861273 | 2.3.1    | IncHI1   | dfrA, sul1, bla-TEM, tetB, strA, strB, sul2, aad | -              |
| 3135STDY5861210 | 2.3.1    | -        | -                | -              |
| 3135STDY5861195 | 2.3.1    | -        | -                | -              |
| 3135STDY5861342 | 2.3.1    | IncHI1   | dfrA, sul1, bla-TEM, tetB, strA, strB, sul2, aad | -              |
| 3135STDY5861334 | 2.3.2    | -        | -                | -              |
| 3135STDY5861294 | 3.1.0    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_30           | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_1057         | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_1060         | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_1063         | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_187          | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB, S83Y | -              |
| PO_227          | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_293          | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_351          | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_355          | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_771          | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_812          | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_919          | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |
| PO_575          | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -              |

(Continued)
| Laboratory name | Subclade | Plasmids | Resistance genes | gyra mutations |
|-----------------|----------|----------|------------------|----------------|
| PO_1255         | 3.1.1    | -        | cat, dfrA, sul1  |               |
| PO_1098         | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| PO_1101         | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| PO_1210         | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| PO_1242         | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| PO_1265         | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861190 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861230 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB, S83Y |
| 3135STDY5861262 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861271 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861184 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861208 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861216 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861224 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861232 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861248 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861256 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861264 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861193 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861201 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861217 | 3.1.1    | -        | -                |               |
| 3135STDY5861225 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861226 | 3.1.1    | IncHI1   | bla-TEM, tetB    |               |
| 3135STDY5861202 | 3.1.1    | IncHI1   | bla-TEM, tetB    |               |
| 3135STDY5861234 | 3.1.1    | -        | -                |               |
| 3135STDY5861242 | 3.1.1    | Kpn3     | bla-TEM, strA, strB, sul1, sul2, dfrA, tetA, tetR, qnrS |               |
| 3135STDY5861218 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB, S83F |
| 3135STDY5861211 | 3.1.1    | IncHI1, IncFilts | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861235 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861243 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861312 | 3.1.1    | -        | cat, dfrA, sul1  |               |
| 3135STDY5861320 | 3.1.1    | -        | cat, dfrA, sul1  |               |
| 3135STDY5861268 | 3.1.1    | -        | cat, dfrA, sul1  |               |
| 3135STDY5861276 | 3.1.1    | -        | cat, dfrA, sul1  |               |
| 3135STDY5861229 | 3.1.1    | -        | cat, dfrA, sul1  |               |
| 3135STDY5861237 | 3.1.1    | -        | cat, dfrA, sul1  |               |
| 3135STDY5861245 | 3.1.1    | -        | cat, dfrA, sul1  |               |
| 3135STDY5861314 | 3.1.1    | -        | -                |               |
| 3135STDY5861330 | 3.1.1    | -        | -                |               |
| 3135STDY5861197 | 3.1.1    | -        | -                |               |
| 3135STDY5861338 | 3.1.1    | IncY     | bla-TEM, strA, strB, sul1, dfrA, tetA, tetR, S83Y |
| 3135STDY5861351 | 3.1.1    | IncY     | bla-TEM, strA, strB, sul1, dfrA, tetA, tetR, S83Y |
| 3135STDY5861282 | 3.1.1    | IncY     | bla-TEM, strA, strB, sul1, dfrA, tetA, tetR, S83Y |
| 3135STDY5861306 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861322 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
| 3135STDY5861326 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB |               |
Africa, with the Nigerian isolates located on a more recent phylogenetic branch. Similarly, genotypes 2.2.0 and 2.3.1 also map close to other African isolates. This general distribution indicates substantial exchange of *S. Typhi* between Nigeria and other parts of Africa. However, the phylogenetic analysis was limited to two sites within Nigeria, with only six *S. Typhi* isolates included in the analysis from Kano, over a five-year period, resulting in a selection bias towards strains from a single study site in Nigeria (Abuja). Therefore, a more comprehensive analysis involving a larger number of strains from multiple regions across Nigeria and surrounding countries over a wider time span would be required to further investigate transmission within the region.

It is notable that none of the Nigerian isolates were of the genotype 4.3.1 (H58), which is now expanding across many other regions with endemic typhoid and is associated with a MDR phenotype. This suggests that the recent expansion of H58 *S. Typhi*, estimated to date from the

| Laboratory name | Subclade | Plasmids | Resistance genes | gyrA mutations |
|-----------------|----------|----------|------------------|---------------|
| 3135STDY5861366 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861279 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861303 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861319 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861327 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861335 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861343 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861367 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861304 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861328 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861336 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861344 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861260 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861189 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861213 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861278 | 3.1.1    | IncHI1   | cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861286 | 3.1.1    | IncHI1   | 5 cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861251 | 3.1.1    | IncHI1   | 5 cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861259 | 3.1.1    | IncHI1   | 5 cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861212 | 3.1.1    | IncHI1   | 5 cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861220 | 3.1.1    | IncHI1   | 5 cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861228 | 3.1.1    | IncHI1   | 5 cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861252 | 3.1.1    | IncHI1   | 5 cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861236 | 3.1.1    | IncHI1   | 4 cat, dfrA, sul1, bla-TEM, | -             |
| 3135STDY5861368 | 3.1.1    | IncHI1, IncQ1 | 5 cat, dfrA, sul1, bla-TEM, tetB | -             |
| 3135STDY5861183 | 3.3.0    | -        | 0                | -             |
| PO_260          | 4.1.0    | -        | 0                | -             |
| PO_1232         | 4.1.0    | -        | 0                | -             |
| 3135STDY5861222 | 4.1.0    | -        | 0                | -             |
| 3135STDY5861246 | 4.1.0    | -        | 0                | -             |
| 3135STDY5861270 | 4.1.0    | -        | 0                | -             |
| 3135STDY5861199 | 4.1.0    | -        | 0                | -             |
| 3135STDY5861247 | 4.1.0    | -        | 0                | -             |
| 3135STDY5861205 | 4.1.0    | -        | 0                | -             |
| PO_1110         | 4.1.1    | Col(RNAI) | 0                | -             |

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mid-1980s, has not yet reached Nigeria, unlike other African countries including Kenya, Tanzania, Malawi and South Africa. The absence of H58 isolates in the sampled area of Nigeria is an important finding. It has been postulated that H58 S. Typhi originally emerged in Asia, but subsequently entered Africa on a number of distinct occasions where they have gone on to cause large typhoid outbreaks [12]. Thus, it is likely that H58 S. Typhi will reach Nigeria in the future, potentially changing the epidemiology of the disease in the region and molecular surveillance could be used to monitor for this.

Nevertheless, MDR S. Typhi are common in the regions of study despite the absence of H58 microorganisms. This is an important observation, as the MDR phenotype in other regions of the world has been driven by the spread of MDR S. Typhi H58. Many of the Nigerian S. Typhi, including those of genotype 3.1.1, harbored IncHI1 plasmids that have been previously associated with S. Typhi of other genotypes, particularly H58 [12, 39]. This is consistent with a genetic compatibility between S. Typhi and such plasmids. Interestingly, genetic analysis indicates that an IncHI1 plasmid recently transferred between 3.1.1 and 0.0.3 Typhi within the study region. However, several other plasmids of distinct incompatibility types were also detected within the sampled S. Typhi and it will be interesting to see if any of these are common elsewhere in Nigeria or whether they solely persist within these study sites.

Mutations associated with resistance to quinolones were relatively rare within the sample set. This could be because fluoroquinolones are not commonly used to treat typhoid in these regions, or alternatively, it may be that such mutations have not become fixed in these non-H58 isolates. Further studies on the use of fluoroquinolones are warranted.

In conclusion, it is clear that typhoid associated with MDR S. Typhi is common in these parts of Nigeria and that the MDR phenotype is evolving independently of haplotype H58, which has emerged elsewhere in the world where typhoid is endemic.

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Supporting Information

S1 Fig. Global distribution of African S. Typhi isolates analyzed in this study. A maximum likelihood tree of 1,960 S. Typhi isolates from 23,300 SNPs surrounded by colored rings representing the geographic origin of 502 African isolates, according to the legend. 128 Nigerian isolates are highlighted in black (122 = Abuja) and grey (6 = Kano); neighboring African countries labeled by black arrows. The genotypes of the Nigerian isolates are labeled in red with the old Roumagnac haplotypes [11] in parentheses (red * denotes untypeable Nigerian strains). The 4.3.1 (H58) subclade is indicated in red italics. Branch lengths are indicative of the estimated substitution rate per variable site.

(TIF)

S1 Table. Details of the 128 Nigerian Salmonella Typhi isolates used in the study (see excel sheet).

(XLS)

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