Host, pathogen, and geographic drivers of major *Salmonella* serovars in rural and urban Kenya

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Abstract

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Introduction: *Salmonella* serotypes are among the leading causes of bacterial infections in Africa, contributing to both diarrheal and blood stream disease. This dissertation comprises two studies to address the clinical epidemiology of non-typhoidal *Salmonella* (NTS) in rural Kenya (Aim 1) and the spatial and environmental epidemiology of typhoid fever in a slum in urban Kenya (Aim 2).

Methods: Both studies involved data from large community-based and clinic surveys. For Aim 1, we selected a sub-cohort of individuals with NTS in whom we compared characteristics of those with NTS bacteremia to those with NTS diarrhea using logistic regression models. Cofactors assessed included those related to host immunity and co-infection (HIV status, malaria, malnutrition, and age) and pathogen-related risk factors (drug resistance, serotype, and multi-locus sequence type). NTS isolates were genotyped to investigate genetic differences in strains causing diarrheal versus bacteremic infection. For Aim 2 we conducted a spatial case-control study to determine host and geographic risk factors for typhoid fever among individuals residing in an informal urban settlement (Kibera) in Nairobi, Kenya. We used both logistic regression and spatial regression models to test whether differences in topography, a proxy for the downstream flow and accumulation of fecal contamination, explain the observed geographic pattern in risk of typhoid fever.

Results: For Aim 1 we found that multi-drug resistant (MDR) non-typhoidal *Salmonella* (NTS) was associated with NTS bacteremia compared to NTS diarrhea, controlling for host-cofactors. NTS bacteremia was also associated with younger age and HIV infection. The association of MDR with NTS bacteremia was present in stratified analyses of HIV-infected and uninfected individuals, with a stronger association among HIV negative individuals. We observed presence of STS313 in both NTS bacteremia and diarrhea. For Aim 2 we found that the risk of typhoid fever was geographically heterogeneous across a small area within the Kibera informal settlement, with greater risk in the lower elevation areas compared to high elevation areas. The association with low elevation was seen in children but not adults.

Conclusion: Our findings from Aim 1 suggest that multi-drug resistance is a key driver of NTS invasiveness, beyond the effects of host-immune function. Whether certain MDR resistant NTS lineages are, in fact, more virulent will need to be confirmed in further study. Our observation of STS313 in diarrhea cases is novel and merits evaluation in a larger group of individuals with NTS diarrhea. Our findings from Aim 2 suggest environmental transmission of typhoid fever in children, but not adults, likely due to more frequent exposure to environmental pathogens. Further investigation is needed to differentiate between possible sources of environmental risk, such as contaminated drinking water versus direct contact with contaminated environmental media like open sewers.

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Dedication

For my grandparents, Abraham and Guta Brum, Esther and Joe Akullian; and for my wonderful parents, David Akullian and Lillie Brum; and for my siblings, Michael Lukas and Anna Akullian; and to Molly:

Thank you for making me who I am.

CHAPTER 1: Introduction

Introduction

This dissertation addresses two major scientific questions related to the clinical and environmental epidemiology of common *Salmonella* serovars causing invasive disease in Africa.

First, non-typhoidal *Salmonella* (NTS) is a leading cause of blood stream infection in Africa despite being commonly associated with self-limited gastroenteritis. The biological mechanisms that contribute to invasiveness in NTS in Africa are unclear. In Chapter 2, we address the following question: *What causes invasiveness in non-typhoidal Salmonella?*

Second, recent data suggest that typhoid fever causes a large burden of disease among children in urban areas of Africa. How typhoid fever is transmitted in these settings, however, has received little attention. In Chapter 3 we address the following question: *Does environmental transmission contribute to the risk of typhoid fever in Africa?*

We use data from two major disease surveillance cohorts, one rural and one urban, to test hypotheses related to these questions.

Salmonella enterica and its pathogenesis

Salmonella enterica is comprised of over 2,400 serovars that span an evolutionary spectrum from broad-host range serovars, associated primarily with self-limited gastro-enteritis, to host-adapted and host-restricted serovars that cause more severe, bloodstream infections. *Salmonella enterica spp.* contain 21 *Salmonella* pathogenicity islands (SPIs), which are clusters of genes involved in host invasion, evasion of the host immune response, persistence, and transmission, and are involved in determining host range [1]. *Salmonella's* ability to produce extra-intestinal infection in warm-blooded hosts is a complex phenotype that cannot be explained by its acquisition or deletion of a single virulence determinant [2]. *S.* Typhi, for example, does not elicit recruitment of neutrophils in the small intestine, which is common to NTS serovars that cause self-limiting diarrhea in the host. *S.* Typhi is able to translocate across epithelial layers of the gut and invade into deeper tissues as a part of systemic infection leading to persistent bacterial infection of the gall bladder and prolonged duration of illness [3]. The inflammatory response produced by NTS in the gut, on the other hand, is key to its competitive advantage over other bacteria [4]. The inflammatory response furthermore induces the shedding of NTS in excreta to ensure its continued transmission.

Salmonella enterica evolved over millennia to cause a diverse array of disease pathologies in a variety of hosts. Distinct virulence properties evolved in both typhoidal and non-typhoidal serovars of *Salmonella* to increase the organism's ability to adapt and survive in the human host. All phylogenetic lineages of *Salmonella* contain virulence factors responsible for intestinal invasion of host epithelial cells [5]. Only typhoidal serovars have adapted to the human host to allow for extra-intestinal, blood stream infection. The genomes of human-host restricted *Salmonella* (vars *S.* Typhi and *S.* Paratyphi A, B, and C) differ remarkably from the broad-host adapted serovars in the large amount of genome degradation present in these host-restricted serovars. These pseudogenes code for pathways involved in gastrointestinal infection and the ability to infect a broad host range. *S.* Typhi, for example, has mutations in a number of fimbrial operons, rendering it unable to adhere to the epithelium of any host except humans. The loss of this pathway is hypothesized to select for virulence factors that cause systematic disease and chronic carriage. About 90% of the genome of *S.* Typhi and *S.* Typhimurium are identical; the remaining 10% of the genes that differ are involved in the unique pathogenic properties of each type of *Salmonella* [6]. These genetic distinctions account for the ability of NTS to invade a broad host range and for typhoidal species to persist in the human adapted niche.

In Africa non typhoidal serovars of *Salmonella* have become a major causes of bloodstream infection, an epidemiologic anomaly that has raised questions about whether certain lineages of NTS are evolving to become more adapted to the human host and hence cause disease in humans through direct human to human transmission.

What causes invasiveness in non typhoidal Salmonella?

Though NTS is normally associated with self-limiting gastroenteritis in humans, it is a leading cause of bloodstream infection in Africa [7]. In some areas of Africa it exceeds typhoid fever and malaria as the primary cause of febrile illness [8]. NTS bacteremia is most common among HIV infected adults and children between 12 and 16 months of age in Africa [9-11], suggesting the potentially important role of host immunity in containing NTS infection. Previous studies have identified multiple host factors associated with NTS invasiveness [10-27], including HIV in adults [10, 21, 22, 26, 27] and malaria in children [12, 14, 18, 23-25]. These studies have been limited in their ability to draw precise or unbiased inference on the association between host/pathogen factors and NTS invasiveness due to methodological constraints, especially selection bias, outcome misclassification, and poorly specified control groups.

Over the past 50 years a novel lineage of *Salmonella enterica* var Typhimurium emerged as a common cause of bacteremia in Africa. The emergent strain, ST313, has undergone genome degradation and many

of the degraded genes are also degraded in human adapted strains [28-37]. The large burden of HIV/AIDS in Africa is hypothesized to have provided a unique immunological niche for NTS to become more human host adapted [29, 30, 35]. Recent evidence, however, has demonstrated a lack of human host specificity for these specific strains [37], and preliminary studies have found ST313 in the stool of patients presenting with diarrhea. Still, the emergence of host specificity may be more of a continual process whereby generalists evolve to first become host-adapted and then host-restricted, with genome degradation as a consequence [9]. Further complicating the epidemiologic narrative of NTS and invasiveness, these emerging strains of NTS have also co-selected for multi-drug resistance. Whether NTS invasiveness is conferred from host co-morbidity, novel virulence properties, or clinical factors related to treating resistant infections is unclear.

Gaps thus remain in our understanding of *Salmonella* pathogenesis in Africa. In Chapter 2 we address specific host and pathogen risk factors leading to blood stream infection from non-typhoidal serovars of *Salmonella* in rural Kenya. Our study was specifically designed to test for associations with invasiveness in NTS. Thus, we compared the distribution of host and pathogen factors between individuals presenting with NTS bacteremia and patients presenting with NTS diarrhea. Data from a large population-based infectious disease surveillance cohort in western Kenya were used to examine the association between multi-drug resistance and NTS bacteremia independently from the effects of host comorbidity, including HIV in adults and malaria in children. Similarly, we evaluated the effect of host-comorbidity on NTS bacteremia independent of MDR. We also assessed for the presence of the emergent genotype ST313.

We found MDR to be associated with NTS bacteremia, controlling for multiple host-factors. This association held across sub-groups defined by host- and pathogen- co-factors. The implications of these results are twofold. First, our results suggest that multi-drug resistant NTS is associated with invasiveness beyond the effects of HIV. HIV is known to be a risk factor for invasive NTS and HIV positive individuals (many of whom receive daily Cotrimoxazole prophylaxis) are more commonly infected with drug resistant bacteria. Despite this, HIV status does not seem to explain the association between MDR and invasiveness. Virulence-related factors associated with MDR are a more likely explanation. Second, the association between MDR and invasiveness exists in both *S.* Typhimurium and *S.* Enteritidis, suggesting that genetically conferred virulence is not specific to one lineage or serotype of *Salmonella* but may be shared across species. Certain emerging lineages of NTS have acquired a virulence plasmid that confers both multi-drug resistance and virulence-associated properties thought to promote the extra-intestinal growth of *Salmonella*e. Virulence plasmids are more commonly found in blood stream isolates than fecal isolates of *S.* Typhimurium and *S.* Enteritidis, highlighting their potential

role in the pathogenesis of *Salmonella* bacteremia. Further investigation is needed to confirm the role of plasmid-mediated virulence in common serotypes of *Salmonella* causing invasive disease in Africa.

Does environmental transmission contribute to the risk of typhoid fever in Africa?

In Chapter 2 we use a spatial case-control framework to map the spatial distribution of typhoid risk in a small, densely populated area of the Kibera slum and test whether the spatial patterns in risk can be explained by differences in environmental factors such as topography and the accumulation of high contaminated waste-water.

The transmission of typhoid fever is dependent on the direct contact with the stool of an infected individual. Recent genetic evidence from urban areas of Asia, however, suggest that *indirect* transmission via exposure to contaminated surface water may also contibute to the risk of infection [38]. Indeed, the greatest risk of typhoid fever has been associated with residing near large water bodies and in low elevation areas [39, 40]. Low elevation areas of drainage basins accumulate high concentrations of water-borne pathogen and water-carried pathogens, [41, 42], drawn from point sources on land as well as those resuspended from attachment surfaces by surface runoff [43]. Recent evidence suggests a large burden of typhoid fever in urban Africa, although data are lacking on its epidemiology in high burden areas. It is unknown whether indirect transmission via hydrological variables plays a roll in driving typhoid risk in densely populated urban slums, where direct transmission of enteric infectious diseases is common.

We used a spatial case-control study design to test for spatial patterns in the risk of typhoid fever across the study area. Case-control studies of spatially referenced data provide an efficient way to estimate the spatial distribution of cases relative to the population at risk, with the advantage of simultaneously adjusting for spatially varying covariates that might explain the observed clustering [44, 45]. Case-control studies in spatial epidemiology have the further advantage of avoiding the problem of ecological bias, in which area-level disease-exposure associations are misinterpreted as occurring at the individual-level [46]. We used a population-based infectious disease surveillance platform to enumerate all cases of typhoid fever presenting to a central clinic and a corresponding realization of controls to estimate the spatial clustering of risk.

Our results suggest a large degree of spatial heterogeneity in the risk of typhoid fever in an urban slum, with higher risk of infection in the lower elevation areas relative to the upstream areas. This pattern was especially pronounced in children under ten and was explained, in part, by differences in elevation across the study area. The fact that a similar spatial pattern was not observed among individuals over ten

suggests differing modes of transmission between children and adults/adolescents. Children may be more likely to be exposed to contaminated environmental media in the immediate surroundings of the household whereas exposure in adults may occur far from the household. These results add to a growing body of literature suggesting that downstream sources of environmental contamination serve as important reservoirs of infection for typhoid fever. Our results furthermore indicate that such contamination poses a disproportionate risk of transmission in children.

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CHAPTER 2: Multi-drug Resistant Non-Typhoidal *Salmonella* Associated with Invasive Disease in Western Kenya, 2007 - 2014

Multi-drug Resistant Non-Typhoidal *Salmonella* Associated with Invasive Disease in Western Kenya, 2007 – 2014

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Abstract

Background: Non-typhoidal *Salmonella* (NTS) is a leading cause of bloodstream infections in Africa, but it is unclear whether invasiveness is driven by host susceptibility or pathogen virulence.

Methods: Using a large population-based surveillance cohort, we compared individuals with NTS bacteremia to those with NTS diarrhea with respect to host factors including HIV, malaria and malnutrition, and pathogen-related cofactors including the presence of multi drug resistance (MDR) (resistant to trimethroprim-sulfamethoxazole (TMP-SMX), ampicillin, and chloramphenicol), serotype, and sequence type. Univariate and multivariate logistic regression models were used to test for associations with invasiveness. *S.* Typhimurium isolates were also genotyped to examine phylogenetic relationships with clinical case type.

Results

Between January 1, 2007 and December 31, 2014, 235 unique episodes of NTS bacteremia (117 under age five) and 70 episodes of NTS diarrhea (15 under age five) were recorded at Lwak Mission Hospital in western Kenya. Children under five years of age with NTS bacteremia were older than those with NTS diarrhea (1.9 vs. 1 year, P = 0.003. Among individuals over five, HIV prevalence was higher among those with NTS bacteremia than NTS diarrhea (69.8% versus 38.5%, P < 0.001). MDR NTS was associated with NTS bacteremia in both children <under age five, [OR = 23.1 95% CI 4.66 – 115), P < 0.001] and older individuals [OR = 11.5 (95% CI 4.86 – 27.0), P < 0.001], adjusted for age and year of diagnosis. This association persisted among both HIV positive [OR = 7.19, 95% CI (1.11 – 46.4), P = 0.038] and HIV negative individuals [OR = 22.1, 95% CI (2.62 – 187), P = 0.004]. *S.* Typhimurium strains corresponded primarily to an emergent sequence type ST313 and clustered into two lineages (one MDR and one drug susceptible). Sequence type ST313 was isolated from both bacteremia and diarrheal patients.

Conclusions

Multi-drug resistance in NTS is independently associated with increased risk of bacteremia compared to diarrhea across dominant serotypes and among both HIV positive and HIV negative individuals. Whether the association between MDR NTS and blood stream infection is the result of virulence properties conferred at the genetic level or results from antibiotic treatment failure warrants further investigation.

INTRODUCTION

Non-typhoidal *Salmonella* (NTS) is a leading cause of bacteremia in Africa. Whereas NTS commonly manifests as self-limiting diarrhea in immune-competent individuals [47], NTS bacteremia occurs at high rates in young children and HIV-infected adults in sub-Saharan Africa [3]. Recent evidence suggests that the increase in NTS bacteremia in Africa is associated with the emergence of novel NTS strains that have undergone considerable genomic changes associated with enhanced virulence and multi-drug resistance [29, 35]. Whether these novel strains cause bacteremia in the absence of host-comorbidity is largely unknown. It remains unclear whether high rates of NTS bacteremia in Africa are associated with diminished host immune function or pathogen-specific resistance and virulence factors.

In sub-Saharan Africa, NTS bacteremia incidence is increased in children (175–388 cases per 100,000) and adults with HIV infection (2000–7500 cases per 100,000) [3], highlighting the important potential role of acquired immunity in limiting invasive disease [11]. HIV infection may predispose to bacteremia via compromised gut mucosa, deregulated cytokine production, and impaired immune responses [9]. In addition, HIV-infected individuals often receive trimethroprim-sulfamethoxazole (TMP-SMX) as a daily prophylactic antibiotic to prevent opportunistic infections [48]. The widespread use of low-dose CTX prophylaxis in HIV-infected individuals may select for multi-drug resistant strains of NTS, a phenomenon that has been shown in other pathogenic as well as commensal bacteria [49, 50].

Among children, NTS bacteremia is most common in those 4-16 months of age, when anti-*Salmonella* IgG titers are at nadir levels [47]. Invasive NTS is less common among infants below 4 months of age, presumably due to the protective effects of transplacental and colostrum antibodies, and lack of exposure to contaminated water when children are exclusively breastfeeding [11]. Pediatric HIV, malnutrition, and malaria [13, 15] are also common among children with NTS bacteremia [12, 25]. Hemolytic anemia, a hallmark of malaria infection, reduces macrophage microbicidal activity, limiting the ability of macrophages to suppress *Salmonella* infection. Malaria infection may also play a role in the dissemination of NTS in the bloodstream by reducing serum Il-12 [12, 14, 51]. No study has tested for independent associations between host co-infection and NTS bacteremia independent of pathogen-specific factors like multi-drug resistance (MDR).

Multi-drug resistant (MDR) NTS (defined as NTS resistant to TMP-SMX, ampicillin, and chloramphenicol) has emerged among *Salmonella* vars Typhimurium and Enteritidis in Africa [7, 13, 15, 20, 29, 30, 32]. Emergent lineages of *S.* Typhimurium (ST 313 and ST 302) have acquired drug

resistance and may cause bacteremia in the otherwise healthy host [29, 30, 37]. However, it is not clear if MDR strains of NTS increase risk of invasiveness [52].

Using a large population-based surveillance cohort in Kenya, we compared individuals with NTS bacteremia to those with NTS diarrhea to elucidate host and pathogen-related cofactors of NTS invasiveness. We specifically evaluated the role of MDR NTS and a unique MDR clone of *S.* Typhimurium (ST313).

METHODS

Study Site

This study was reviewed and approved by the Institutional Review Boards of the Centers for Disease Control and Prevention (CDC) and the Kenya Medical Research Institute (KEMRI). This study was nested within an ongoing population-based infectious disease surveillance system conducted since late 2005 by the Centers for Disease Control and Prevention's (CDC) International Emerging Infections Program (IEIP) and the Kenya Medical Research Institute (KEMRI). The cohort consists of approximately 25,000 individuals of all ages residing in Asembo, a rural region of western Kenya located in Bondo District on Lake Victoria (Figure 1). Asembo has a low overall population density of 325 persons per square kilometer, with houses distributed in small clusters alongside cultivated fields. The region is characterized by intense, year-round malaria transmission and an HIV prevalence of 15–17% [15]. Participants enrolled in the surveillance system can access free health care at the Lwak Mission Hospital, a centrally located clinic staffed mostly by study-supported and trained personnel.

The design of the IEIP surveillance system and inclusion criteria into the longitudinal study have been described previously [53]. In brief, inclusion into the surveillance study requires the following: 1) currently reside within a village whose epicenter is located no more than 5 kilometers from the Lwak Mission Hospital in Asembo, 2) have resided permanently in the area for more than four calendar months, 3) have been registered into the KEMRI/CDC Demographic Surveillance System (DSS) [54] and 4) have provided written informed consent/assent. In 2006, at the beginning of the study, the enrolled population consisted of approximately 25,000 people residing in 6,000 households within 33 villages.

Inclusion criteria

The sample flow chart for inclusion into our study is shown in Figure 2. All individuals in the IEIP surveillance cohort (n = 25,000) are visited once every fourteen days by community interviewers (CIs) at their households and are administered a household morbidity survey (HMS) to check for signs and

symptoms related to a number of infectious diseases, including respiratory symptoms, influenza-like illness, diarrhea, and fever. Individuals reporting symptoms from the previous 2 weeks prior to interview are encouraged to visit Lwak Mission Hospital for clinical follow-up. Screening criteria for suspected bloodstream and diarrheal infection have been described previously for the CDC disease surveillance cohort [15].

NTS bacteremia:

Any one of the following criteria was necessary to meet indication for blood culture: 1) severe acute respiratory illness (SARI), 2) acute febrile illness (temperature > 38.0 degrees C), 3) jaundice, or 4) hospital admission (whether or not fever was present). Due to the large number of individuals with febrile illnesses reporting to Lwak Mission Hospital, only the first two individuals over/under age five presenting with acute febrile illness each day were screened by blood culture. Some individuals meeting inclusion criteria did not receive a blood culture for the following reasons: 1) the physician did not recognize that the patient met eligibility criteria, 2) the patient refused blood or stool culture, or 3) an inpatient had already received intravenous antibiotics. NTS bacteremia cases were defined as those who tested blood culture positive for at least one NTS serotype. Individuals who tested positive for typhoidal serotypes were excluded from the analysis.

NTS diarrhea:

All individuals presenting with diarrhea (three loose/bloody/watery stools in a 24 hour period) were offered a stool culture and those testing positive for NTS were included in this analysis. Individuals who tested blood and stool culture positive for NTS were classified as an NTS bacteremia cases. Repeat visits within one month of the original NTS diagnosis that were NTS culture positive were also excluded.

Clinical measurements

Malnutrition:

Height for age z-scores (HAZ) and weight for height z-score (WHZ) cut-offs of -2.0 were used to classify wasting and stunting, respectively, in children under 5 years of age based on 2006 WHO child growth standards [55]. Z-scores above 10.0 or below -10.0 were excluded due to likely recording errors of biometric measurements.

HIV:

HIV status was ascertained on a subset of NTS patients through two community-level HIV surveys conducted on all consenting individuals > age 13 present in the disease surveillance cohort during either of the two HIV surveys in 2008/2009 and in 2013. Children under 13 years of age were only tested if their parent was HIV positive. HIV data from these two community-level surveys were then linked to the clinic database through patient records.

Current malaria parasitemia:

Blood smears for malaria were offered to all individuals presenting with acute febrile illness. Blood smears were read by trained KEMRI/CDC microscopists on site. Current malaria at the time of NTS diagnosis was identified as the presence of asexual stages of *Plasmodium falciparum* on microscopy using thick smear.

Bacterial culture and identification

Laboratory methods for culturing bacterial pathogens from blood have been described previously [15]. Briefly, blood (7–10 ml for adults and 1–3 ml for children) was inoculated into BACTEC culture vials and incubated in an automated BACTEC 9050 at 35uC for 1–5 days. Any solution with growth was then plated, Gram-stained, and sub-cultured onto standard selective media. Stool specimens were processed according to standard protocols described elsewhere [56]. Colonies of *Salmonella* were identified by morphology and confirmed by biochemical typing and serotyping.

Antimicrobial susceptibility:

Antimicrobial susceptibility was measured on each isolate based on the minimum inhibitory concentration (MIC) to prevent growth of the bacteria after incubation for 16 hours. Susceptibility was classified according to three MIC cutoff values (resistant, intermediate, and susceptible) for non-typhoidal *Salmonella* as defined by CLSI [57]. Susceptibility testing was performed for the following anti-microbials: chloramphenicol, trimethroprim-sulfamethoxazole (TMP-SMX), tetracycline, ciprofloxacin, nalidixic acid, ampicillin, sulfisoxazole, streptomycin, kanamycin, gentamycin, teftiazone, and amoxicillin-dayulinic acid. Multi-drug resistance (MDR) was defined as resistance to chloramphenicol, TMP-SMX, and ampicillin [15]. For purposes of analysis, NTS isolates were classified as resistant versus non-resistant (which included susceptible and intermediate resistance).

Genotyping:

To isolate genomic DNA for sequencing, strains were grown overnight at 37°C with shaking in 3 mL of Luria Broth (BD Biosciences, USA). Genomic DNA was isolated using Gentra Puregene Yeast/Bact. Kit (Qiagen, Valencia, CA), according to manufacturer's directions. For each genome standard Illumina Nextera or Nextera XT libraries were constructed according to manufacturer's guidelines (Illumina Inc., San Diego, CA). Prior to the Nextera XT library normalization/denaturation step, double stranded libraries were normalized with the Invitrogen SequalPrep Normalization Plate Kit (Thermo Fisher Scientific/Life Technologies, Grand Island, NY). Paired-end libraries for each genome were used to generate 100 bp or 150 bp reads with the Illumina HiSeq 2000 or MiSeq,. Sequencing of libraries was performed according to manufacturer's standards (Illumina Inc., San Diego, CA). Sequence reads were aligned to the seven reference gene fragments used in the *Salmonella enterica* multilocus sequence typing scheme (MLST) described by Achtman et al. (2012) [58] to determine sequence types as described previously [59].

Statistical analysis

Logistic regression with robust standard errors was used to compare the odds of host and pathogen exposures between NTS bacteremia and NTS diarrhea cases in univariate and multivariate models. Multivariate models were stratified on age over and under five years and were adjusted for potential confounders defined *a priori,* including age (continuous), year of diagnosis, and multi-drug resistant phenotype. Adjusted odds ratios were further stratified on HIV status to estimate the independent effects of pathogen-specific factors on clinical outcome in both HIV positive and HIV negative groups.

RESULTS

Study population

Between January 1, 2007 and December 31, 2014 there were 140,941 visits to Lwak Mission Hospital (24,748 unique individuals), of which 22,182 (15.7%) presented with acute febrile illness without diarrhea, 2,376 (1.7%) presented with both acute febrile illness and acute diarrhea, 9,711 (6.9%) presented with acute diarrhea without fever, and 106,672 (75.7%) presented with neither. Figure 1 shows the numbers of clinic visits in which an individual was screened by blood and/or stool culture, as well as rates of culture positivity and NTS positivity in each of the four symptom groups. Among 15,114 individuals screened by blood culture, 1,549 (10.3%) tested positive for at least one bacterial pathogen, of which 273 (17.6%) were NTS. Among 1,856 individuals screened by stool culture, 648 (34.9%) were culture positive for one or more bacterial pathogens, of which 101 (15.6%) were NTS.

Over the 8-year surveillance period, 305 visits (from 297 unique individuals) met the inclusion criteria for NTS, of which 70 were diarrheal NTS isolates and 235 bloodstream NTS isolates. Twenty-two secondary episodes of NTS cultured from blood, (occurring within one month of the index episode), were excluded. Sixteen additional episodes originally classified as NTS bacteremia by serotyping were excluded based on testing positive for *S.* Typhi in subsequent genotypic analysis. Among stool culture positive NTS isolates, 14 were excluded due to a secondary case of NTS bacteremia occurring in the same individual within one month of the initial index case, and 17 were excluded due to a secondary case of diarrheal NTS occurring within one month of the initial case.

Comparison of demographic, clinical, and pathogen-specific factors between NTS bacteremia and NTS diarrhea cases

Demographics and presenting symptoms:

NTS bacteremia cases occurred in similar frequency among females and males (54.0% versus 45.7%, P = 0.221). Self-reported fever was more common in patients presenting with NTS bacteremia (95.7%) than in those with NTS diarrhea (62.9%) while documented fever (≥ 38°C) was more common among those presenting with NTS bacteremia (77.8%) than among those presenting with NTS diarrhea (77.8% versus 14.3%). Although diarrhea at presentation was an indication for stool culture, not all individuals who received a stool culture reported diarrhea associated with their illness. However, almost all (92.9%) NTS diarrhea patients reported diarrhea, as compared to 25.2% of patients presenting with NTS bacteremia.

Malnutrition and malaria:

Stunting and wasting among children under five was comparable between those with NTS diarrhea and NTS bacteremia (stunting: 42.9% versus 30.4%, respectively, p = 0.343 and wasting: 23.1% versus 14.6%, respectively, p = 0.422), and differences were not statistically significant.

HIV:

HIV status was ascertained in 87 individuals (60 with NTS bacteremia and 27 with NTS diarrhea), of whom 83.9% were over age five. Those who received an HIV test tended to be older (median age 29.3 years, IQR 12.1–41.3) compared to those who were never tested (median age 4.0, IQR 1.7–16.3). HIV positive individuals tended to be older than those who tested HIV negative (median age 30.9 versus 19.1 years, p = 0.184). NTS bacteremia cases were more likely to be HIV positive compared to NTS diarrhea cases (65.0% versus 37.0%, respectively, P = 0.015) (Table 2). As compared to HIV negative individuals, HIV positive individuals over age five were more likely to be infected with NTS resistant to

TMP-SMX (84.8% versus 52.6%, P = 0.001), and with multi-drug resistant (MDR) NTS (71.7% versus 47.4%, P = 0.023).

NTS serogroup:

Salmonella groups B (*S.* Typhimurium) and D (*S.* Enteritidis) were the most common serogroups isolated from individuals presenting with NTS bacteremia (66.0% and 33.2%, respectively) and individuals presenting with NTS diarrhea (45.7% and 20.0%, respectively) (Table 2). *Salmonella* groups C1 and C2 were exclusively isolated from NTS diarrhea cases and comprised 24.3% of the diarrheal NTS serogroups (Table 2).

Antibiotic resistance:

Multi-drug resistance was a common phenotype in both *Salmonella* vars Typhimurium and Enteritidis and absent in *Salmonella* serogroups C1/C2. MDR NTS was more likely to be isolated from cases of NTS bacteremia as compared to cases of NTS diarrhea (80.3% versus 23.1%, respectively, P < 0.001) (Table 2),

Over the 7-year study period, the prevalence of MDR among blood culture group D isolates increased substantially, from 0% in 2008 to over 90% in 2010, and then decreased to 50% by 2014 (Table 1). This increase was temporally associated with increases in the crude number of blood culture positive *S.* Enteritidis isolates, which increased from 1 case in 2008 to 31 cases in 2010. The prevalence of MDR *S.* Typhimurium from blood cultures vacillated between 60 – 100% over the course of the study period and displayed no temporal association with the crude number of cases of *S.* Typhimurium bacteremia.

Age-stratified analysis

Under age five:

There were 117 cases of NTS bacteremia and 15 cases of NTS diarrhea identified in children under five. In this subset of individuals, NTS bacteremia patients were older than NTS diarrhea patients (median age 1.9 versus 1.0 years, P = 0.003) (Table 2). There was no association with HIV or malaria in this subset, although few of these children were tested for HIV (only 13 children under five with NTS bacteremia and one child with NTS diarrhea) (Table 3). The prevalence of multi-drug resistance (MDR) was higher in children with NTS bacteremia than NTS diarrhea (83.3% versus 15.4%, P < 0.001). In multivariate analysis, after adjusting for age and year of diagnosis, MDR NTS was more highly associated with NTS bacteremia than NTS diarrhea [OR = 23.1, 95% CI 4.66 – 115, P < 0.001] (Table 3). The association

between MDR and NTS bacteremia furthermore appeared to be modified by serotype. Among those under age five who were infected with *S.* Typhimurium, 83.1% of the NTS bacteremia isolates and 33.3% of the NTS diarrheal isolates were MDR, [aOR = 9.96, 95% CI (1.31 – 75.6), P = 0.026]. In contrast, the prevalence of MDR in *S.* Enteritidis was 88.6% among NTS bacteremia isolates, while no MDR was detected from NTS diarrheal isolates in this group (Table 3).

Age five and over:

Among individuals age five and over, there were 118 cases of NTS bacteremia and 55 NTS diarrhea cases. NTS bacteremia cases tended to be younger in this subset (median age 23.0 versus 30.5 years, p=0.044). Age was not preferentially associated with NTS bacteremia versus NTS diarrhea [aOR = 0.99, 95% CI 0.98–1.01, p= 0.479], however, after adjusting for year of diagnosis and multi-drug resistance (Table 2). HIV-infection was more common among NTS bacteremia cases as compared to NTS diarrhea cases (69.8% versus 38.5%, P < 0.001).

MDR NTS was more highly associated with bacteremia as compared to diarrhea in multivariate analysis, adjusting for age and year of diagnosis [aOR = 11.5 95% CI 4.86 – 27.0, P < 0.001] (Table 2). Similar to those under age five, the magnitude of the association between MDR and NTS bacteremia in those over age five was greater among those infected with *S.* Enteritidis, [OR = 26.5 (2.75 – 256), P = 0.005] as compared to those infected with *S.* Typhimurium, [4.97 (1.74 – 14.2) , P = 0.003]. The association between MDR and NTS bacteremia held among both HIV positive and HIV negative groups. MDR was more common among NTS bacteremia cases as compared to NTS diarrhea cases in HIV positive (86.7% versus 50.0%, P = 0.016) and HIV negative subsets (76.9% versus 12.5%, P < 0.001). The association between MDR and NTS bacteremia furthermore held in multivariate analysis adjusting for age and year of diagnosis, in both HIV positive, [aOR = 7.19 (1.11 – 46.4), P = 0.038] and HIV negative individuals [aOR = 22.1, 95% CI 2.62–187, P = 0.004] (Table 4).

Genotype results

Among 88 genotyped *S.* Typhimurium strains that met a clinical case definition for NTS bacteremia or NTS diarrhea, 87 corresponded to multi-locus sequence type ST313 and one to ST19 (from a bacteremic patient) (Supplemental Table 1). ST313 isolates clustered into two clades represented by strains A130 and D23580 (Figure 3), both of which have been described previously in Malawi and Kenya [35]. There were three identified ST313 strains that corresponded to the chloramphenicol-susceptible clade A130, all of which were isolated from bacteremic patients. Among 84 ST313 isolates clustering with the multi-drug resistant D23580 clade, 81 were from bacteremic patients and 3 were from diarrheal patients.

Almost all (71 of 77) D23580 bacteremia isolates tested for drug susceptibility were multi-drug resistant. The three D23580 diarrheal isolates were all MDR.

DISCUSSION

Infection with non-typhoidal *Salmonella* (NTS) is commonly associated with self-limiting gastroenteritis, although a large burden of NTS bacteremia exists in sub-Saharan Africa [60]. Whether NTS invasiveness is conferred through host or pathogen-specific factors is unclear. Using a large population-based infectious disease surveillance system in western Kenya we evaluated host and pathogen determinants of NTS invasiveness. Consistent with other studies, we found evidence that younger age and HIV infection were associated with NTS bacteremia. We found that the presence of multidrug resistance among NTS isolates was associated with NTS bacteremia. The association between MDR NTS persisted across subgroups defined by age, HIV status, and NTS serotype. These findings suggest that pathogen-specific factors may contribute significantly to NTS invasiveness in Africa and that these factors are not specific to one serotype of NTS.

The emergence of MDR *Salmonella* vars Typhimurium and Enteritidis in Africa parallels a dramatic increase in the incidence of NTS bacteremia in both serotypes, with no changes in incidence of other bloodstream pathogens [30]. In this study the prevalence of MDR varied over time and across serotypes. The prevalence of MDR among *S.* Typhimurium isolates from blood cultures was consistently high, varying from 60 – 100%. The prevalence of MDR *S.* Enteritidis isolates from blood culture, on the other hand, was initially low (0%) and increased to over 90% by 2010. This increase was temporally associated with an order of magnitude increase in the crude number of MDR *S.* Enteritidis isolates, underscoring the possible emergence of an invasive MDR *S.* Enteritidis clone in western Kenya in 2009-2010. Multi-drug resistance in NTS is conferred via a large plasmid that is easily transferable across species [32], and evidence suggests this plasmid has been circulating in Kenya for decades.

There appeared to be some differential association between MDR and NTS bacteremia by serotype, with a larger effect in *S.* Enteritidis compared to *S.* Typhimurium. While this observation has not been shown previously, it may be indicative of the multiple biological mechanisms by which infection with NTS leads to blood stream infection. MDR and its associated virulence may be less important as a determinant in clinical outcome for emerging lineages of *S.* Typhimurium that have been shown to confer virulence at the chromosomal level. In contrast, MDR *S.* Enteritidis may be largely associated with bloodstream infection due to plasmid-mediated virulence. Additional genotypic investigation of both MDR and non-MDR *S.* Enteritidis isolates will be necessary to better understand possible virulence determinants.

This study adds to a growing body of literature suggesting that ST313 is a dominant genotype of *Salmonella* Typhimurium circulating in East Africa. We found clustering of *S.* Typhimurium into two clades, both of which correspond to a unique sequence type ST313 described previously [9, 30, 61]. The two clades of ST313 emerged over the past 50 years - the first clade is represented by strain A130 and is defined by its antibiotic susceptibility and partial genome degradation. The second clade, represented by strain D23580, emerged later and acquired multiple-linked antibiotic resistance determinants encoded by a virulence-associated plasmid necessary for systemic disease [29]. Both clades have been associated with outbreaks of NTS bacteremia in Malawi and Kenya. Genome degradation in *Salmonella* is thought to select for genes involved in blood-stream infection. In our study, MDR D23580 was the most common lineage of *S.* Typhimurium associated with blood-stream invasiveness, and our results support the hypothesis that selection for multi-drug resistance in emerging NTS lineages contributed to the clonal replacement of clade A130 with D23580 [29].

ST313 was also isolated from diarrheal patients in our study, an observation consistent with other recent studies from sub-Saharan Africa [62, 63]. While our study provides further evidence that ST313 is not exclusively an invasive pathotype, the extent to which ST313 contributes to the burden of NTS diarrhea in Africa is not clear [9]. Sequence type ST19 (traditionally associated with self-limited gastroenteritis), was not a common cause of either diarrhea or bacteremia in our study, indicating that ST313 may have replaced ST19 as the dominant lineage of *S.* Typhimurium in Kenya. These results suggest that ST313 can cause bacteremia in HIV negative individuals, a result that has not been previously reported. The occurrence of invasive ST313 has been described almost exclusively in young children and HIV positive individuals, and some hypothesize that ST313 emerged as a result of its adaptation to the immune-compromised host [9]. ST313 may have evolved to occupy a unique ecological niche within HIV positive individuals in Africa, though these results suggest that ST313 can cause invasive disease even in the absence of HIV-related immunosuppression.

Certain emerging lineages of NTS have acquired a virulence plasmid [29, 64] that confers both multi-drug resistance and virulence-associated genotypic properties thought to promote the extra-intestinal growth of *Salmonella*e [65]. Virulence plasmids are more commonly found in blood stream isolates than fecal isolates of *S.* Typhimurium and *S.* Enteritidis [66], highlighting their potential role in the pathogenesis of *Salmonella* bacteremia. Although the selection for antibiotic resistance is often thought to be associated with reduced fitness and impaired virulence in an organism, co-selection for both virulence and resistance determinants is not uncommon in pathogenic bacteria [67] and may occur among emerging lineages of NTS in Africa under the pressure of antibiotic use [29]. The rapid scale-up of low-dose, prophylactic

TMP-SMX use among HIV positive individuals in sub-Saharan Africa over the past 20 years may have contributed, in part, to the selection for community acquired MDR NTS with enhanced virulence properties. Our results suggest that individuals with HIV are disproportionately infected with MDR strains of NTS, a phenomenon that we attribute to individual-level TMP-SMX use. In addition, widespread use of TMP-SMX for prophylaxis may have contributed to the co-selection for MDR and virulence-associated invasive disease at the population-level.

Whether bacteremia from NTS is conferred from virulence properties or antibiotic treatment failure remains unclear, and differentiating between the two biological mechanisms has been a challenge for epidemiologic and genetic studies alike. Several epidemiologic studies, mostly from industrialized countries, suggest that complications from the failure to adequately treat drug resistant NTS infections are important in determining clinical outcomes for NTS. Two studies from industrialized countries, for example, showed that NTS strains resistant to more than one antimicrobial agent were associated with increased risk of severe clinical outcomes, including hospitalization, duration of hospitalization, invasive disease, and death [68, 69]. Evidence from Africa, however, does not support such findings. Another study from Kenya, for example, found no differences in antimicrobial resistance profiles between NTS isolates from bacteremic and diarrhea patients [13], although this study applied a looser definition for NTS bacteremia that included patients presenting with diarrhea and fever and therefore may be subject to outcome misclassification. Another study from outside Africa found no difference in overall mortality between those infected with MDR versus pan susceptible bacterial pathogens [70].

HIV

HIV was more common among patients with NTS bacteremia than those with NTS diarrhea. Multiple previous studies have observed a large burden of NTS bacteremia among HIV positive individuals [7, 21, 71], and the biological mechanisms by which HIV predisposes to disseminated infection are well established. No previous study, however, investigated independent association between HIV and NTS bacteremia compared to NTS diarrhea. Aside from the immune-modulating mechanisms predisposing HIV positive individuals to invasive infection from NTS, selection for highly virulent MDR strains may also contribute to the excess risk of NTS bacteremia among HIV positive individuals in Africa. The association between HIV and NTS invasiveness observed in previous studies may thus be attributed, in part, to the selection for MDR strains among those with HIV, who are more likely to receive prophylactic antibiotics. The smaller association between MDR and NTS bacteremia in HIV positive individuals compared to HIV negative individuals appears to be explained by the higher prevalence of MDR in NTS diarrheal isolates from HIV positive individuals.

We found no evidence of an association between current malaria parasitemia and invasiveness in either children or adults, despite previous study suggesting an association. Several methodological differences may explain this discrepancy. First, we used cases of diarrheal NTS as a comparison group to assess the association between host co-factors and invasiveness from NTS. Other studies, in contrast, have compared NTS bacteremia cases to either non-bacteremia hospital admissions or cases of non-NTS bacteremia. One study from Kenya, for example, compared the prevalence of current malaria parasitemia between pediatric NTS bacteremia patients and pediatric non-bacteremia patients and found a negative association between current malaria parasitemia to be *negatively* and NTS bacteremia. Recent malaria, on the other hand, was found to be positively associated with NTS bacteremia [25]. Clinic-based studies in areas with high rates of malaria hospitalization in children may overestimate the prevalence of current malaria in non-bacteremic patients, thus resulting in a negative association, a phenomenon known as Berkson's bias [72]. In other studies, NTS bacteremia has been compared to other causes of bacteremia with respect to malaria co-infection. One study from the Gambia found that malaria co-infection was more frequent among children presenting with NTS bacteremia (42%) versus typhoid fever (11%) or other causes of bacteremia (6%), $p < 0.001$ [24]. Such studies highlight the specificity of malaria as a correlate of NTS invasiveness compared to other causes of bacteremia.

Our results must be considered in light of some inherent limitations of the study design. There may be misclassification of NTS diarrhea and bacteremia. Secondly, HIV status was ascertained in two community-based HIV prevalence studies, one conducted between 2008-2009 and another conducted in 2013, consisting of a random sample of consenting adults over age 13 and children under 13 with an HIV positive parent. A number of individuals in this study lacked HIV status at NTS diagnosis. We also lacked sufficient sample size on children to test whether HIV is a risk factor for invasiveness. Finally, because this study relied on clinic-based surveillance, differential referral to the study clinic may result in selection bias.

Conclusion

In this study we provide strong evidence of an association between multi-drug resistant NTS and blood stream infection in Kenya. Multi-drug resistant (defined as resistant to TMP-SMX, ampicillin, and chloramphenicol) strains of *S.* Typhimurium and *S.* Enteritis were independently associated with bloodstream infection in children and adults, as well as in HIV positive and HIV negative groups. Consistent with previous study, the genotypic results presented here suggest that *S.* Typhimurium sequence type ST313 is a common cause of NTS bacteremia. This study also highlights the occurrence of ST313 in NTS diarrhea cases. MDR is a major concern for the control of highly pathogenic NTS

serotypes, and whether MDR increases the risk of invasive disease as a result of virulence properties conferred at the genetic level or as a result of antibiotic treatment failure warrants further investigation.

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Figure 1. Study site with geographic extent of the surveillance population shown.

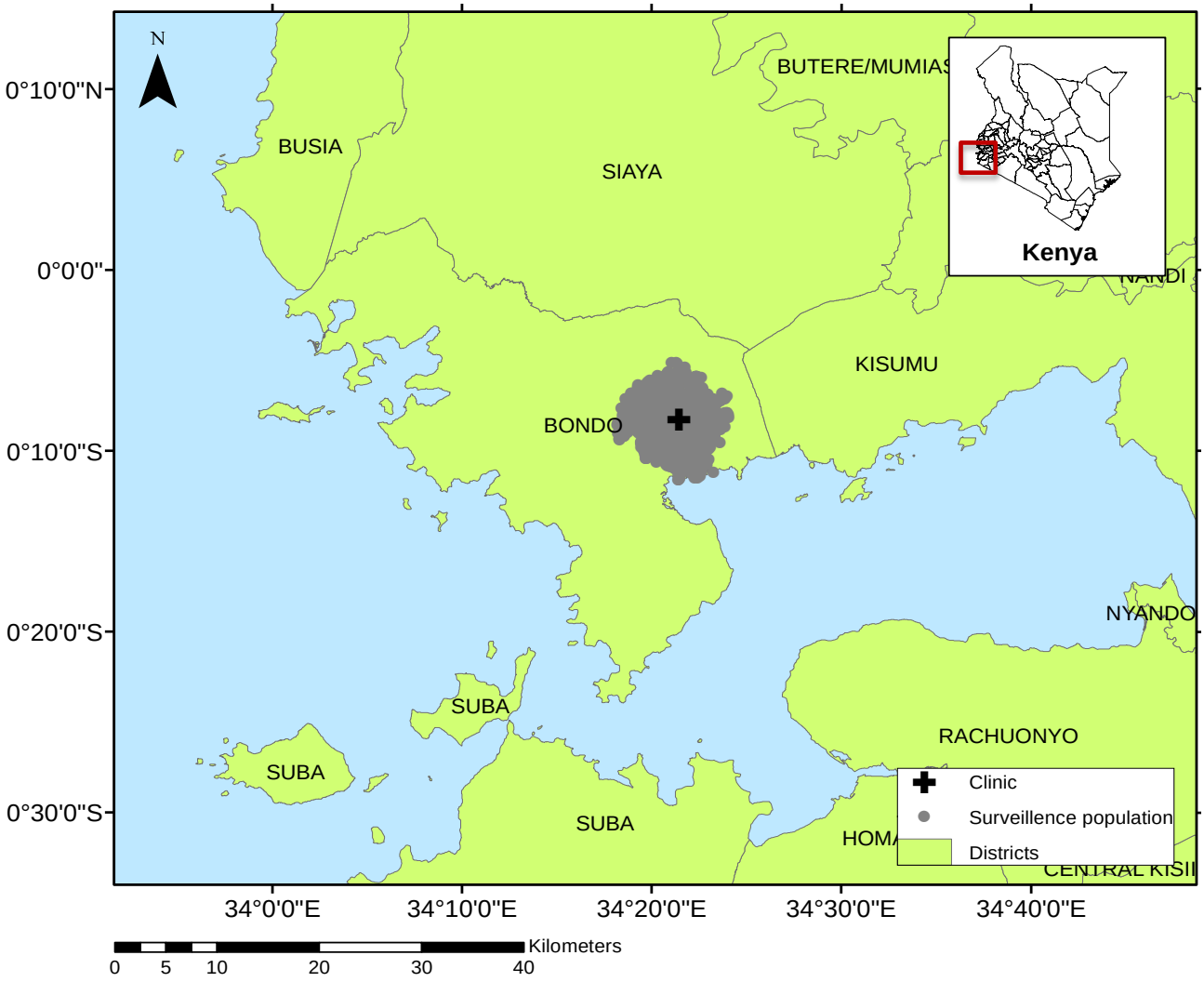

Figure 2. NTS sample flow chart

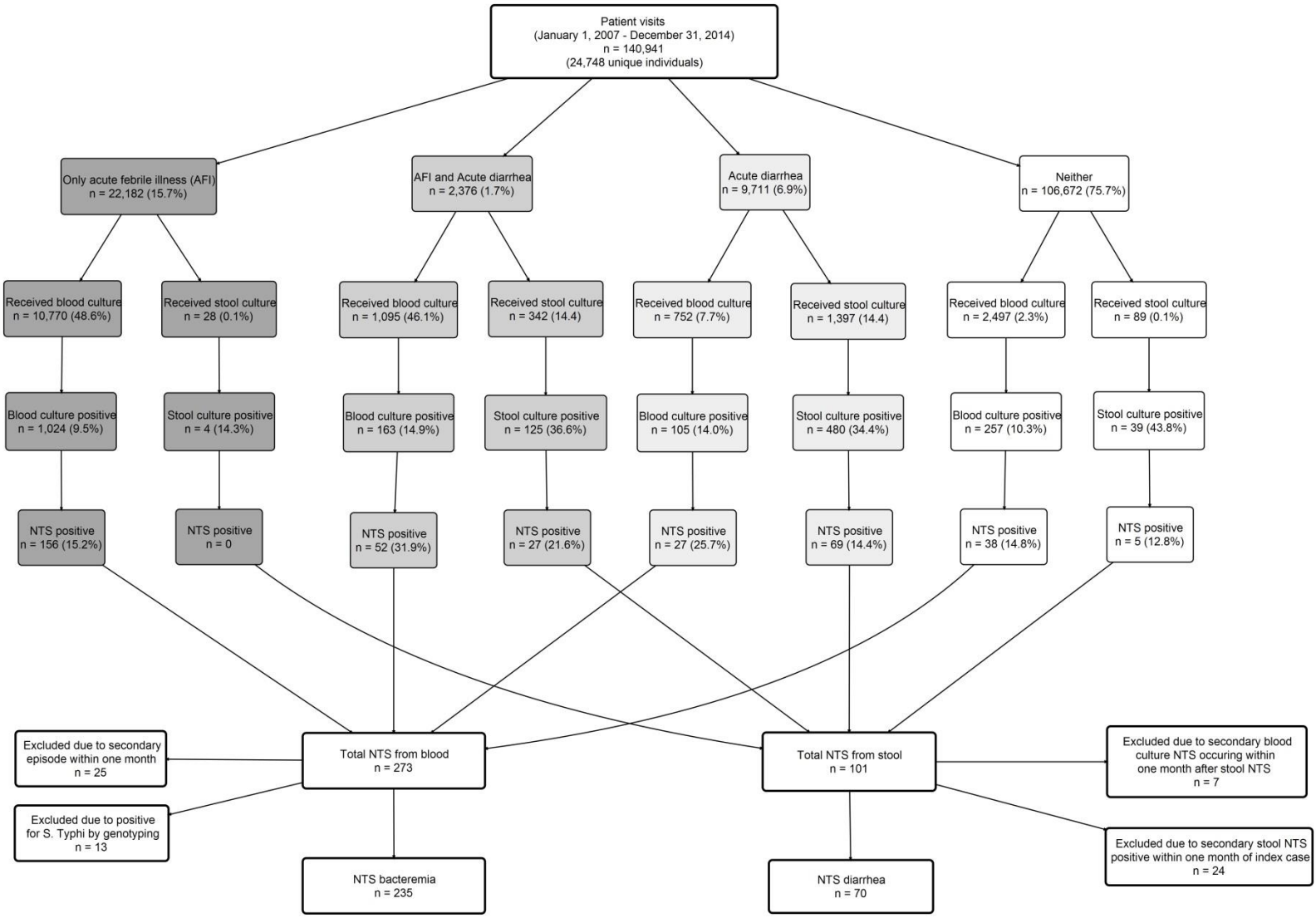

Table 1. Blood and stool culture positive NTS isolates and percent multi-drug resistant S. Typhimurium and S. Enteritidis, 2007 – 2014.

Visit Year	2007	2008	2009	2010	2011	2012	2013	2014	Total/average
Total blood cultures done	1,298	1,285	1,715	2,855	1,951	2,204	2,044	1,762	15,114
Blood culture positive	86	110	238	412	167	219	191	126	1,549
Blood culture positive NTS	7	31	43	68	23	16	33	14	235
Total *S.* Typhimurium	6	29	35	36	12	5	16	6	145
% MDR *S.* Typhimurium	83	93	66	94	75	60	75	100	82
Total *S.* Enteritidis	0	1	4	31	9	8	12	6	71
% MDR *S.* Enteritidis	.-	0	50	94	89	63	75	50	79
Total stool cultures done	327	286	337	278	160	164	88	216	1,856
Stool culture positive	92	86	113	93	71	65	42	86	648
Stool culture NTS	12	9	13	10	8	10	2	6	70
Total *S.* Typhimurium	6	5	6	4	6	2	1	2	32
% MDR *S.* Typhimurium	67	60	17	0	33	100	0	100	44
Total *S.* Enteritidis	2	1	2	3	0	5	0	0	13
% MDR *S.* Enteritidis	0	0	0	33	-	0	-	-	7.7

Table 2. Host-characteristics by NTS clinical outcome and among all clinic visits to Lwak Clinic for reference, 2007 – 2014

	All clinic patients[1] N = 140,941 N (%)/ Median (iqr)	NTS Bacteremia N = 235 N (%) / median (iqr)	NTS Diarrhea N = 70 N (%) / median (iqr)	p-value[2]
Host factors				
Age in years (median/iqr)				
Age < 5 years [3]	2.2 (1.1 – 3.5)	1.9 (1.4 – 3.0)	1.0 (0.8 – 1.5)	**0.003**
Age ≥ 5	17.6 (10.3 – 36.0)	23.0 (10.3 – 36.9)	30.5 (14.4 – 45.4)	**0.044**
Sex (% female)	82,440 (58.6)	127 (54.0)	32 (45.7)	0.221
Nutritional status (children < 5)[3]				
Height for age Z-score (HAZ)	-1.3 (-2.2 – -0.4)	-1.3 (-2.2 – -0.7)	-1.9 (-2.3 – -1.0)	0.162
Weight for height Z-score (WHZ)	-0.3 (-1.2 – 0.6)	-0.6 (-1.4 – 0.3)	-0.8 (-1.1 – 0.1)	0.840
Stunted (HAZ < -2.0)	11,709 (29.7)	34 (30.4)	6 (42.9)	0.343
Wasted (WHZ < -2.0)	4,396 (11.2)	16 (14.6)	3 (23.1)	0.422
Severe acute malnutrition (WHZ < -3.0)	1,611 (4.1)	7 (6.4)	1 (7.7)	0.609
Antibiotics taken for illness[5]				
TMP-SMX	4,144 (8.8)	12 (11.9)	3 (9.7)	1.000
Other antibiotic	1,460 (3.1)	4 (4.0)	5 (16.7)	**0.029**
HIV				
Received an HIV test[6]	5,914 (4.2)	60 (25.5)	27 (38.6)	**0.034**
HIV positive (tested at any point)[7]	1,991 (34.1)	39 (65.0)	10 (37.0)	**0.015**
Malaria				
Received malaria smear	115,978 (82.3)	214 (91.1)	52 (74.3)	**0.001**
Malaria positive[9]	49,205 (42.4)	53 (24.8)	6 (11.5)	0.039
Pathogen factors for NTS				
Salmonella O-antigen group				
Group B (*S.* Typhimurium)	-	155 (66.0)	32 (45.7)	**<0.001**
Group D (*S.* Enteritidis)	-	78 (33.2)	14 (20.0)	
Group C1/C2	-	0 (0.0)	17 (24.3)	
Other *Salmonella* spp.	-	2 (0.9)	7 (10.0)	
Antibiotic resistance				
TMP-SMX	-	198 (90.4)	21 (32.3)	**<0.001**
Ampicillin	-	194 (89.4)	22 (33.9)	**<0.001**
Chloramphenicol	-	178 (81.6)	16 (24.6)	**<0.001**
Multi-drug resistance (MDR)[10]	-	175 (80.3)	15 (23.1)	**<0.001**
MDR (Group B)		119 (82.1)	14 (43.8)	**<0.001**
MDR (Group D)		56 (78.9)	1 (7.7)	**<0.001**

Numbers and percentages out of all non-missing records

[1] Total visits (sick and well) to Lwak clinic between 2007 – 2014 include 43,191visits from children under five and 97,750 visits from those five and older

[2] P-value testing for difference in co-variates between NTS bacteremia and NTS diarrhea, calculated using a chi-squared statistic for comparing differences in means and Wilcoxon signed rank sum for comparing medians.

[3] Among 117 children with NTS bacteremia and 15 children with NTS diarrhea

[4] Based on self-report diarrhea at presentation

[5] Among those who answered "yes" to having taken any medication for the current illness

[6] HIV status ascertained through two community surveillance surveys conducted in 2008-2009 and 2013 and from HIV provider initiated testing at Lwak Clinic.

[7] Among 93 individuals who were tested any point between 2007 and 2014

[8] To ensure HIV status was known within a year of NTS diagnosis, only those individuals over 13 years of age who tested positive prior to one year after NTS diagnosis or negative after one year before NTS diagnosis were included. Individuals under 13 years of age were included regardless of date of HIV test.

[9] Among those presenting with fever or history of fever

[10] Resistance to TMP-SMX, Ampicillin, and Chloramphenicol

Table 3. Multivariate- adjusted odds ratios, stratified on age group (< / ≥ 5 years)

	Age < 5				Age ≥ 5			
	NTS-Bact. N = 117 (88.6%) n (%)	NTS-Diar. N = 15 (11.4%) n (%)	aOR[1] (95% CI)	P-value	NTS-Bact. N = 118 (68.2%) n (%)	NTS-Diar. N = 55 (31.8%) n (%)	aOR[1] (95% CI)	P-value
Host factors								
Age (median/iqr)	1.9 (1.3 – 3.0)	1.2 (0.8 – 1.5)	1.95 (0.74 – 5.16)	0.180	22.5 (10.0 – 36.5)	30.4 (14.1 –45.6)	0.99 (0.98 – 1.01)	0.479
Malnutrition								
Stunting (WHZ < -2)	30 (29.1)	4 (33.3)	0.87 (0.22 – 3.52)	0.864	-	-	-	-
Wasting (WAZ < -2)	15 (15.0)	2 (18.2)	0.49 (0.06 – 4.02)	0.508	-	-	-	-
Co-morbidity								
Malaria[2]	30 (30.8)	3 (27.3)	1.32 (0.27 – 6.49)	0.728	19 (20.2)	3 (7.9)	2.18 (0.56 – 8.49)	0.263
HIV positive[3]	4 (40.0)	0 (0.0)	-	-	30 (69.8)	10 (38.5)	2.16 (0.56 – 7.38)	0.265
Pathogen factors								
NTS serogroup								
Group B	71 (65.7)	6 (46.2)	2.65 (0.59 – 11.8)	0.202	74 (67.3)	26 (50.0)	1.44 (0.64 –3.24)	0.384
Group D	35 (32.4)	3 (23.1)	0.82 (0.18 – 3.72)	0.798	36 (32.7)	10 (19.2)	2.43 (0.95 – 6.25)	0.065
Antibiotic resistance								
Cotrimoxazole	98 (89.9)	4 (30.8)	19.8 (4.67 – 84.0)	**<0.001**	100 (90.9)	17 (32.7)	25.6 (9.70 – 67.8)	**<0.001**
Ampicillin	96 (88.9)	3 (23.1)	22.8 (5.33 – 97.8)	**<0.001**	98 (89.9)	19 (36.5)	16.6 (6.94 – 39.8)	**<0.001**
Chloramphenicol	91 (84.3)	2 (15.4)	24.5 (4.81 – 124)	**<0.001**	97 (79.1)	14 (26.9)	11.4 (4.90 – 26.4)	**<0.001**
Multi-drug resistance (MDR)	90 (83.3)	2 (15.4)	23.1 (4.66 – 115)	**<0.001**	85 (77.3)	13 (25.0)	11.5 (4.86 – 27.0)	**<0.001**
MDR (Group B)	59 (83.1)	2 (33.3)	9.96 (1.31 – 75.6)	**0.026**	60 (81.1)	12 (46.2)	4.97 (1.74 – 14.2)	**0.003**
MDR (Group D)	31 (88.6)	0 (0.0)	-	-	25 (69.4)	1 (10.0)	26.5 (2.75 – 256)	**0.005**

Numbers and percentages out of all non-missing age, year of diagnosis, and multi-drug resistance

[1] Adjusted for age, year of diagnosis, multi-drug resistance

[2] Among 108 children under 5 who received a malaria test at NTS diagnosis.

[3] Among 11 children under 5 with an HIV status

Table 4. HIV-stratified, age- and year- adjusted odds ratios, among 73 individuals over age five

	NTS bacteremia N = 47 (64.4%) n (%)	NTS diarrhea N = 26 (35.6%) n (%)	aOR (95% CI)	P-value
HIV negative	N = 14 (46.7%)	N = 16 (53.3%)		
Antibiotic resistance				
TMP-SMX	12 (92.3)	2 (12.5)	87.3 (3.49 – 2,190)	**0.007**
Ampicillin	11 (84.6)	2 (12.5)	38.2 (3.32 – 439)	**0.003**
Chloramphenicol	10 (76.9)	2 (12.5)	22.1 (2.62 – 187)	**0.004**
Multi-drug resistance (MDR)	10 (76.9)	2 (12.5)	22.1 (2.62 – 187)	**0.004**
HIV positive	N = 33 (76.7%)	N = 10 (23.3%)		
Antibiotic resistance				
TMP-SMX	30 (100.0)	6 (60.0)	-	-
Ampicillin	30 (100.0)	5 (50.0)	-	-
Chloramphenicol	26 (86.7)	6 (60.0)	4.28 (0.69 – 26.6)	0.119
Multi-drug resistance (MDR)	26 (86.7)	5 (50.0)	7.19 (1.11 – 46.4)	**0.038**

Supplementary table 1. List of 88 genotyped S. Typhimurium strains from 2007 – 2014 in western Kenya.

							Antibiotic resistance			
Serovar	Clade	Case type	Year	Malaria	Age (years)	HIV status	TMP-SMX	Amp	Chl	MDR
Salmonella Group B	A130	bacteremia	2008	negative	1.9		resistant	resistant	susceptible	no
Salmonella Group B	A130	bacteremia	2008	negative	34.4	positive	resistant	resistant	susceptible	no
Salmonella Group B	A130	bacteremia	2009	negative	52.8	positive	resistant	resistant	susceptible	no
Salmonella Group B	D23580	bacteremia	2007	negative	34.7		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2007	negative	54.6		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2007	negative	1.2		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2007	negative	6.1	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	positive	1.5		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	46.8	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	positive	6.2		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	4.1	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	39.3	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	20.6		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	18.4		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	12.1	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	46.9	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	5.7		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	7.1	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	positive	2.2		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	1.8		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	positive	0.8		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	4.5	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2008	negative	10	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	positive	2.8		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	21.8		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009		0.3		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	41.3	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	22.9	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	positive	3.2	negative	resistant		resistant	
Salmonella Group B	D23580	bacteremia	2009	negative	67.5	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	53.1	positive	resistant	resistant	susceptible	no
Salmonella Group B	D23580	bacteremia	2009	negative	1.7		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	1.6	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	19.3	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	2.2	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	3.9		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	positive	40.3		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	28.5		resistant	resistant	resistant	yes

Salmonella Group B	D23580	bacteremia	2009	negative	22.5	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2009	negative	1.1		susceptible	susceptible	susceptible	no
Salmonella Group B	D23580	bacteremia	2010	negative	5.6		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	64.7	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	1.7		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	0.6		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010		11.6	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	1.7		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	0.6		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	positive	8.8		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	positive	5.7		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	1.6		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	41.8	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	16.7	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	38.8		susceptible	susceptible	resistant	no
Salmonella Group B	D23580	bacteremia	2010	negative	3.9	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	positive	1.9		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	16.3		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	positive	3.7		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	1		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	3.3		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	0.6		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	positive	0.6		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	28.2	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	positive	8.6		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	positive	11.7	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	4.5		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	negative	1.4		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	positive	1.8		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2010	positive	2.9		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2011	negative	1.5					
Salmonella Group B	D23580	bacteremia	2011	positive	3.1		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2011	negative	4		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2011	negative	37.5	positive	resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2011	positive	7.5		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2013	negative	4.2					
Salmonella Group B	D23580	bacteremia	2013		36.4		resistant	resistant	susceptible	no
Salmonella Group B	D23580	bacteremia	2013	negative	6		resistant	resistant	susceptible	no
Salmonella Group B	D23580	bacteremia	2013	negative	2.7		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2013	positive	15.2		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2013	positive	2.9		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2013	positive	2.2		resistant	resistant	resistant	yes

Salmonella Group B	D23580	bacteremia	2013	negative	33.1		resistant	resistant	resistant	yes
Salmonella Group B	D23580	bacteremia	2013	negative	40.3					
Salmonella Group B	D23580	bacteremia	2013		7.3		resistant	resistant	resistant	yes
Salmonella Group D	D23580	bacteremia	2010	positive	13.8		susceptible	susceptible	susceptible	no
Salmonella Group B	D23580	diarrhea	2008		48.4		resistant	resistant	resistant	yes
Salmonella Group B	D23580	diarrhea	2012	negative	12.9	negative	resistant	resistant	resistant	yes
Salmonella Group B	D23580	diarrhea	2014		11.3	positive	resistant	resistant	resistant	yes
Salmonella Group B	ST19	bacteremia	2011		28.4		resistant	resistant	susceptible	no

CHAPTER 3: Environmental transmission of typhoid fever among children in an urban slum: a population-based, spatial case-control study

Environmental transmission of typhoid fever among children in an urban slum: a population-based, spatial case-control study

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ABSTRACT

Background: Enteric fever due to *Salmonella* Typhi (typhoid fever) occurs in urban areas with poor sanitation. While direct fecal-oral transmission is thought to be the predominant mode of transmission, recent evidence suggests that indirect environmental transmission may also contribute to disease spread.

Methods: Data from a population-based infectious disease surveillance system (28,000 individuals followed biweekly) managed by the Kenya Medical Research Institute and the US Centers for Disease Control (KEMRI/CDC) were used to map the spatial pattern of typhoid fever in Kibera, an urban slum in Nairobi Kenya, between 2010-2011. Cases were defined as individuals with fever and positive blood culture for *Salmonella* Typhi. Controls were selected randomly from the population-based cohort. We used a spatial modeling framework to map the geographic distribution of typhoid fever cases and population-based controls, and to detect whether geographic distribution of risk was explained by variations in topography and accumulation of surface water using multivariate logistic regression models.

Results: The median age of the included population of cases and controls was 14·4 years (IQR=6·1 – 26·1). Cases were significantly younger than controls, (8·4 versus 16·4 years, p<0·001) and were also more likely to reside in larger households (> 5 individuals), (70·0% versus 48·6%, p < 0·001). Among children < 10 years of age, risk of typhoid fever was geographically heterogeneous across the study area (p=0·016) and was positively associated with lower elevation, OR = 1·87, 95% CI (1·36 – 2·57), p <0·001. In contrast, the risk of typhoid fever did not vary geographically or with elevation among individuals >10 years of age.

Conclusions: Our results provide evidence of indirect, environmental transmission of typhoid fever among children, a group with high exposure to fecal pathogens in the environment. Spatially targeting sanitation interventions may decrease enteric fever transmission.

Key words: Spatial analysis, geo-statistics, typhoid fever, environmental transmission, pediatric infectious disease, geographic information systems (GIS), environmental health

INTRODUCTION

Typhoid fever is a systemic, enteric disease caused by *Salmonella enterica* serovars Typhi and Paratyphi and has an estimated annual global incidence of 26·9 million cases, and causes 200,000 deaths per year.[73] Morbidity and mortality due to typhoid fever occurs primarily in young children in Africa and Asia.[40, 74] Children lack natural immunity and experience high levels of exposure to fecal pathogens.[75] If untreated, the case fatality rate of typhoid can exceed 10%, although appropriate antibiotic treatment can reduce mortality to 1% or less.[76]

Transmission of typhoid fever depends primarily on direct contact with the stool of an infected individual,[77-79] and risk is highest in densely populated areas that lack proper sanitation and access to safe drinking water.[40, 80] Household-level hygiene and food/water safety and handling practices, as well as close contact with an index case, are associated with the direct transmission of typhoid in endemic areas.[80-82] Because *S.* Typhi is exclusively human host-adapted, reservoirs of infection exist solely within groups of infected humans, a small number of which (1 – 6%) develop a chronic carrier state,[83, 84] which has allowed the disease to persist during inter-epidemic periods.[85]

Recent evidence suggests that environmental reservoirs of infection may also support disease transmission. The risk of typhoid fever is associated with environmental factors, including proximity to open sewers and highly contaminated water bodies, residence in low elevation areas, and rainy season.[38-40, 86] Major outbreaks of *S.* Typhi have been linked to contaminated municipal water sources, and suggest waterborne transmission as an important environmental pathway.[81, 87] Whether environmental sources contribute to endemic transmission during non-outbreak periods is unclear.

There is, furthermore, limited study on the epidemiology and environmental drivers of *S.* Typhi infection in Africa,[88] where the incidence in some urban areas parallels that of high burden regions of Asia.[74] Additional data is needed to investigate the role that environmental reservoirs play in the endemic transmission of typhoid fever in Africa, particularly among children who are at an elevated risk of infection.[74] Such information can be used to predict where risk is greatest and can inform targeting for vaccination programs, water and sanitation improvements, or other community interventons.[89]

We utilized a spatial modeling framework with climatic and remotely sensed data to estimate the geographic distribution of typhoid fever risk among a large disease surveillance cohort in Kibera, a densely populated, urban informal settlement in Nairobi Kenya. We examined the contribution of

environmental exposures to transmission by testing for associations between typhoid fever risk and variations in the hydrologic landscape. These data suggest that environmental transmission is an important contributor to the risk of typhoid fever in young children but may not be important in adults and adolescents.

METHODS

The protocol was reviewed and approved by the Institutional Review Boards of the United States Centers for Disease Control and Prevention (US-CDC) and the Kenya Medical Research Institute (KEMRI). We conducted a spatial case-control analysis to identify geographic and environmental risk factors for *S.* Typhi infection in Kibera, an informal urban settlement of between 220,000 – 250,000 residents in Nairobi, Kenya (Central Bureau of Statistics. 2009). We used disease surveillance data obtained from an ongoing, KEMRI/CDC-Kenya, population-based, household and clinic surveillance system in Kibera. Details of the surveillance have been described previously.[74, 90] Briefly, about 28,000 individuals have been followed biweekly since 2006 by trained community interviewers, and those with fever during these household visits were advised to seek medical attention at the surveillance site clinic. Blood cultures were conducted on all consenting individuals presenting to the clinic with an axillary temperature of ≥38.0. The area lacks adequate sanitation infrastructure, as indicated by open sewers and limited access to clean water,[91] and has a large burden of many infectious diseases, including an adult HIV prevalence at 12·6%.[92] The surveillance area covers 0·40 km^2, with high population density (70,000 individuals/km^2).[74] The study area lies at an altitude of 1700-1740 m, with terrain gently sloping towards the Motoine River in the southeastern portion of the study area (Figure 1a). Kibera experiences two seasons: wet, characterized as long rains from March–May and short rains from October–November and dry, which runs June – September and December–February). The average monthly temperature is 19°C .[90]

Case Selection

Incident cases of symptomatic typhoid fever were ascertained between Jan 1, 2010 and Dec 31, 2011 at Tabitha Clinic, a free-clinic in Kibera centrally located within 0·60 km of the entire study population. Cases were defined as individuals who presented to Tabitha Clinic with acute febrile illness ≥ 38°C and from whom *S.* Typhi was isolated through blood culture. Only the first episode of typhoid fever during the 2-year study period was included. Cases whose residential location could not be verified 14 days prior to a typhoid fever episode were excluded from the analysis.

Control Selection

Controls were randomly selected from the underlying surveillance cohort (~28,000 individuals) in order to estimate the geographic distribution of the population at risk. A pool of eligible controls from the surveillance cohort was identified as meeting the following criteria: 1) not identified as a case of typhoid fever between 2010 and 2011 (whether or not fever had been reported), 2) enrolled in the study with at least one household study visit between 2010 and 2011, and 3) non-missing GPS coordinates at the time of study visit. Four controls per case were selected at random, and one study visit from each control was randomly selected from the series of study visits over the study period 2010–2011.

GIS and Hydrological Variables

Handheld Garmin GPSMap76CSx units were used to capture Global Positioning Systems (GPS) points on case households 14 days prior to diagnosis (to account for the average incubation period), and at the time of interview for the control households. Household elevation was estimated for each case and each control using a 90 X 90 meter digital elevation model (DEM) downloaded from NOAA.gov.[93] The DEM was used to generate a continuous 90 X 90 meter flow accumulation surface using the ArcHydro Toolset in Arc GIS 10.0,[94] with values at each location corresponding to the upstream contributing area draining to that point (e.g., lower-values are higher in the watershed and have less upstream area draining to that location, whereas high-value cells are lower in the watershed and have more upstream contributing area). DEM data are freely available, provide good approximations of the dominant flow direction of surface water at the watershed scale,[95] and have been used to model the hydrologic diffusion and geographic distribution of different infectious diseases.[42, 96-98] We used DEM-derived elevation and flow accumulation surfaces to approximate the level of exposure to accumulated fecal contamination at each across the study area. We also measured the Euclidean distance from each case/control to the nearest point along two heavily polluted streams that bound the study area to test for associations between typhoid fever risk and exposure to point source contamination along the streams.

Rainfall data recorded at a weather station at Nairobi's Jomo Kenyatta International Airport (JKA) between 2010 and 2011 were downloaded from https://data.noaa.gov/dataset/global-surface-summary-of-the-day-gsod, "The Global Surface Summary of Day" product, produced by the National Climatic Data Center (NCDC).[99] The total accumulation of rainfall over the previous 3, 7, and 30 days was calculated for each case from the date 14 days prior to the case diagnosis (to account for the average incubation period for typhoid following exposure) and 14 days prior to the control interview for comparability.

Statistical Analysis

We compared the distribution of demographics (age, gender, household size) and environmental/hydrological variables (elevation, flow accumulation, distance to stream, and daily rainfall) between cases and controls in both univariate and multivariate logistic regression models. Multivariate analyses were stratified on age group (children < 10 and adults/adolescents > 10) in order to test for differential effects of environmental risk factors on typhoid transmission in each age-stratified risk group. Children under 10 years of age experience the highest incidence of typhoid fever in our study area,[74] and may also have unique exposure pathways that warrant further investigation. Multivariate analyses were adjusted for confounding factors identified *a priori*. All logistic regression analyses were performed using STATA 11 (STATACo, Texas 77845 USA).

Spatial Analysis

We used spatial regression to compare the geographic distribution of cases relative to controls after adjusting for individual-level variables. Under the null hypothesis, the distribution of cases relative to controls is expected to be equivalent to the case-control ratio at every location across the study area. The risk surface, when plotted on a map, is expressed as the log-odds of being a case versus control – where a value of zero corresponds to the expected case-control ratio. A log-odds above (below) zero indicates a more (less) than expected proportion of cases to controls. We tested for significance of the log-odds surfaces against the null hypothesis. Maps of the adjusted odds were produced using a locally weighted regression smoother in a general additive model (GAM) framework for case-control data[44] using a logistic link function and a non-parametric component for the residual spatial surface. All spatial analyses were performed in the R-statistical software[100] using the mgcv package for fitting GAMs (methods described elsewhere[101]) and visualized using the splancs package.[102] GAM plots were stratified on age < and > 10 years of age.

RESULTS

Study population:

A total of 118 cases of typhoid fever were confirmed at Tabitha clinic between Jan 1, 2010 and Dec 31, 2011. Of those, 111 had confirmed residence and household-level GPS coordinates at the time of infection and were included in the analysis. One case was excluded as it was a repeat episode of typhoid on the same individual within a one month period, which we consider a recrudescent case as opposed to two separate transmission events. The 110 incident cases included were unique individuals who resided

in 103 unique households, with 7 secondary cases within the same household. Among the 7 secondary cases at the household level, 5 occurred within 30 days of an index case in the same household, which we classify as intra-household transmission events. Four hundred and forty controls were randomly selected from the underlying population at risk, comprising 416 households, with interview dates spanning from January 6, 2010 to December 29, 2011.

Comparison of socio-demographic characteristics of cases and controls:

The distribution of demographic and environmental characteristics between cases and controls is shown in Table 1. Cases were significantly younger than controls (mean age of 8·4 years versus 16·4 years, respectively, ($p<0·001$). More than half of cases (56·4%) were under age 10 versus 34·6% of controls ($p<0·001$, Figure 3). Cases resided in households with more individuals (70·0% of cases lived in households with more than 5 individuals compared to 48·6% of controls, $p<0·001$). There was no evidence that cases differed from controls with respect to gender (52·7% female versus 49·8% female, $p=0·579$).

Comparison of spatial, temporal, and climatic characteristics of cases and controls:

The spatial distribution of the 110 incident cases and 440 controls is displayed in Figure 1b. Compared to controls, cases were concentrated in the eastern, lower elevation region of the study area. Only one case was observed in the westernmost part of the study area. The incidence of typhoid fever over the 2-year study period did not follow any seasonal pattern, nor was there any discernable association between monthly rainfall and risk (Figure 2).

Individuals with typhoid fever resided in lower elevation areas (19·1% of cases resided in the lowest elevation area, 1,695 – 1,707 meters, compared to 12·5% of controls, $p<0·001$) with higher flow accumulation (33·6% of cases resided in areas with the highest flow accumulation area compared to 18·0% of controls, $p<0·001$). There was no evidence that cases differed from controls with respect to season (43·6% of cases occurred in the wet season versus 51·1% of control interviews, $p=0·161$), or total precipitation in the three days prior to infection/interview (68·2% of cases and 65·2% of controls occurred after three days with no precipitation, $p=0·205$).

Age-stratified analyses:

Household size (measured as number of inhabitants) was positively associated with risk of typhoid in both children (under age 10) and adults/adolescents (over age 10), OR = 1·27, 95% CI (1·11 – 1·46), p < 0·001; OR = 1·20, 95% CI (1·10 – 1·31), p < 0·001, respectively. Among children under age 10, those who resided at lower elevation had significantly greater risk of typhoid fever compared to those in higher elevation areas, OR = 1·87, 95% CI (1·36 – 2·57), p <0·001, corresponding to a 10 meter decrease in elevation (Table 2). Similarly, those children who resided in areas with higher flow accumulation had greater risk of typhoid fever compared to children in areas with less flow accumulation, OR = 1·27, 95% CI (1·00 – 1·62), p = 0·05. Among adults/adolescents over age 10, there was no evidence of an association between typhoid risk and elevation, OR = 1·23, 95% CI (0·89 – 1·71), p = 0·205 or flow accumulation, OR = 1·11, 95% CI (0·91 – 1·37), p = 0·305. Long periods of dryness (>30 days of no rain) prior to exposure/interview were positively associated with higher risk of typhoid in adults/adolescents, OR = 2·88, 95% CI (1·05 – 7·87), p=0·040], with no evidence of an association in children.

Among children < 10 years of age, the risk of typhoid fever was geographically heterogeneous across the study area (p = 0·016) and generally followed a linear geographic gradient, with risk increasing from the west to east (Figure 4). There was a weak, and non-statistically significant (p = 0·150) spatial pattern in the risk of typhoid fever among adolescents/adults > 10 years of age.

DISCUSSION

In this large, population-based, case-control study, risk of typhoid fever was spatially heterogeneous across a small geographic area. The observed spatial pattern in risk was especially pronounced among children under 10 years of age, who experienced an almost doubling of risk for every 10 meter decrease in elevation. In contrast, the spatial distribution in the risk of typhoid fever among adults and adolescents varied only slightly across the study area. Our results suggest distinct modes of transmission of typhoid fever between children and adolescents/adults in a Kenyan urban slum. Transmission in children may be more environmentally mediated than that in adults.

Low elevation areas with high flow accumulation aggregate bacterial pathogens from diffuse sources upstream via the overland flow of surface waters,[41, 103-109] and may serve as environmental reservoirs for a number of water-borne and water-related infectious diseases in children, including soil transmitted helminthes and schistsomiasis.[97] Our results build on previous studies that have shown

environmental heterogeneity in the risk of typhoid fever[38, 39, 110] by highlighting differences in environmental drivers of risk between children and adults.

The global health implications of environmental transmission of typhoid fever among children in Africa are significant. Young children are at the greatest risk of typhoid fever in densely populated urban areas with poor hygiene and sanitation infrastructure.[74, 80, 111] Unlike adults and adolescents, children have underdeveloped natural immunity conferred by previous infection and are unable to fight systemic bacterial colonization.[112] Furthermore, young children are likely to be exposed to fecal contamination in the immediate environment surrounding their household.[113] Based on estimates from 2006-2009 in Kibera, children between 2-4 years of age and 5-9 years of age experienced the highest incidence of typhoid fever (2242.6 cases per 100,000 person years and 1,788 per 100,000 person years, respectively), compared to 821.5 cases per 100,000 person years among 0-1 year olds. The higher incidence among children two and over is consistent with behaviors associated with outdoor play and exposure to fecal pathogens in the environment. Based on our results, it is plausible that in typhoid-endemic areas children contact *S.* Typhi from environmental reservoirs at substantially higher rates than do adults. Environmental transmission may therefore account for a large part of the burden of typhoid fever in children and may in turn play an important role in its continued transmission.

We observed a negative association between precipitation and risk of typhoid fever in adults/adolescents > 10 years of age, who experienced a 3 fold-higher risk of infection after long dry periods. The effect of rainfall on risk of enteric pathogens, including typhoid fever, is unclear. Heavy rainfall is associated with increased risk of enteric disease transmission as a result of washing fecal pathogens into drinking water sources.[114, 115] One study from Nepal showed an increase in typhoid fever incidence in parallel with seasonal peaks associated with the rainy season.[38] On the other hand, long dry periods may increase the risk of diarrheal pathogens as a result of the limited availability of clean water for proper hygiene.[116, 117]

A primary strength of our study was the use of population-based controls to estimate the underlying geographic distribution of the population at risk. The selection of controls in a clinic or hospital-based setting, a practice used in many spatial epidemiology studies, can obscure true disease-exposure associations if the controls do not adequately represent the geographic distribution of the underlying population at risk. A second strength is the stratification of our data into under and over 10 years of age, which allowed us to compare the effects of environment on typhoid transmission across age groups.

The results of our study must also be considered in light of certain limitations. First, we lacked individual-level socioeconomic status (SES) needed to adjust for small-scale variation in access to resources, improved sanitation, and hygiene practices. Considering that our population was restricted to small geographic area with a relatively homogenous distribution of SES and sanitation, we don't expect strong confounding by SES across the study area. Next, though DEM-derived surfaces have been used to model hydrological drivers of waterborne and water-related infectious diseases[97, 118], they are limited in their ability to capture fine scale heterogeneity in the flow of water and thus only crudely measure the accumulation of water-borne contamination. Finally, our study was unable to confirm environmental transmission via bacteriologic evidence indicating the presence of *S.* Typhi in the environment.

In summary, this study provides evidence of environmental transmission of typhoid fever in young children in an urban slum in Africa. Implementation of interventions to reduce transmission should include targeted sanitation improvements in areas of high geographic risk, particularly in low elevated areas where fecal waste tends to concentrate. Children living in these areas are at increased risk of typhoid transmission and may benefit from environmental interventions and targeted vaccination campaigns, as has been emphasized previously [74, 89].

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Figure 1a. The Kibera informal settlement in Nairobi, Kenya, with the study area highlighted in red.

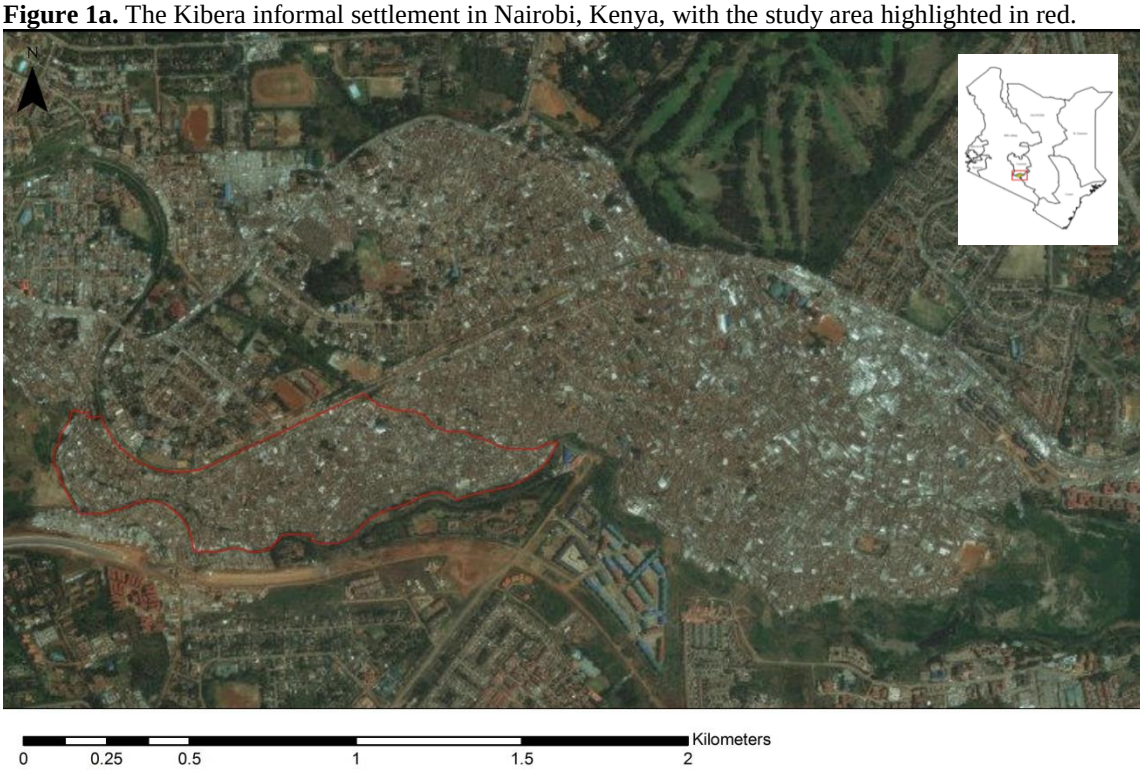
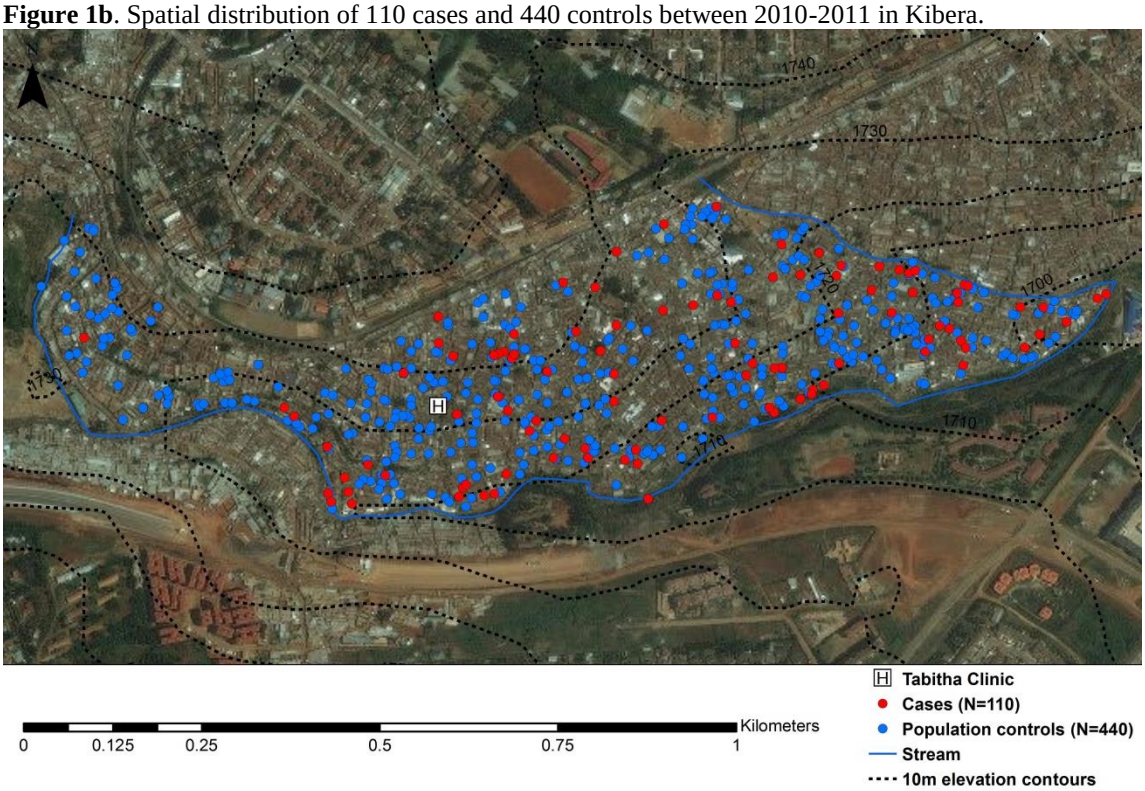


Figure 1b. Spatial distribution of 110 cases and 440 controls between 2010-2011 in Kibera.



Base layer data source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aeorgrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 2. Monthly distribution of 110 incident cases between 2010-2011 in Kibera with monthly rainfall data overlaid.

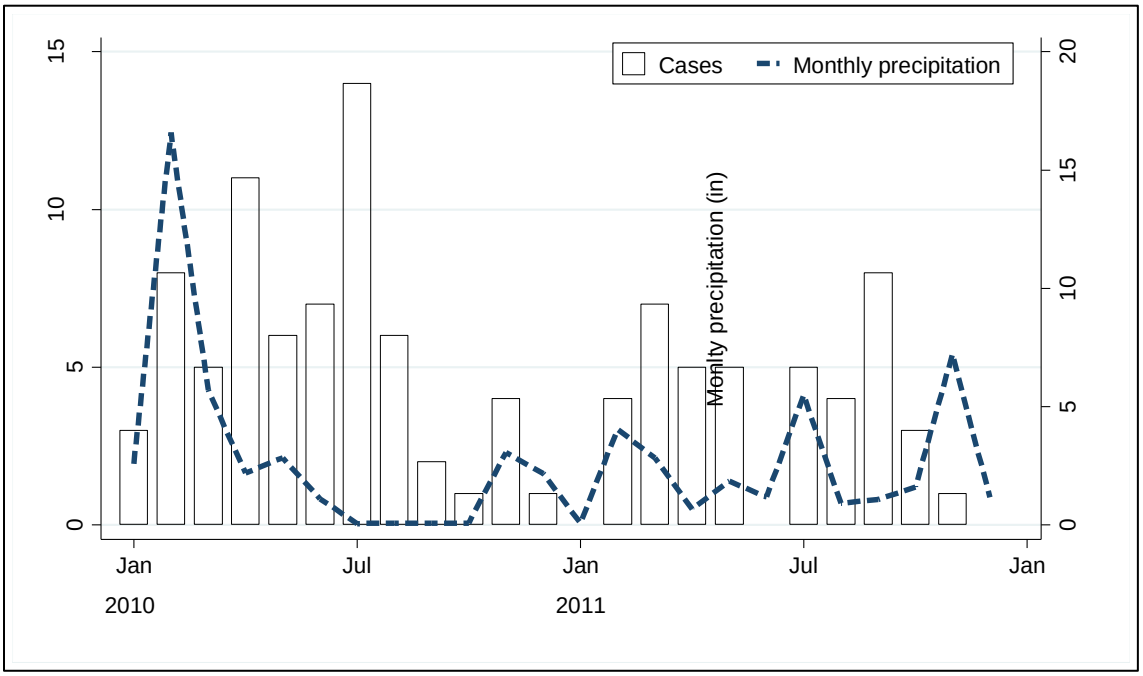


Table 1. Demographic and environmental variables by case-control status

	Cases n = 110 Number (percent)		Controls n = 440 Number (percent)		p-value[1]
Age (median/IQR)					
0-4 years	29	(26·4)	78	(17·7)	**<0·001**
5-9 years	33	(30·0)	74	(16·8)	
10-14 years	16	(14·6)	52	(11·8)	
15-24	15	(13·6)	104	(23·6)	
25+	17	(15·5)	132	(30·0)	
Gender					
(% female)	58	(52·7)	219	(49·8)	0·580
Number of inhabitants in household					
1-2	0	(0·0)	23	(5·2)	**<0·001**
3-5	33	(30·0)	203	(46·1)	
>5	77	(70·0)	214	(48·6)	
Season[2]					
Wet	45	(40·9)	213	(48·4)	0·160
Dry	65	(59·1)	227	(51·6)	
Distance to Tabitha Clinic (meters)					
< 100	6	(5·5)	56	(12·7)	**0·019**
100-500	64	(58·2)	257	(58·4)	
> 500	40	(36·4)	127	(28·9)	
Elevation (meters)[3]					
1,695 – 1,707	21	(19·1)	55	(12·5)	**0·007**
1,708 – 1,720	41	(37·3)	111	(25·2)	
1,721 – 1,733	21	(19·1)	143	(32·5)	
1734 – 1,747	27	(24·6)	131	(29·8)	
Flow Accumulation[4]					
0	30	(27·3)	164	(37·3)	**0·001**
1-5	38	(34·6)	176	(40·0)	
6-10	5	(4·6)	21	(4·8)	
> 10	37	(33·6)	79	(18·0)	
Distance to stream (meters)					
0 – 24	34	(30·9)	74	(16·8)	0·078
25 - 99	45	(40·9)	242	(55·0)	
> 100	31	(28·2)	124	(28·2)	
Total precipitation in past three days (inches)[5]					
No precipitation	75	(68·2)	287	(65·2)	0·205
> 0 – 0·1	14	(12·7)	41	(9·3)	
0·1 – 0·4	11	(10·0)	49	(11·1)	
> 0·4	10	(9·1)	63	(14·3)	

[1] Based on linear test of trend for ordered categorical variables

[2] Determined by approximate month when exposed (e.g., diagnosis date – 14 days to account for typhoid fever incubation period). Wet season is characterized by long rains from March–May and short rains from October–November. Dry season runs June – September and December–February.

[3] Measured using Shuttle Radar Topography Mission Digital Elevation Model (DEM) data with 90 meter resolution

[4] A 90 meter resolution hydrological surface generated from a digital elevation model (DEM), used to estimate the amount of accumulated flow draining to every location in the study area.

[5] Measured as the sum of all rainfall over a three day window 14 days (the incubation period for typhoid) prior to either date of diagnosis for cases or interview date for controls

Figure 3. Distribution (by percentage of total) of 110 typhoid fever infections and 440 population-based controls by age in Kibera.

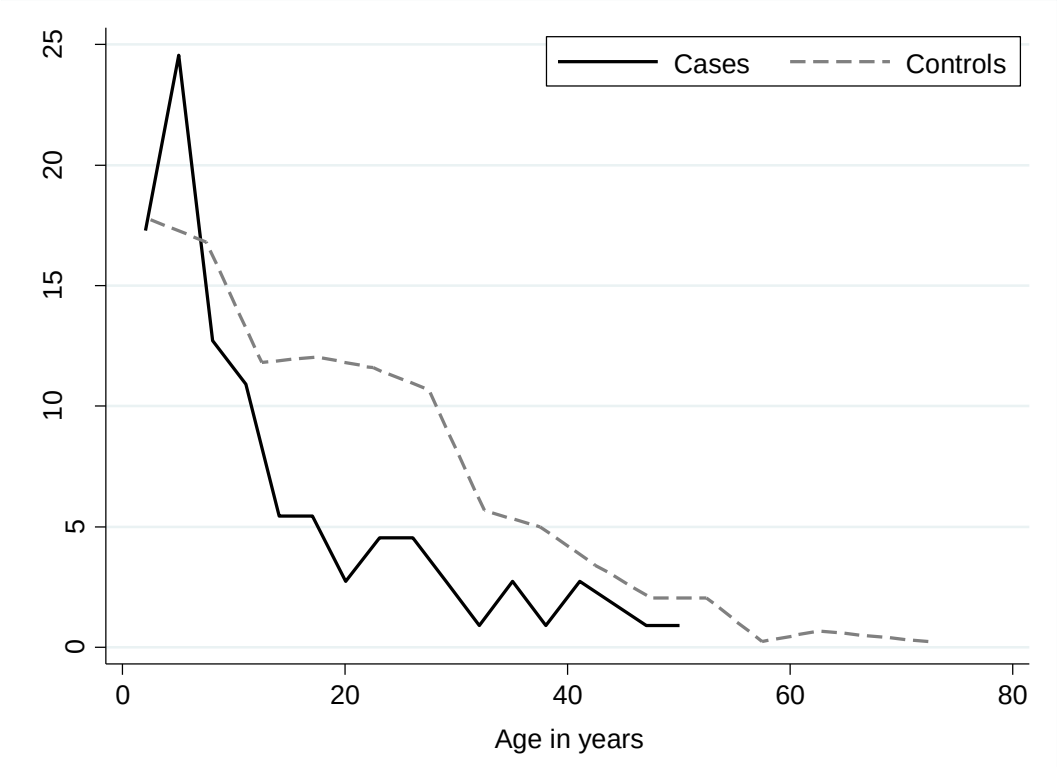


Table 2. Adjusted odds ratios by demographic and environmental variables

	Children < 10 years				Adults/adolescents > 10 years			
	Cases *N (%)*	Controls *N (%)*	aOR (95% CI)	p-value	Cases *N (%)*	Controls *N (%)*	aOR (95% CI)	p-value
Demographics								
Age (years)[1]	62	152	1·02 (0·91 - 1·15)	0·695	48	288	0·98 (0·95 – 1·01)	0·125
Household size (# inhab.)[1]	62	152	**1·27 (1·11 - 1·46)**	**<0·001**	48	288	**1·20 (1·10 – 1·31)**	**<0·001**
Environment								
Distance to streams (m)[1]	62	152	1·00 (1·00 - 1·01)	0·199	48	288	1·00 (0·99 – 1·01)	0·471
Elevation (10m decrease)[1,2]	62	152	**1·87 (1·36 – 2·57)**	**<0·001**	48	288	1·23 (0·89 – 1·71)	0·205
Flow accumulation[3]	62	152	**1·27 (1·00 - 1·62)**	**0·050**	48	288	1·11 (0·91 – 1·37)	0·305
Climate[4]								
Season (dry) [4]	34 (54·8)	75 (49·3)	1·24 (0·68 – 2·26)	0·467	31 (64·6)	152 (52·8)	1·63 (0·86 – 3·08)	0·131
No precip. in prev. 3 days[4]	36 (58·1)	100 (65·8)	0·65 (0·34 – 1·24)	0·196	39 (81·3)	187 (64·9)	2·13 (0·96 – 4·74)	0·063
No precip. in prev. 30 days[4]	5 (8·1)	7 (4·6)	1·67 (0·49 – 5·73)	0·411	7 (14·6)	14 (4·9)	**2·88 (1·05 – 7·87)**	**0·040**

[1]Adjusted for age, household size, elevation, and distance to streams (excluding co-variate of interest).
[2]*DEM* Shuttle Radar Topography Mission Digital Elevation Model, (expressed as per 10 meter decrease in elevation).
[3]Upstream contributing area in units of 10 90X90 square meters.
[4]Calculated from 14 days prior to case diagnosis or control interview to account for the incubation period. No adjustment.

Figure 4a. Smoothed log odds of typhoid fever by age group across the study area, adjusted for age and household size. P-values test for significance of the observed spatial pattern in log odds against a homogenous risk surface (i.e., where the log odds is zero at all locations).

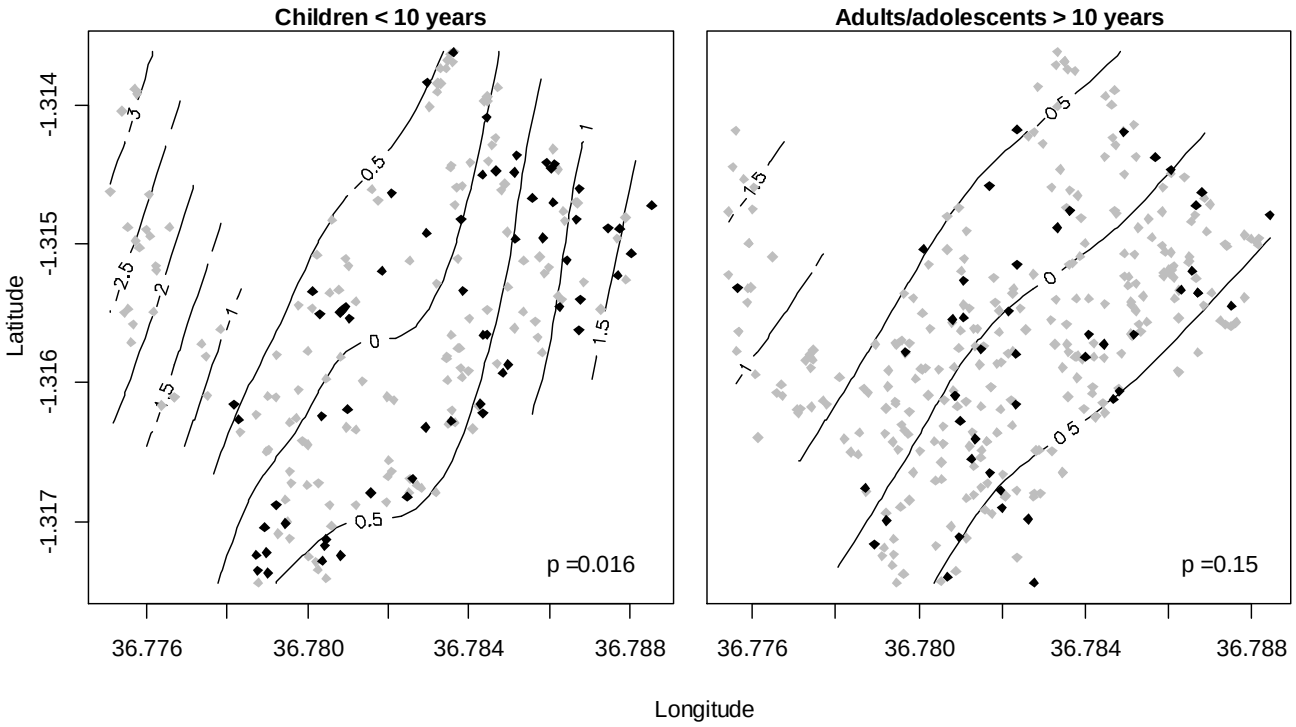
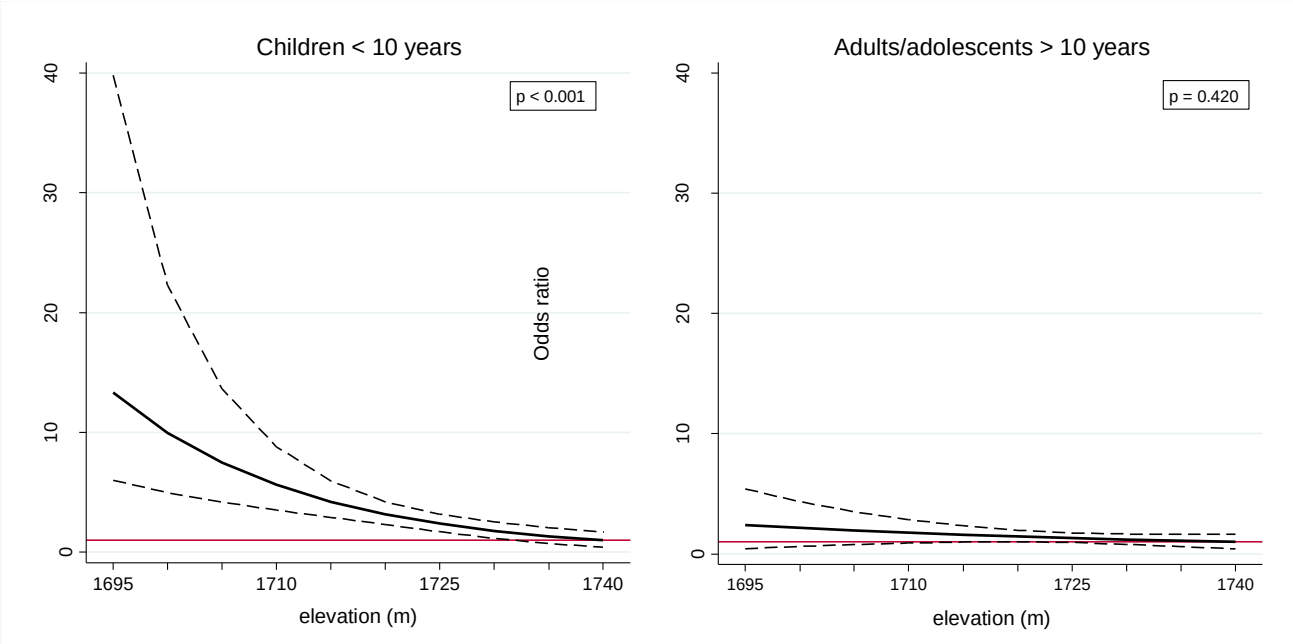


Figure 4b. Adjusted odds ratios from the multi-variate logistic model (table 2) for the association between elevation and typhoid fever for both children (<10 years) and adults/children (>10 years), with 95% CI overlaid (dashed lines). Odds ratio of 1 indicated by horizontal red line.



CHAPTER 4: CONCLUSION

CONCLUSION

This dissertation addresses gaps in our understanding of the transmission dynamics and clinical epidemiology of major *Salmonella* serovars causing blood stream infection in Africa. This work, in two parts, has a number of important findings discussed below.

Host versus pathogen drivers of NTS bacteremia in rural Kenya

In Chapter 2 we found that infection with a multi-drug resistant (MDR) strain of NTS was associated with bacteremia compared to diarrhea. This relationship persisted in further analysis stratified on HIV status. Though HIV was more common in patients with NTS bacteremia compared to those with NTS diarrhea, it was not significantly associated with NTS bacteremia after adjusting for multi-drug resistance. Malaria was also not significantly associated with NTS bacteremia in children under age five after also controlling for MDR. Host factors, while important in determining the clinical progression of disease from NTS infection, were not as strongly associated with NTS bacteremia versus diarrhea as the multi-drug resistant phenotype. Though a number of previous studies have observed associations between host co-infection and invasive NTS, none adjusted their estimates for pathogen-specific attributes that may explain differences in clinical outcome, which may have resulted in confounded effect estimates.

We observed positive associations between MDR and NTS bacteremia across both *Salmonella* serovars Typhimurium and Enteritidis, the two most common causes of NTS bacteremia in Africa. The prevalence of multi-drug resistance has increased among major NTS serovars across Africa [32], presenting a major challenge to the empiric management of blood stream infections from NTS. The putative "invasive" strain of *S.* Typhimurium, sequence type 313 (strain D23580), contains multiple-linked antibiotic resistance determinants encoded by a plasmid associated with systemic disease [29]. This clone was isolated from bacteremic patients during the epidemic increase in MDR NTS bacteremia in Malawi and Kenya between 1997 and 2007. Chloremphenicol was the primary treatment for NTS bacteremia in these settings proceeding the outbreak [30] and is thought to have contributed to the clonal replacement of ST313 clones with a chloramphenicol-resistant variant [29].

Whether the association between MDR and NTS bacteremia can be attributed to enhanced virulence or treatment failure is unclear. While antibiotic resistance is often thought to be associated with reduced fitness and impaired virulence in an organism, co-selection for both virulence and resistance determinants is not uncommon [67]. The biological cost associated with the acquisition of antibiotic resistance determinants is dependent on the bacterial species, level of resistance, and number of resistance mutations

conferred. Therefore, the opposite has also been observed, with resistance and virulence co-evolving to increase fitness and the persistence of pathogenic bacteria in the population [67].

Aside from innate virulence properties, the same MDR NTS strain may be associated with invasive disease in certain settings and gastro-enteritis in others as a result differences in clinical practice, host-immunity, and access to treatment options. Drug resistant bacteria may not respond empiric first-line therapy, and evidence suggests that improper initial antimicrobial therapy resuls in treatment failure and worse clinical outcomes [119]. A cohort study in Denmark, for example, found that infection with a Fluoroquinolone resistant strain of *S.* Typhimurium was associated with more than a 3-fold increased risk of invasive disease or death compared to infection with pan resistant strains of *S.* Typhimurium [120].

Regardless of the biological mechanism, the emergence of MDR NTS poses a major challenge for the management of blood-borne disease, and furthermore strains local health systems with limited access to more effective treatments, resulting in increased morbidity and mortality. Increases in MDR NTS have prompted a change to alternative therapies in the treatment of Salmonellosis [30]. The rise in MDR strains of *S.* Typhi, for example, has shifted clinical practice in high-burden areas of South and Southeast Asia to treat with ceftriaxone, which has resulted in a decrease in case-fatality associated with typhoid fever in clinical settings where this practice is standard protocol [121].

Spatial patterns of typhoid fever in an urban slum

In the second paper, we mapped the occurrence of typhoid fever across a small area in the Kibera slum and tested for associations between risk and variations in topography, a proxy for the overland flow and accumulation of contaminated waste. We found that, after controlling or individual-level factors such as age and household size, there was a strong geographic gradient in the risk of typhoid fever among children under 10 years of age. The geographic heterogeneity observed in the risk of typhoid fever among children is especially intriguing given that it occurred over a small geographic area of a slum with pervasively inadequate sanitation infrastructure. We secondarily found that the spatial pattern in risk of typhoid fever was associated with the topographic gradient, which we used to approximate the overland flow and accumulation of contaminated water. The association between elevation and risk of typhoid was observed in a previous study from Katmandu [38]. Our study furthermore showed that the elevation-typhoid association was only present in children under 10. For individuals over age 10, the risk of typhoid fever did not depend as much on location of residence.

We hypothesize that children are more likely to come into direct contact with contaminated environmental media outside of the household. Because Kibera lacks proper sanitation infrastructure to remove waste, those children residing in the lower elevation areas will be exposed not only to fecal contamination from their immediate environment, but will also be exposed to contaminants from upstream areas. There were no cases of typhoid fever among children under 10 in the upstream area, a finding that is consistent with the hypothesis that exposure to fecal contaminants is lower in the upstream area. Still, we were unable to draw a causal inference between exposure to environmental media and risk of typhoid fever, given that 1) we did not have environmental samples to validate our environmental risk model and 2) we were unable to control for important spatial confounders that might explain the geographic gradient in risk, including contaminated water sources, household hygiene practices, and behavioral patterns and patterns of play in children. Despite these limitations, our results add to a growing body of evidence indicating that typhoid fever is an environmentally mediated infectious disease that can be transmitted not only directly between humans, but also from highly contaminated environments where indirect exposure to fecal contamination occurs.

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2. Matheson AI, Manhart LE, Pavlinac PB, Means AR, **Akullian A**, Levine GA, Jacobson J, Shutes E, Walson JL. (2013). Tools to prioritize countries for mass drug administration interventions: A case study of azithromycin for reducing child mortality. *Plos One*

3. **Akullian A**, Ding L, McDowell J, Davis G, Spear R, Remais J. (2012). Modeling the combined influence of host dispersal and waterborne fate and transport on pathogen spread in complex landscapes. *Water Quality, Exposure and Health*

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In Preparation:

8. **Akullian A**, Ng'eno E, Matheson A, Macharia D, Cosmas L, Bigogo G, John-Stewart G, Walson J, Wakefield J, Montgomery J. Environmental transmission of typhoid fever among children in an urban slum in kenya: a population-based, spatial case-control study (In preparation).

9. **Akullian A**, Walson G, Cosmas L, John-Stewart G, Wakefield. Control selection in spatial epidemiology (In preparation).

10. **Akullian A**, Walson J, Bigogo G, Audi A, Miller S, John-Stewart G, Montgomery J. Multi-drug resistant non-typhoidal *Salmonella* associated with invasive disease in western Kenya. (In preparation).

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REASEARCH EXPERIENCE:

Research Assistant, University of Washington Department of Global Health (September, 2010 – Present)
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Dissertation research focuses on: 1) Geo-statistical analysis of typhoid fever in an urban slum; 2) bacterial pathogenesis of novel *Salmonella* strains using landscape genetics, geographic information systems, and epidemiologic methods; and 3) a critique of control selection in spatial epidemiology. Masters thesis research (published in *AIDS*) focused on

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Research Assistant, Strategic Analysis and Research Training Program (Summer, 2011)
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Develop a report on the geographic distribution of childhood mortality and disease burden for prioritizing mass drug administrative (MDA) interventions.

Research Scientist, Schistosomiasis in China Research Group (June, 2007 – June, 2010)
School of Public Health, Division of Environmental Health Science, University of California at Berkeley.
Conduct spatial analysis on epidemiological and environmental data using geographic information systems (GIS) and remote sensing (RS) platforms. Develop geo-statistical models that predict inter-village disease spread. Manage GIS and RS data bases. Coordinate international field work under an NSF R01 research grant. Advise masters-level thesis research.

Honors Thesis in Environmental Science (September, 2006 – May, 2007)
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Thesis title: "Spatial Patterns of Schistosomiasis: A Watershed Approach to Measuring *Schistosoma japonicum* Environmental Risk and Human and Animal Disease Outcomes." Analyzed spatial data on disease estimators, ecological variables, and raster data sets from fifty villages using ArcView 9.1 and STATA 9.

Research Assistant, Narragansett Bay Hypoxia Project (June, 2006 – September, 2006)
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Collected sediment samples and conducted microscopic fossil identification of benthic foraminifera in order to reconstruct historical nutrient pollution into Narragansett Bay, Rhode Island.

Graduate Student Assistantship (June, 2005 – August, 2005)
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Conducted literature reviews and internal interviews with principal investigators to develop a framework for incorporating environmental justice into OEHHA's risk assessment policies.

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Invited Guest Lecture to Faculty Fellows on Global Health and Environment (October, 2010)
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Akullian A, Ng'eno E, Matheson A, Macharia D, Cosmas L, Bigogo G, John-Stewart G, Walson J, Wakefield J, Montgomery J. "Hydrologic drivers of typhoid fever in an urban slum" Oral presentation at the American Society for Tropical Medicine and Hygiene, New Orleans (November, 2014)

Akullian A, Walson J, Bigogo G, Audi A, Miller S, John-Stewart G, Montgomery J. "Multi-drug Resistant Non-Typhoidal *Salmonella* Associated with Invasive Disease in Western Kenya" Poster at the American Society for Tropical Medicine and Hygiene, New Orleans (November, 2014)

Oral presentation at the Enterics Research Investigation Network Meeting, entitled "Epidemiology and co-factors for non-typhoidal *Salmonella* bacteremia in Kenya." (June, 2014)
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Matheson AI, Manhart LE, Pavlinac PB, Means AR, **Akullian A**, Levine GA, Jacobson J, Shutes E, Walson JL. "Tools to prioritize countries for mass drug administration interventions: A case study of azithromycin for reducing child mortality." Presented at the 62nd Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 13-17, 2013, Washington DC. Abstract No. 140.

Invited Participant to the Meaningful Modeling of Epidemiologic Data (MMED) Cape Town, South Africa (June, 2013)
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Akullian A, Remais JV, Spear R. "Linking Molecular Genetics and Environmental Modeling to Identify Diffusion Pathways of the Human Parasite *Schistosoma japonicum*." Poster at The National Institute of General Medical Sciences (NIGMS) Ecology and Epidemiology of Infectious Disease (EEID) PI meeting, Albuquerque, NM. (November, 2007).

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