TYPHOIDAL *SALMONELLA* DISEASE IN MUKURU INFORMAL SETTLEMENT, NAIROBI KENYA; CARRIAGE,STRAIN DIVERSITY, AND ANTIMICROBIAL RESISTANT GENES

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Typhoidal *Salmonella* Disease in Mukuru Informal Settlement, Nairobi Kenya;Carriage, Strain Diversity, and Antimicrobial Resistant Genes

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for Examination with our approval as University Supervisors.

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DEDICATION

I dedicate this work to God Almighty who has guided me throughout my master's study and research. I also dedicate this work to my colleagues and family especially my father Charles Wandera for his financial aid and encouragement.

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ABBREVIATIONS AND ACRONYMS

µg	microgram
µl	microliter
AMR	Antimicrobial Resistance
ASTs	Antimicrobial Susceptibility Tests
CLSI	Clinical Laboratory Standard Institute guidelines
CTX-M	Cefotaxime Munich
DCS	Decreased CiprofloxacinSusceptibility
DNA	Deoxyribonucleic Acid
ESBL	Extended Spectrum Beta-lactamase
GLASS	Global Antimicrobial Resistance Surveillance System
GPS	Global Positioning System
gyrA	gyrase A
gyrB	gyrase B
iNTS	invasive Non-Typhoidal *Salmonella*
MDR	Multi-Drug Resistance
MIC	Minimum inhibitory concentration

PCR	Polymerase Chain Reaction
RAPD	Random Amplification of polymorphic DNA
SERU	Scientific Ethics Research Committee
SHV	Sulfhydryl Variant
WHO	World Health Organization
WASH	Water, Sanitation, and hygiene
XDR	Extended Drug Resistance
LMICs	Low Middle-Income Countries
WGS	Whole Genome Sequencing
RDTs	Rapid Diagnostic Tests
Pyo	Person years of observation

ABSTRACT

Typhoid fever remains a significant public health concern globally, with approximately 26 million cases and 210,000 deaths annually. In Mukuru informal settlement—characterized by high population density, poor sanitation, and inadequate access to clean water—typhoid fever is known to be endemic. This study aimed to determine the isolation rate, antimicrobial resistance patterns, and genetic diversity of *Salmonella* Typhi among cases and asymptomatic individuals in Mukuru between 2021 and 2022. In total, 1014 outpatients presenting with typhoid-like symptoms were recruited from selected health centers, with blood and stool samples collected. Asymptomatic individuals, identified from contacts living in the households of positive cases, provided only stool samples to assess their potential role in disease transmission. Isolation and identification of *S*.Typhi were performed using standard microbiological methods, including culture on selective media, biochemical tests, the API 20E system, and serotyping with specific antisera. Antimicrobial susceptibility was assessed using the Kirby-Bauer disc diffusion method, and multi-drug resistant (MDR) strains were characterized through conventional PCR and whole-genome sequencing (WGS). Bioinformatics analysis of WGS data provided detailed phylogenetic relationships and antimicrobial resistance determinants. Of the participants, 54 (5%) tested positive for *S*.Typhi through serotyping, with 70% of isolates from stool samples and 30% from blood samples; 0.2% were from asymptomatic individuals. Multi-drug resistance was observed in 37% (20) of isolates, with high resistance rates to ciprofloxacin (43%) and nalidixic acid (52%). The $bla_{TEM\text{-}1B}$ gene was detected in 95% (19/20) of MDR isolates, all carrying IncQ1 plasmids harboring multiple resistance genes. Mutations in *gyrA* and *gyrB* genes associated with quinolone resistance were also identified. Phylogenetic analysis revealed the dominance of sub-lineage 4.3.1.2 EA3 at 46% and identified three major clusters. The prevalence of MDR *S*.Typhi and reduced susceptibility to first-line antibiotics in Mukuru underscores the urgent need for effective antimicrobial stewardship, routine AMR surveillance, and improved sanitation measures. The genetic relatedness between isolates from cases and carriers suggests asymptomatic carriage as a potential source of infection, highlighting the importance of comprehensive public health strategies to control typhoid fever in high-risk settings.

CHAPTER ONE

INTRODUCTION

1.1. Background of the Study

Salmonella enterica serovar Typhi (*S*.Typhi) is a Gram-negative bacillus in the Enterobacteriaceaefamily and is predominantly a human pathogen (Chua et al., 2015). The pathogen is responsible for causing typhoid fever a systemic illness characterized by prolonged fever, headache, dry cough,and alteration of bowel habits (Crawford et al., 2010). Other common symptoms of the disease include fatigue, high fever (> 39°C), vomiting, coughing, and rapid pulse (Dougan & Baker, 2014). This disease poses a significant global health threat. According to recent estimates, typhoid fever affects approximately 5.6 billion people worldwide, and the disease results in about 210,000 deaths annually (Id et al., 2018; Parry et al., 2010). This substantial number of deaths underscores the severe impact of typhoid fever and highlights the urgent need for effective prevention and treatment strategies.

Salmonella Typhi is a human-restricted bacterial pathogen maintained in a population through asymptomaticindividuals who develop a carrier state after an infection (Wong et al., 2015). There are vaccines currently available for control and prevention of typhoid but these are not widely available in Kenya and the region (Kariuki et al., 2019). The emergence of resistance to commonly used antimicrobials and, in most cases, multiple drug resistance (MDR) is causing significant challenges with the available options for treatment (Kavai et al., 2018).

Living in densely populated areas, with poor sanitation and lack of safe drinking water, predisposespeople living within urban slums in Africa and Asia to typhoid fever (Kariuki et al., 2021). An incidence of 520 per 100,000 person-years of observation was found in children 8 years and below in Kenya's largest slum, Kibera (Breiman, et al., 2012). Mukuru settlement is one of the largest nd most overcrowded urban slums in Nairobi,

Kenya, with a population of approximately 250,000 (Kariuki et al., 2020). As an informal settlement, it is characterized by poor infrastructure, sanitation, and a lack of safe water (Kariuki et al., 2019). In addition, the patients can easily accessantimicrobials over the counter perpetuating the emergence and spread of antimicrobial resistance(Kavai et al., 2018).

Even after successful treatment, approximately 1-6% of typhoid infections become carriers(Waldner et al., 2012). The long-term persistence of *S.*Typhi in carriers explains why typhoid feverremains endemic in regions of the world with poor-quality drinking water and limited sewage treatment (Chua et al., 2015). *S.*Typhi dissemination occurs through ingesting food or water contaminated with feces from typhoid patients and human carriers (Douesnard-Malo & Daigle, 2011). Acute infections and asymptomatic carriage caused by *S.*Typhi can be treated using effective antibioticssuch as fluoroquinolones, however, the selection and spread of a highly resistant *S.*Typhi clade prevents such measures in areas with limited resources and irrational use of antibiotics (Id et al., 2018). The rise and spread of MDR *S.*Typhi, strains resistant to 3 classes of antimicrobials: ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim, is a major public health concern. This is because it increases patients' hospital stays, is costly to treat, and increases the chance of mortality (Dyson et al., 2019). Over the years, most typhoid cases in Kenya have been MDR coupled with reducedsusceptibility to fluoroquinolones (Parry et al., 2010). Fluoroquinolones are the recommended drugs for the treatment of MDR typhoid in Kenya but the indiscriminate use of fluoroquinolones has led to a rapid increase in strains that are resistant to the antimicrobial. To prevent clinical treatment failure, it is critical to know the current trend of antimicrobial resistance in an endemic setting of typhoid fever like the Mukuru settlement.

For instance, Kavai *et al.,* (2018) reported a Multi-Drug Resistance (MDR) prevalence of 55.5% of *S.*Typhi isolated from outpatient clinical samples from Mukuru villages in Nairobi (Kavai et al.,2018). Regular monitoring of the emergence of MDR *S.*Typhi and the dissemination of AMR-associated genes is crucial to gather essential information for making informed decisions on antibiotic usage (Yusof et al., 2022). It is therefore essential

to investigate the typhoid carriage andcirculating MDR strains in Mukuru. Our study aimed to determine the isolation rate of *S.*Typhi from patients and asymptomatic individuals and the MDR prevalence in Mukuru Informalsettlement.

Genotype 4.3.1, previously known as H58, has been frequently associated with carrying the MDRdeterminants and is mainly divided into two major lineages, I and II (Kariuki et al., 2021). It hasits origins in Southeast Asia and has, over the last 30 years, spread to other continents, including Africa where it is prevalent in Kenya, Tanzania, Malawi, and Southern Africa (Smith et al., 2023)(Wong et al., 2015) while Multi-Drug Resistant (MDR) clades associated with outbreaks in Nigeriaand Ghana include the *S.*Typhi H56 (Id et al., 2018). From causing epidemics across Asia, haplotype 58 has spread throughout Africa due to its ability to displace antibiotic-sensitive isolates(Dougan & Baker, 2014). One study reported at least three haplotypes that have spread throughout East Africa and the African continent (Kariuki et al., 2010). Because of this diversity,genotype 4.3.1 sub-lineages have been further classified into East African 1(EA1), EA2, and EA3 (Kariuki et al., 2021).

MDR phenotype of H58 strains is likely influenced by different regional antibiotic usage. The H58MDR is on the path to becoming the dominant strain because of the selective pressure from antimicrobial agents (Dougan & Baker, 2014). Dyson *et al*., (2019) also described H58 associated with the MDR phenotype as an evolutionary success and that virulent MDR clades are still in circulation in low-resource areas where antibiotics are misused.

Genotype 4.3.1 isolated in endemic regions such as urban informal settlements in Kenya was observed to have MDR and Decreased Ciprofloxacin Susceptibility (DCS) determinants (Kariuki et al., 2010). Fluoroquinolone resistance occurs in two ways: chromosomal mutations at the quinolone resistance determining regions (QRDR) of topoisomerase genes (*gyrA*, *gyrB*, *parC*, and *parE* genes) and resistance mediated by plasmids (Acheampong et al., 2019). Antibiotic resistance phenotypes exhibited by *S.*Typhi with DCS, MDR, and nalidixic acid resistance pose a massive threat. They take

time to be cleared from the body with the available prescribed antibiotics and often lead to clinical treatment failure (Dyson et al., 2019b). From the evidence gathered that ASTs affect clinical outcomes, this study proposes to evaluate AST profiles on the common antibiotic drugs used in the treatment of typhoid fever in Mukuru e.g., ampicillin, sulfamethoxazole-trimethoprim, chloramphenicol, nalidixic acid, fluoroquinolones, and β-Lactams.

Clusters of *Salmonella* Typhi represent genetically related isolates that originate from a common source of infection. Identifying these clusters is crucial for understanding the epidemiology of typhoid fever, as they can indicate potential outbreaks and help pinpoint sources of infection (Smith et al., 2023). Genotypic clustering of *S.*Typhi isolates involves examining genetic similarities among strains, which can reveal patterns of transmission and guide targeted public health interventions.

Recent advancements in whole-genome sequencing (WGS) have enhanced our ability to investigate these clusters with greater precision. By analyzing the genetic makeup of *S.*Typhi isolates, researchers can identify specific clusters and their associated resistance mechanisms. This approach enables a detailed understanding of the phylogenetic relationships between strains, highlighting the diversity within *S.*Typhi populations and their geographical distribution.

1.2. Problem Statement

The average incidence of typhoid fever among all age groups in Kenya is 263/100,000 (95% CI: 199–347) pyo (Kariuki et al., 2020). A high prevalence of both invasive Non-Typhoidal *Salmonella* (iNTS) and typhoid fever have been observed in Mukuru where school-going children are the most affected with 72% of positive isolates being from children of 5-16 years (Kariuki et al., 2019). Recent studies have highlighted similar trends in other urban settlements, such as Kibera, where a resurgence of typhoid fever was reported in 2019, showing high multidrug-resistant (MDR) prevalence (Ng'eno et al., 2023).

The transmission efficiency of *S.*Typhi has been linked to increased levels of fecal bacteria, with high-shedding hosts. Fecal shedding happens without the manifestation of clinical signs and it is a vital reason why carriers need to be identified (Crump, 2019).

The utilization of antibiotics in the treatment of both asymptomatic carriers and those with acute typhoid disease has been implicated in the emergence of resistance (Dyson et al., 2019). Previous studies in Kenya have reported a multidrug-resistant *S.*Typhi prevalence of 82% (Ng'eno et al., 2023). In 2018, a study in Nairobi County reported an 18.2% resistance of *S.*Typhi to fluoroquinolones and 15.4% for beta-lactam drugs. The recommended drugs for the treatment of typhoid fever countrywide (Kavai et al., 2018).

The data indicates that the bacteria is persistently evolving into highly resistant strains that cause clinical failure and typhoid outbreaks. Genotype 4.3.1 (formerly haplotype H58), which has spread globally from Southeast Asia, is now prevalent in Kenya and other regions, including Tanzania and Malawi (Kim et al., 2022). This genotype is associated with MDR traits and has displaced antibiotic-sensitive strains due to selective pressures from regional antibiotic use (Breiman et al., 2022). Despite the availability of vaccines, their limited coverage in Kenya and neighboring areas underscores the need for improved public health strategies and enhanced surveillance (Kavai et al., 2018).

1.3. Justification

Typhoid fever is endemic in Mukuru. Investigation of the current isolation rate of *S.*Typhi in thearea will inform policy-makers about the burden of the illness in the area hence supporting the identification of priorities in healthcare, prevention, and policy development. The cases and individuals found to be carriers will be recommended to start treatment with effective drugs after analyzing Antimicrobial Susceptibility Tests (ASTs) data to avoid clinical treatment failure. Current resistance phenotypes data from this study will help manage acute and severe typhoid infectionsamong typhoid patients. Knowledge of the type of *S.*Typhi genotypes harbored by human hosts and circulating in the region is useful in epidemiological surveillance of typhoid infections. It also elaborates on which

strain is more likely associated with carriage and helps identify any introductions.

1.4. Research Questions

1. What is the isolation rate of *S.*Typhi from cases and asymptomatic individuals in Mukuru informal settlement?
2. What is the antibiotic susceptibility of *S.*Typhi to most commonly used antibiotics?
3. What are the resistance genes encoding resistance to the commonly used antibiotics for treating typhoid?
4. What are the *S.*Typhi genotypes circulating in Mukuru?

1.5. Objectives

1.5.1. General Objective

The main objective of this study was to determine the isolation rate of *S.*Typhi among cases and asymptomatic individuals in Mukuru settlement and to identify and characterize antibiotic resistance genes within the same population.

1.5.2. Specific Objectives

i. To determine the isolation rate of *S.*Typhi from cases and asymptomatic individuals in Mukuru informal settlement between 2021-2022.
ii. To determine the antibiotic susceptibility of *S.*Typhi to the most commonly used antibiotics.
iii. To determine the resistance genes encoding resistance to the most commonly used antibiotics for treating typhoid.
iv. To determine the genotypes of *S.*Typhi in cases and carriers in Mukuru settlement.

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview of Typhoid Fever

Salmonella enterica subspecies *enterica* serovar Typhi is a gram-negative bacillus (Crump et al.,2019). It is an exclusively human-adapted pathogen and the etiological agent of enteric fever (Khan et al., 2022).

2.1.1. Global Burden of Typhoid Fever

Globally, approximately 26 million people are infected with typhoid fever with about 210,000 deaths occurring yearly (Id et al., 2018). This enteric fever is a major global health concern as 5.6billion people worldwide are at risk of contracting the disease (Sapkota et al., 2022). In highly endemic regions like Southeast Asia and sub-Saharan Africa, the years of lives lost from this illness are comparable to breast cancer, prostate cancer, and leukemia in North America (John et al., 2014). Enteric fever, as in the past years, continues to be a global health issue mainly confined to Africa and Asia (Smith et al., 2023). Important scientific efforts and studies have been directed towards reducing the burden of the disease worldwide. *Salmonella* Typhi is a highly virulent strain mostly causing infections in children, adolescents, and the elderly in developing countries (Tack et al., 2019). Moreover,typhoid fever is more prevalent in children above five years where a global estimate of cases has been reported at 55.9% in children less than 15 years (95% CI, 50.3–61.6), and 12.6% among children younger than 5 years (95% CI, 8.7–17.7) (Kim et al., 2022).

2.1.2. The Burden of Typhoid in Africa

In 2017 it was reported that the typhoid incidence burden in Africa had increased by 6.1% and 1.6% in low-middle-income countries (Kim et al., 2017). In sub-Saharan Africa, the burden of typhoid fever is estimated at 762 per 100,000 person-years higher than incidence

findings in Southeast Asia,East Asia, and Oceania with an estimated incidence of 108 per 100,000 person-years (Kim et al., 2023).Severe complications from typhoid infection progress to death if left untreated. The overall prevalence of complications and case fatality rate in Africa are 37.7% and 3.82% (95% CI, 1.97–7.26%) respectively (Kim et al., 2022)

Salmonella Typhi has been identified as the most common cause of bloodstream bacterial infections amongchildren in Nigeria while more than 200 cases per 100 000 pyo in a rural village of Agogo, was the reported incidence of typhoid in Ghana (Consortium et al., 2016).

2.1.3. The Burden of Typhoid in Kenya

A study conducted in Kenya in 2012 reported endemic incidence of typhoid bacteremia in an urbanslum to be 247 cases per 100,000 person-years of observation (pyo), and the rate of *S.*Typhi to be596 per 100,000 pyo. among children (Slayton et al., 2013). A Typhoid Fever Surveillance in Africa Program initiative also reported an incidence of more than 520 per 100 000 pyo in an informal settlement in Nairobi Kenya (Kalckreuth et al., 2016).

Within nineteen years the population in an urban center in Nairobi had grown by 300,000 (Kariukiet al., 2021). As population growth and migration into urban centers peaks, scarcity of the available resources affects such population hence maintaining cycles of infectious diseases. It has been implied that the urban population in Sub-Saharan Africa will be at 800 million by 2030 (Kim et al., 2022). This boom in population will likely increase the rate of typhoid infections.

2.2. Pathogenesis and Transmission of *S.*Typhi

2.2.1. Entry Into the Host and Reservoir

A reservoir can be defined as the site where an organism can effectively live and flourish throughmultiplication. For *S.*Typhi, humans are its only known reservoirs. People infected

with *S.*Typhi mainly shed it through feces and sometimes urine (John et al., 2014). Asymptomatic infected individuals experiencefecal shedding and bacteremia. Entry is through the mouth during ingestion of food or water contaminated with fecal matter (Crump, 2019). *Salmonella* Typhi incubation period and disease development is usually between 7-14 days after infection with the infection dose being the main determinant of the incubation period (de Jong et al., 2012). The infectious dose has been estimatedto be 10,000 organisms or lower differing in various settings (Dougan & Baker, 2014). Crump (2019), suggests studies be done on the contribution of temporary and chronic carriers to *S.*Typhi transmission in endemic settings.

2.2.2. Pathogenesis of Typhoid Fever

Ingested bacteria reach the small intestine where, through microfold (M) cells of Peyer's patches,the bacteria migrate to the mesenteric lymph nodes, multiply, and are released into the bloodstreamfor the first time during infection (Rahman et al., 2013). *Salmonella* Typhi disseminates causing transient primary bacteremia. Macrophages lined in the spleen, liver, and bone marrow remove the bacteriafrom blood hence presenting an opportunity for them to replicate in the macrophages (Khan & Shamim, 2022) . The bacteria reenter circulation causing secondary bacteremia which is usually the onset of clinical disease (Johnson et al., 2018). Removal of *S.*Typhi from the blood via the gallbladder exposes thePeyer's patches to the bacteria for a second time. This, coupled with the localization of *S.*Typhi to the small intestine, causes inflammation, ulceration, and typhoid ulcers (Rahman et al., 2013)

2.2.3. Typhoid Carrier State and Transmission

A typhoid carrier is an individual who after infection with the bacteria, recovers from the illness with or without treatment but continuously sheds the bacteria in feces and urine and does not showany clinical symptoms of the disease. Unlike most pathogens implicated in enteric infections, *S.*Typhi lacks an environmental reservoir explaining its adaptation to maintain transmission through human carriers (John et al., 2014). In regions of

endemicity, 3-5% of Typhoid infections lead to typhoid carriage and fecal shedding (Gonzalez-Escobedo & Gunn, 2013). Fecal Shedding is more prominent in untreated infected individuals which is why carriers shed more (de Jong et al., 2012). Fecal shedding can be temporary or chronic depending on the course of infection (Crawford et al., 2010). After the onset of acute illness,a convalescent carrier sheds *S.*Typhi for ≥3-12 months while a chronic carrier sheds typhoid bacillus for more than 12 months (Crump, 2019). As they shed bacilli in the local environment, carriers keep up with the reservoir of infection which affects the community at large (John et al., 2014). *Salmonella* Typhi remains a human-restricted pathogen that survives poorly in the environment andis trapped in the human population (Dougan & Baker, 2014).

Carriers that progress to the chronic stage are usually highly contagious as they shed a large number of bacilli in feces (Crawford et al., 2010). Apart from being at an 8-fold greater risk of developing cancer of the bladder when compared to non-carriers, chronic carriers maintain a highincidence of typhoid fever in endemic settings (John et al., 2014). Usually, 25% of chronic carriershave no history of contracting typhoid fever (Crawford et al., 2010). The persistence of *S.*Typhi in the form of carriage occurs in the following ways; through the formation of gallstones, biofilm formation, and intracellular colonization on the epithelium of the gallbladder (Gonzalez-Escobedo & Gunn, 2013). The presence of long-term carriers in the population has been associated with outbreaks (Mogasale, et al., 2016) hence identification and treatment of *S.*Typhi carriers are crucial for the control of typhoid outbreaks (Gopinath et al., 2012)

Salmonella Typhi transmission to healthy persons is majorly vehicle-borne (Crump, 2019a). *S.*Typhi can survive in contaminated food and water for extended periods but multiplication is mostly achieved in the human host (Dougan & Baker, 2014). Transmission primarily occurs via the fecal-oral route (Gopinath et al., 2012). Crump et al., (2019) observed that transmission cycles are categorized into 2; short-cycle and long-cycle. Short-cycle transmission is limited to the immediate environment. Here, food and water are contaminated through fecal shedding, and transmission is enabled by inadequate hygiene and sanitation measures (Gonzalez-Escobedo & Gunn, 2013). Chronic carriers

have been identified to cause outbreaks in the short cycle in low-incidence regions. Long-cycle transmission involves the broader environment (Johnson et al., 2018). This happens when human feces pollute untreated water sources and application of raw human feces or untreated sewage as fertilizers (Kim et al., 2022). Chronic carriers have been implicated in causing water-borne-associated transmissions in high-incidence settings (Sapkota et al., 2022).

The gold standard for the identification of carriers has been the isolation of *S.*Typhi in fecal samples (Goay et al., 2016). One study determined that the identification of carriers of *S.*Typhi can be accomplished by serial stool culture (Crump et al., 2015). In a different study to detect and find the prevalence of *Salmonella* excretion among carriers in an endemic setting, stool cultures were used from individuals in households and none were carriers (Im et al., 2016). The recommendation from the study was to do follow-up periods to determine carriers in the population.

2.3. Isolation and Identification Methods of *S.*Typhi

The isolation and identification of *S.*Typhi from clinical involves combining culture-based techniques and biochemical tests to ensure accurate detection and identification of this pathogen.

2.3.1. Culture-Based Methods

Culture-based techniques are important for isolating *S.*Typhi (Mogasale et al., 2016). When the bacterial load is low or when a high yield of the bacteria is required enrichment broths such as Selenite F broth and Tetrathionate broth are normally used (Kaur et al., 2018). These broths enhance the growth of *S.*Typhi while suppressing competing flora and coliforms, increasing the likelihood of pathogen isolation (Tack et al., 2019). After overnight incubation, the enriched samples are sub-cultured onto selective media for further identification (Mogasale et al., 2016) .

Clinical samples such as blood and stool are first cultured on selective and differential

media, including MacConkey agar and Xylose Lysine Deoxycholate (XLD) agar (Kariuki et al., 2020). On MacConkey agar, *S*.Typhi colonies are usually pale or colorless due to their inability to ferment lactose (Kavai et al., 2018). This contrasts with lactose-fermenting bacteria, which produce pink colonies, thus aiding in differentiation. On XLD agar, *S*.Typhi colonies exhibit a brick-red color with black centers due to hydrogen sulfide (H_2S) production (Khan & Shamim, 2022). This distinctive feature makes XLD agar a crucial medium for differentiating *S*.Typhi from other *Salmonella* and *Shigella* species (Mutai et al., 2018).

2.3.2. Biochemical Identification of *S*.Typhi

The identification of *Salmonella* Typhi is achieved through traditional (conventional) biochemical tests and commercial biochemical panels (Kariuki et al., 2020). These methods are essential for accurately distinguishing *S*.Typhi isolates from other *Salmonella* serovars.

The Triple Sugar Iron (TSI) Agar test is particularly important, where *S*.Typhi shows an alkaline slant and an acid butt with hydrogen sulfide (H_2S) production (Jahan et al., 2022). This result indicates glucose fermentation without the fermentation of lactose or sucrose. Other standard tests, such as the Urease Test, are also performed to confirm the absence of urease enzyme activity, which is characteristic of *S*.Typhi. The Indole and Simmons Citrate tests further support identification, as both yield negative results for *S*.Typhi, indicating no indole production and the inability to utilize citrate as the sole carbon source (Kaur et al., 2018) .

In addition to conventional methods, commercial biochemical panels like the API 20E system are extensively used in both clinical and research environments. The API 20E panel assesses bacterial metabolic reactions to a range of twenty substrates, providing a detailed biochemical profile (Samuel et al., 2019). This panel has been validated across numerous studies, demonstrating high accuracy in identifying members of the Enterobacteriaceae family, including *S*.Typhi (Argimón et al., 2016). By employing these tests and panels,

laboratories ensure precise identification of *S.*Typhi, facilitating effective diagnosis and research.

2.3.3. Antimicrobial Resistance Detection Methods

Antimicrobial resistance detection in *Salmonella* Typhi has evolved, driven by advancements in both phenotypic and genotypic methods. Conventional Antimicrobial Susceptibility Testing (AST) methods, such as the Kirby-Bauer Disk Diffusion Method and Broth Microdilution, are widely used to evaluate resistance profiles against a broad spectrum of antibiotics (Acheampong et al., 2019). These phenotypic approaches remain the cornerstone of clinical diagnostics, offering reliable data on how bacterial isolates respond to various drugs (Sharma et al., 2019). The Kirby-Bauer method involves measuring the zone of inhibition around antibiotic disks, while Broth Microdilution assesses the minimum inhibitory concentration (MIC) needed to inhibit bacterial growth (Kariuki et al., 2020).

In parallel, Polymerase Chain Reaction (PCR) assays are extensively employed to detect known resistance determinants. For instance, PCR can detect plasmid-mediated resistance genes such as $bla_{TEM\text{-}1B}$, which confers beta-lactam resistance (Goay et al., 2016). These methods provide detailed insights into the genetic basis of resistance.

Whole Genome Sequencing (WGS) represents a cutting-edge approach that has significantly advanced resistance detection. WGS offers a comprehensive view of the entire genome, allowing for the identification of both established and novel resistance mechanisms (Cuypers et al., 2018). This high-resolution technique facilitates the tracking of resistance gene evolution and the spread of resistant strains, therefore enhancing our understanding of antimicrobial resistance dynamics and guiding more effective control strategies (Hasman et al., 2014).

2.3.4. Genotyping and Phylogenetic Analysis Techniques

The exploration of genetic diversity and the phylogenetic relationships of *Salmonella* Typhi strains has been greatly refined through the application of advanced molecular genotyping methods. Historically, techniques such as Multilocus Sequence Typing (MLST) and Pulsed-Field Gel Electrophoresis (PFGE) were employed to classify *S*.Typhi strains based on variations in their DNA sequences (Harbottle et al., 2006). The MLST technique involves sequencing multiple housekeeping genes, while PFGE separates large DNA fragments after restriction enzyme digestion, providing a fingerprint of the bacterial genome (Devanga Ragupathi et al., 2016). Although these methods have been instrumental in understanding *S*.Typhi genetic variation, they often fall short in resolution compared to newer technologies.

The introduction of Whole Genome Sequencing (WGS) has marked a significant leap forward in genotyping and phylogenetic analysis. WGS provides a comprehensive view of the entire bacterial genome, facilitating high-resolution phylogenetic reconstruction and the identification of strain clusters (Cuypers et al., 2018). This method enables the detailed examination of genetic relatedness, which is crucial for tracking the transmission pathways of *S*.Typhi during outbreaks and in endemic regions (Hasman et al., 2014).

Additionally, core genome MLST (cgMLST) has become a prominent tool in modern genotyping. Unlike traditional MLST, which examines a limited number of loci, cgMLST focuses on the core genome, the set of genes present in all strains of a species (Smith et al., 2023). This approach offers a more nuanced understanding of genetic relationships and is particularly useful for tracing the evolution and dissemination of drug-resistant strains (Yap et al., 2016). These advanced techniques are pivotal for elucidating the global epidemiology of typhoid fever and informing public health strategies to combat this persistent pathogen.

2.4. Antibiotic Resistance and Genes

Antibiotic resistance genes can be defined as determinants in the nucleic acid component of an organism that has undergone mutations to enable it to survive in the presence of an antimicrobial agent (Yousafzai et al., 2019). Typhoid in the African region is known to be driven by the MDR *S.*Typhi. The MDR has resistance genes in the incH1 plasmid such as; *catA (*chloramphenicol resistance*), sul1, sul2* (sulfamethoxazole resistance), *dfrA* (trimethoprim resistance), and $bla_{TEM\text{-}1B}$ (ampicillin resistance) (Hendriksen et al., 2015). These genes collectively contribute to resistance against first-line treatments like chloramphenicol, ampicillin and sulfamethoxazole-trimethoprim (Id et al., 2018). The IncH1 plasmid, predominantly associated with the MDR phenotype, has also been reported to encode resistance genes to tetracyclines (*tetA, tetB, tetC,* and *tetD*) and *strA, strB* (streptomycin resistance) (Das et al., 2017).

2.4.1. Quinolones/Fluoroquinolones Resistance

Quinolones, including fluoroquinolones (FQs), are antibiotics that target bacterial DNA gyrase and topoisomerase IV, enzymes responsible for controlling DNA supercoiling (Amiri et al., 2017). Nalidixic acid is a first-generation quinolone, while fluoroquinolones, such as ciprofloxacin, have a fluorine atom that enhances their efficacy by improving cell penetration and increasing their inhibitory effect on DNA gyrase (Adesegun et al., 2020) Ciprofloxacin has been particularly effective against *Salmonella* species and other Gram-negative bacteria (Mutai et al., 2018).

Several mechanisms contribute to quinolone resistance. Mutations in the quinolone resistance-determining regions (QRDRs) of the *gyrA, gyrB, parC,* and *parE* genes reduce quinolone binding to DNA gyrase and topoisomerase IV (Amiri et al., 2017). Among the most common mutations is the substitution of serine with phenylalanine at codon 83 in *gyrA* (Ser83Phe), which is linked to high-level resistance (Tadesse et al., 2018).

Additionally, plasmid-mediated resistance occurs through the acquisition of *qnr* genes

(*qnrA, qnrB, qnrS, qnrC, qnrD*), which produce proteins that protect DNA gyrase and topoisomerase from quinolone inhibition. Other mechanisms include the *aac(6')-Ib-cr* gene, which encodes an enzyme that acetylates ciprofloxacin, reducing its activity, and efflux pump genes such as *oqxAB* and *qepA* that remove quinolones from bacterial cells (Amiri et al., 2017). Efflux pumps like *AcrAB-TolC* and porin alterations further lower intracellular concentrations of fluoroquinolones, contributing to resistance (Adesegun et al., 2020).

Resistance to fluoroquinolones is phenotypically assessed using the minimum inhibitory concentration (MIC) of ciprofloxacin. MIC values ≥1 μg/ml indicate resistance, while values ≤0.06 μg/ml suggest susceptibility. Intermediate MIC values are associated with decreased ciprofloxacin susceptibility (DCS), often leading to treatment failure (Tack et al., 2019).

2.4.2. Beta-Lactams Resistance

Beta-lactam antibiotics, such as amoxicillin, are commonly resisted by the production of beta-lactamases, enzymes that hydrolyze the beta-lactam ring, rendering the antibiotic ineffective (Id et al., 2018). The bla_{TEM-1B} gene is the most frequently identified beta-lactamase gene responsible for amoxicillin resistance (Kariuki et al., 2020). Resistance to third-generation cephalosporins, such as ceftriaxone, is primarily due to extended-spectrum beta-lactamases (ESBLs), which degrade these antibiotics.

In India and Pakistan, ESBL-producing *S*.Typhi isolates have been identified carrying bla_{SHV-12}, bla_{CMY-2}, bla_{TEM-1B}, and bla_{DHA-1} genes (Yousafzai et al., 2019). Similarly, studies in Kenya have identified bla_{TEM-1B}, bla_{CTX-M}, and bla_{INT} genes in isolates resistant to beta-lactams and fluoroquinolones (Kavai et al., 2018). In the Democratic Republic of Congo, an IncY plasmid carrying the $bla_{CTX-M15}$ gene, associated with resistance to third-generation cephalosporins, has been detected in *S*.Typhi strains (Lunguya et al., 2012). Extensively drug-resistant (XDR) strains of *S*.Typhi have been found to contain both $bla_{CTX-M15}$ and *qnrS* genes on a composite transposon and an additional IncY plasmid,

further complicating treatment options (Id et al., 2018).

2.4.3. Macrolides (Azithromycin Resistance)

Azithromycin, a macrolide, is increasingly used to treat typhoid fever, particularly in cases resistant to fluoroquinolones or ceftriaxone. Although azithromycin resistance is still rare, the emergence of resistant strains poses a growing concern (Marchello et al., 2020). Mechanisms of azithromycin resistance include chromosomal mutations and efflux pump activity, which reduce intracellular drug concentrations. Point mutations in the AcrB efflux pump (R717Q/L) have been implicated in resistance, as these mutations increase the pump's ability to expel azithromycin from bacterial cells (Sharma et al., 2019).

In addition to efflux mechanisms, resistance genes such as *mphA, mefA, ereA*, and *ermB* contribute to azithromycin resistance. These genes modify or methylate ribosomal targets, thereby preventing the antibiotic from binding to its target (Hooda et al., 2019). Resistance is typically confirmed when the MIC for azithromycin reaches or exceeds 32 mg/ml, the Clinical and Laboratory Standards Institute (CLSI) breakpoint (Crump et al., 2015).

2.5. Whole Genome Sequencing

The use of whole-genome sequencing (WGS) to analyze bacterial genomes for antibiotic-resistance markers and to comprehend the phylogeny of bacteria in connection to their antibiotic-resistance profiles is growing in popularity (Liaquat et al., 2018). Whole genome sequencing (WGS), which analyzes a bacterium's entire DNA sequence, has been made easier by developments in next-generation sequencing technology, which makes it a perfect method for surveillance (Hendriksen et al., 2015). Whole genome sequencing offers the finest resolution for characterizing a singleorganism and delivers unambiguous genotype information (Hasman et al., 2014). Moreover, it is possible to distinguish between strains that have the same resistance characteristics but are imparted by distinct mechanisms (Cuypers et al., 2018). Whole genome sequencing-based determination of antibiotic resistance can be used in conjunction with conventional laboratory-based

surveillance to offer direct insights into the evolution and cross-species transmission of antibiotic resistance (Kudirkiene et al., 2018). While traditional antimicrobial susceptibility tests produce the strain phenotypes, current genome sequencing methods provide improved and comprehensive data related to the genotypic characteristics of the pathogen along with the identification of virulence determinants, antimicrobial resistance genes, and serotypes (Hasman et al., 2014). For medications that are not regularly tested or for which the mechanisms of antimicrobial resistance are not yet known, WGS data can assist in illuminating the antibiotic resistance mechanism (Liaquat et al., 2018).

2.6. Clinical Management and Laboratory Diagnosis

2.6.1. Clinical and Laboratory Diagnosis

Clinical diagnosis of typhoid fever is challenging because care must be taken to distinguish it from other gastrointestinal diseases and febrile illnesses like malaria, amebiasis, typhus leishmaniasis, and tuberculosis (Sapkota et al., 2022). This is because, at the onset of typhoid infection,the disease is normally characterized by non-specific symptoms like headache, malaise, and delirium (Johnson & Mylona, 2018).

Salmonella Typhi can be isolated from bone marrow, blood, intestinal secretions, and stool. Cultures differ in the sensitivity rates where less than 50% in stool, 40-60% in blood, and 90% in bone marrow (Adesegun et al., 2020). The gold standard for the diagnosis of typhoid fever is a bone-marrow culture but it is highly invasive and impractical in most settings (Kim et al., 2022). Thus, the mostpreferred method is blood culture. Specimens collected for culture are done using selective bacterial media in multiple series of cultures to increase sensitivity (Sapkota et al., 2022).

The polymerase chain reaction is a more sensitive test in the detection of *S.*Typhi genetic sequences in specimens. However, the use of this test is limited especially in resource-poor regionsof Africa (Waldner et al., 2012).

Serologic techniques available for the diagnosis of enteric fever include the tube/slide agglutination test (Widal test), indirect hemagglutination, indirect fluorescent Vi antibody and enzyme- linked immunosorbent assay (ELISA), (Dougan & Baker, 2014) . In Africa, majority ofsettings use the widal test for the diagnosis of typhoid fever because of its cost effectiveness (Adesegun et al., 2020). However, studies have shown that this test can lead to misdiagnosis since the presence of cross-reactive antibodies to other illnesses such as non-typhoidal *Salmonella* infections, malaria, dengue fever, and endocarditis, can confound the result (Id et al., 2018).

As a result, alternative tests have been widely adopted, especially in LMICs. Despite several reports of low sensitivity and specificity, which in certain circumstances have resulted in multiplemisdiagnoses, disease outbreaks, treatment delays, and even deaths, the Widal test remains the most often used rapid diagnostic test (RDT) (Khanam et al., 2013). Other typhoid RDTs, including Tubex (IDLBiotech), Test-it Typhoid IgM (LifeAssayDiagnostics), and Typhidot (ReszonDiagnostics), have also been found to have limitations (Mather et al., 2019). Even thoughthese are better than the Widal test, their sensitivity and specificity are still relatively moderate (Khanam et al., 2013).

A recent study set to evaluate the sensitivity and specificity of nine commercially available *Salmonella* Typhi rapid diagnostic tests (RDTs) observed that the best-performing RDT was the Enterocheck WB, with 72.7% sensitivity and 86.5% specificity (Sapkota et al., 2022). While the Typhoid IgG/IgM Combo Rapid Test CE showed a higher sensitivity for IgG (Sapkota et al., 2022).

2.6.2. Management of Typhoid Fever and Antibiotic Resistance

From a microbiological perspective, antibiotic resistance is when a pathogenic organism's growthcan no longer be hindered or inhibited by the application of a drug previously used. This can be due to the acquisition of molecular determinants over time that helps it survive and flourish in thepresence of the antimicrobial agent. Multi-drug resistant *S*.Typhi is a term used to describe *S*.Typhi strains whosegrowth is not inhibited by chloramphenicol,

ampicillin, and sulfamethoxazole-trimethoprim drugs while XDR (extensively drug-resistant) *S.*Typhi are strains that are cephalosporin-resistant in addition to having MDR and fluoroquinolone resistance properties (Galán, 2016).

The choice of antibiotics depends on the susceptibility of circulating *S.* Typhi strains in an endemicarea. Before the antibiotic era, the average mortality rate of untreated typhoid was 13.5% (Adesegun et al., 2020).

The discovery of chloramphenicol in the treatment of clinical complications caused by *S.*Typhi was a major clinical breakthrough because it reduced the mortality rate to <1% (Hooda et al., 2019). This however was followed by resistance to the drug (Dyson et al., 2019). In the 1960s, resistance to the first-line antibiotics against *S.*Typhi i.e. chloramphenicol, sulfamethoxazole-trimethoprim, and ampicillin was frequently observed. This phenomenon was described as MDR *S.*Typhi (Crump et al., 2015). With the emergence of the MDR phenotype against these drugs, fluoroquinolones and 3rd generation cephalosporins were considered. However, *S.*Typhi strains resistant to these drugs have already been reported (Id et al., 2018) Poor diagnosis of typhoid fever coupled with unregulated administration of the classic first-line antibiotics and fluoroquinolones has progressively led to resistance traits in *S.*Typhi (Kariuki et al., 2010).

Multi-drug resistance is declining in Asia while increasing in Africa (Dyson et al., 2019). This could be due to the stringent measures applied in antimicrobial use in Asia while careless use of antibiotics in Africa continues without restrictions (Kariuki et al., 2019). *S.*Typhi resistance patterns are evolving at different rates with diverse phenotypic characteristics in various endemic areas (Yousafzai et al., 2019). Extensively drug-resistant *S.*Typhi has been isolated in Asia showing resistance to 3rd generation cephalosporins (Id et al., 2018). This is a looming clinical problem with treatment using the available antibiotics. The discovery of the XDR strain in Pakistan is a huge public health concern as it may take us in the pre-antibiotic era where typhoid fever remained untreatable and caused a staggeringly large number of deaths especially in low-middle income countries (Levine, 2018).

The World Health Organisation recommends the treatment of uncomplicated typhoid fever caused by both sensitive and MDR strains using fluoroquinolones (Acheampong et al., 2019). Studies have been done to find out if susceptibility tests affect clinical outcomes, for example, Parry *et al*., (2011) conducted susceptibility tests of *S*.Typhi to fluoroquinolones and discovered a clear relationship in the ofloxacin susceptibility and clinical outcomes from patients. The same study also found that because of the spread of MDR strains that prompted the usage of fluoroquinolones, emergence has risen especially to ciprofloxacin and ofloxacin across parts of Africa and Asia (Parry et al., 2011). From random clinical trials, data analyzed showed that elevated MIC of ciprofloxacin and ofloxacin implicated failure of treatment using these drugs (Acheampong et al., 2019). Antibiotic Susceptibility Tests (ASTs) on *S*.Typhi in Asia have shown a marked increase in resistance to nalidixic acid and fluoroquinolones, from 20% in 2001–2005 to 65% in 2011-2015 (Id et al., 2018).

Typhoid outbreaks reported in Africa are caused by the MDR *S*.Typhi with the MDR phenotype being up to 90% in some regions (Kariuki et al., 2019). Between 2004 and 2006 the prevalence of MDR in Kenya was 70% and 61% in Nigeria (Dyson et al., 2019). Because of the high prevalence of the MDR phenotypein endemic settings, ciprofloxacin was introduced in the treatment of typhoid fever. Shortly after, *S*.Typhi isolates resistant to fluoroquinolones were quickly observed (Cuypers et al., 2018). Areas that reported both MDR and fluoroquinolone resistance initiated treatment with azithromycin and ceftriaxone, which was followed by reports of extended-spectrum beta-lactamase enzymes e.g. SHV-12, CTX-M, AmpC-producing *S*.Typhi isolates(Crump et al., 2015). MDR *S*.Typhi's resistance to fluoroquinolones has been linked to causing outbreaks in Southeast Asia and here in Kenya. Fluoroquinolones have already been rendered ineffective in some parts of Southeast Asiaand West Africa (Kariuki et al., 2010). Seeing this imminent threat, prevention strategies should include the identification of carriers that continually supply the bacteria to the population and breakthe chain of transmission.

Multi-drug resistant clades associated with outbreaks in Nigeria and Ghana include the *S*.Typhi haplotype H56 (Id et al., 2018). Kariuki *et al.*, (2010) reported that the *S*.Typhi

clone of the H58 haplotype isolated from past typhoid outbreaks in Kenya has its origins in South East Asia and has been observed to be a multi-drug and partially resistant clone. Phylogenetic analysis from single nucleotide polymorphism methods showed that the haplotype H58 is an expanding node (Dougan & Baker, 2014). It has been discovered that *S.*Typhi evolved from a single populationand likely entered the human population only recently (Galán, 2016). From causing epidemics across Asia, the haplotype H58 has entered Africa and is more stable and long-lived than MDRphenotypes (Dougan & Baker, 2014). One study reported at least 3 haplotypes that have spread throughout East Africa and the African continent (Kariuki et al., 2010). The H58 haplotype that is MDR is on the path of becoming the dominant strain because of the selective pressure from the usage of antimicrobial agents (Dougan & Baker, 2014). Dyson et al., (2019) also described H58 associated with the MDR phenotype as an evolutionary success and that virulent MDR clades are still in circulation in low-resource areas where there is careless use of antibiotics. The *S.*Typhi haplotype H58 isolated in endemic regions in Kenya was observed to have MDR and Decreased Ciprofloxacin Susceptibility (DCS) determinants (Kariuki et al., 2010). Antibiotic resistance phenotypes exhibited by *S.*Typhi with DCS, MDR, and nalidixic acid pose a huge threat as they take time to be cleared off from the body with the available prescribed antibiotics and often lead to clinical treatment failure (Dyson et al., 2019).

Salmonella Typhi is known to cause chronic infections associated with relapse and in some individuals' asymptomatic carriage both of which can be successfully stopped by the use of effective antibiotics (Chua et al., 2015). The selection and spread of the MDR *S.*Typhi clade have been demonstrated to occur in areas with limited resources and careless use of antibiotics hence limit treatment options (Dyson et al., 2019). Kariuki et al., (2019) found that inappropriate usage of antibiotics in Mukuru settlement is enhancing more resistance patterns. Dyson et al., (2019) also determined that utilization of antibiotics in the treatment of both asymptomatic carriers and those with acute typhoid disease have been implicated with the emergence of resistance. In such endemic settings, the spread of antimicrobial-resistant strains has and will continuously impair treatment options if proper

antibiotic stewardship is not applied (Kariuki et al., 2010).

In Kenya infections caused by MDR *S*.Typhi are normally treated by the fluoroquinolones ciprofloxacin and norfloxacin as has been the practice for the past years (Kavai et al., 2018). However, the prevalence of MDR *S*.Typhi resistance to nalidixic acid is increasing, implying strains resistant to fluoroquinolones are establishing themselves. This will lead to other less readily available and expensive drugs being sought (Kariuki et al., 2019). In a recent study using archived hospital and clinical isolates from villages in Mukuru, the classic first-line antibiotics in the treatment of typhoid i.e. ampicillin, sulfamethoxazole-trimethoprim, chloramphenicol, and tetracycline drugs had a 55.5% resistance from typhoid cases while resistance to fluoroquinolones and 3rd generation cephalosporins was 18.2% and 15.4% respectively from the antibiotic susceptibility tests performed (Kavai et al., 2018).

2.6.3. Prevention and Control of Typhoid Fever

Given that humans are the only known host of *S*.Typhi and the fecal-oral transmission channel for typhoid fever, it is imperative to implement effective WASH approaches. This will include approaches such as improving food hygiene and water sanitation, as significant critical strategic control measures (Adesegun et al., 2020). The complete adoption of sanitation and hygiene has not been attained due to high levels of poverty, particularly in endemic areas of LMICs, which has caused a slowdown in socioeconomic progress (Kariuki et al., 2020).

Low-middle-income countries are unable to fully rely on antimicrobial treatment to control typhoid fever, mainly becauseof the deficiency of functional medical services and the rising antimicrobial resistance (AMR) in *S*.Typhi (Khan & Shamim, 2022).

Accordingly, vaccination must be considered an essential precautionary strategy against typhoid fever for residents of endemic areas and visitors to these parts of Southern and Eastern Africaas well as the continent of Africa (Crump et al., 2015).

WASH practices, such as improved sanitation, clean water, and hygiene, are effective ways to prevent typhoid fever (Kim et al., 2023). Boiling water before consuming it is always recommended. Ice for drinks that can be consumed with it should be prepared from boiling water. Avoiding popsicles and flavored ice is advised since they might have been produced with contaminated water. Among the household, precautions include making sure that all raw edible foods are well cleaned with clean water and that other meals are cooked to the highest degree possible and consumed hot (Solomon et al., 2018).

It is advisable to refrain from consuming food and beverages from street sellers who lack the necessary accreditation to handle food, as it might be challenging to maintain food cleanliness (Bafa et al., 2019). According to Solomon et al. (2018), individual governments can support health professionals by making sure that suitable drain and sewerage systems are in place, providing safe drinking water in public schools, and pushing for widespread typhoid vaccination campaigns.

2.6.4. Vaccine Intervention

Currently, two commercial vaccines are available for the prevention of typhoid fever: the live oral vaccine (Ty21a) and the Vi polysaccharide vaccine (ViCPS or Vi) (Milligan et al., 2018). Ty21a is an attenuated vaccine derived from a strain of *S.*Typhi, which induces both cell-mediated and humoral immunity. It is typically administered to individuals over five years of age (Mastroeni et al., 2001). The ViCPS vaccine, based on the Vi antigen expressed by *S.*Typhi, induces a T-cell-independent humoral immune response and can be administered to children under two years of age, who are at higher risk of *S.*Typhi infection in endemic areas (Milligan et al., 2018). Both vaccines are available in Kenya, with an efficacy of 55% for the ViCPS vaccine and 51% for the Ty21a vaccine (Kariuki et al., 2019).

2.7. Research Gap and Rationale for the Study

Despite significant progress in understanding the burden, transmission, and antimicrobial

resistance of *Salmonella* Typhi, gaps remain in key areas that need further investigation.

While various studies have documented the rise of multidrug-resistant *S*.Typhi strains and the prevalence of quinolone, beta-lactam, and macrolide resistance genes globally, including in Kenya, there is limited local data on the genotypic diversity and specific resistance determinants present in informal urban settlements like Mukuru. Most previous studies have focused on general population dynamics, but there is insufficient data on the role of asymptomatic individuals in the transmission of resistant strains and how these carriers contribute to the persistence of typhoid fever within endemic areas.

Furthermore, although the use of whole-genome sequencing (WGS) has enhanced our understanding of antimicrobial resistance patterns, it is underutilized in Kenya's public health surveillance, particularly in community-based settings. The genetic linkage between cases and carriers in informal settlements has not been fully explored.

This study seeks to address these gaps by investigating the genotypic diversity of *S*.Typhi isolates and determining the antimicrobial resistance genes from both clinical cases and carriers within an endemic informal settlement. It will also explore the potential role of carriers in maintaining the transmission cycle of resistant *S*.Typhi strains, which could provide crucial insights for future prevention and control strategies.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Site

Mukuru is one of the largest and overcrowded informal settlements in Nairobi city, Kenya (Reuben et al., 2017). It lies at longitude 1°19'27.91" S and latitude 36°53'35.72" E. Mukuru informal settlement is located 25km east of the central business district with a population of about 250,000 people. The settlement constitutes 86 villages (Kariuki et al., 2019). The general population is approximately 60% male and 40% female. Mukuru is characterized by poor infrastructure and severely limited options for sanitation and safe water for drinking (Reuben et al., 2017). The livingconditions residents of the settlement are exposed to do not meet the sustainable development goals in areas of water and sanitation. The residents are exposed to major health risks especially enteric diseases because of inadequate toilets, pollution, solid-waste management, and poor drainage systems (Kariuki et al., 2019).

The study participants were recruited from three clinics in Mukuru informal settlement: Medical Missionaries of Mary (MMM), Municipal County Council Clinic (MCC), Mukuru Kwa Reuben Clinic (MR), and one inpatient facility, Mama Lucy Kibaki Hospital.

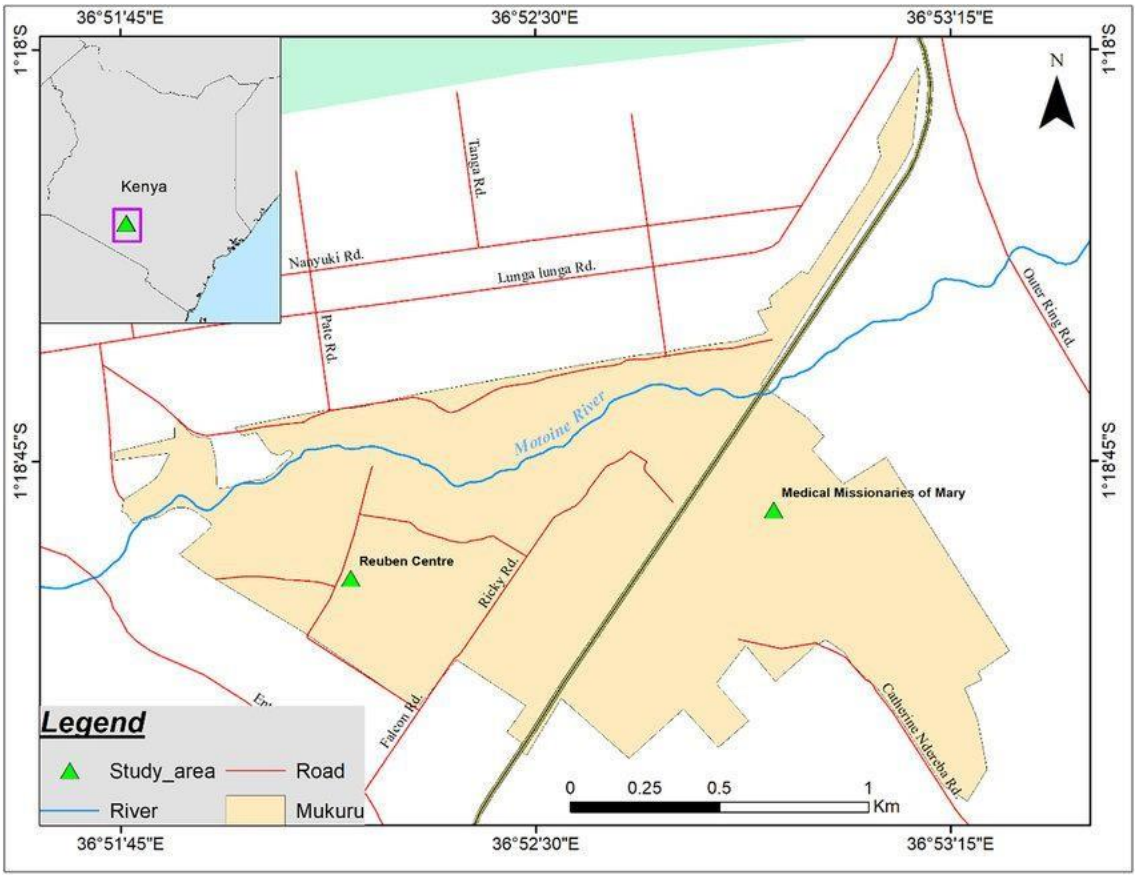

FIGURE 3.1: A Map Showing the Study Areas in Mukuru Settlement, Nairobi Kenya (Mulinge et al., 2021)

3.2. Study Design

A cross-sectional study design was used to determine the isolation rate of *S.*Typhi among cases and asymptomatic individuals in the Mukuru settlement and to identify and characterize antibiotic resistance genes within the same population in 1 year.

3.3. Study Population

The study participants were residents of Mukuru informal settlement aged 0-80 years (0 years= infants less than 1 year) and in-patients at Mama Lucy Kibaki Hospital.

3.3.1. Inclusion Criteria

- Individuals with fever with a body temperature ≥37.5 ^{o}C

- Individuals who have passed three or more watery stools in the 24 hours before presentation at any of the 3 clinics.
- Individuals who would not have used any antibiotic for the current episode of diarrhea to increase the chances of isolating *S*.Typhi.

3.3.2. Exclusion Criteria

- Participants who do not meet the inclusion criteria
- Individuals who do not give consent to participate in the study.

3.4. Sample Size and Sampling Technique

Fisher's sample size calculation method will be used to establish the sample size in the study (Jung 2014).

N = Z2 P (l-P)/d2

Where N = Minimal sample size:

Z =Standard normal deviation corresponding to 95% confidence interval (=1.96);

P = Estimated prevalence of 4% (*S*.Typhi annual isolation rate from fecal samples, (Kariuki et al., 2020)

d = degree of precision (2%)

= (1.96)20.04(1-0.15)/ (0.02)2 = 368

N = 368 required sample size

3.5. Recruitment

Study participants were recruited from July 2021 to July 2022. These were outpatients who visited the four health facilities and presented with typhoid-like symptoms such as headache, diarrhea/constipation, fatigue, and a temperature > 37.5℃. After evaluation of the patient's symptoms and inclusion criteria, the attending clinicians filled out the patients' case report forms.The patients were then introduced to the study and made to understand its objectives by the research team. Patients who were willing to participate were taken through the informed consent or assent forms in their preferred language i.e., English or Kiswahili, and those who gave their consent were given a unique study identifier that was filled in on questionnaires and the same ID used on sample collection tubes. For every confirmed *S.*Typhi case, at least one contact of the patient living in the same household was requested to give a stool sample to check for carriage. Before sample collection, the contact was taken through the same ethical considerations describedabove and if found to be a typhoid carrier, the individual was treated.

3.6. Specimen Collection

In this study, both blood and rectal swabs were collected from cases while rectal swabs only were collected from carriers. The field research team guided the participants on how to use rectal swabs. For children, their guardians were provided with rectal swabs and instructed on how to use them correctly. Rectal swabs were collected and dipped into Cary Blair media (Oxoid, Basingstoke, UK). Aseptically, blood specimens were drawn from the arm by venipuncture and transferred directly into the blood culture bottle by qualified phlebotomists. 1-3ml and 8-10ml of blood were collected for children and adults respectively. Following the collection of the samples, a unique study identification number/barcode was labeled on the sample. Blood was transported at room temperature while rectal swabs were transported at 4-8℃ in cooler boxes to the CMR-KEMRI laboratory within 6 hours of collection.

3.7. Isolation and Identification

Upon receipt in the laboratory, rectal swabs were placed in selenite fecal enrichment broth and incubated at 37 degrees overnight for 18-24 hours. From the enriched selenite F broth, streaking was done onto Xylose Lysine Deoxycholate agar (XLD) agar and Mac Conkey agar (Oxoid). Incubation was done at 37˚C for 24 hours. *Salmonella* Typhi isolates were initially identified using distinguishing colony morphology characteristics such as pale colonies on MAC and brick red with black centers on XLD. Non-duplicate colonies from standard biochemical tests such as citrate, indole, urease, and Triple Sugar Iron (TSI) were examined with API20E (Biomeriux) and confirmed with serology. Serotyping tests were done using the slide agglutination technique using polyvalent antisera O, and monovalent anti-sera O- 9, vi and d (Murex Diagnostics, Dartford, UK) (Kavai et al., 2018).

Blood culture bottles were incubated in a BACTEC 9050 Culture System (Becton Dickinson, USA) at 37ºC for up to seven days. A positive culture bottle with the reference strain was used to validate the results. Samples flagged as negative by the BACTEC were discarded while those flagged as positive were cultured onto MacConkey agar, Blood agar and Chocolate agar. Isolates showing growth characteristics of *S*.Typhi were subcultured onto Mueller Hinton (Oxoid, Basingstoke, UK) for the growth of single discrete colonies.

3.8. Identification of *S*.Typhi Tube Biochemical Tests

Using *Salmonella* suspect colonies, four biochemical tests namely; Simmon's citrate, Urea, Triple Sugar Iron, and Sulphur Indole Motility were done. *Salmonella* Typhi is unable to utilize citrate as a source of energy resulting in no color change in the medium.

Urea is the product of the decarboxylation of amino acids. Hydrolysis of urea produces ammonia and Carbon IV Oxide. The pathogen produces no color change or yellow as a result of acid production. Triple sugar iron is a differential medium with three carbohydrates glucose, sucrose, and lactose. For *S*.Typhi, the media appears with a red slant and yellow butt (because of the bacteria's ability to utilize glucose) with slight

production of H_2S.

The indole test demonstrates the ability of certain bacteria to decompose the amino acid tryptophane to indole, which accumulates in the medium. When indole is combined with Kovac's Reagent the solution turns from yellow to cherry red. *Salmonella* Typhi is unable to decompose tryptophane to indole. Motility- *S.*Typhi has flagella and in the media, it's observed to grow from the stab line.

3.9. Analytical Profile Index Biochemical Test

An analytical profile index, or API 20E, is a biochemical panel used to distinguish and identify members of the Enterobacteriaceae family. Twenty mini-test chambers with dehydrated media that have chemically determined compositions for every test are contained on the API 20E's plastic strip. Typically, they identify enzymatic activity, primarily associated with the inoculated organisms' fermentation of carbohydrates or catabolism of proteins or amino acids.

3.10. Serology

For the serological identification of *S.*Typhi, pure colonies identified by API20E were subjected to the slide agglutination technique using polyvalent O, followed by monovalent antisera O-9, Vi, and d (Murex Diagnostics, Dartford, UK). A pure colony was picked from a Mueller Hinton agar plate and mixed with a drop of sterile normal saline on a glass slide to create a milky suspension. An antisera drop was added to the suspension, and the mixture was observed for agglutination. The presence of agglutination confirmed the identification of *S.*Typhi.

3.11. Antibiotic Susceptibility of Isolates

The Kirby-Bauer disc diffusion technique was used in testing the antimicrobial susceptibility of *S.*Typhi isolates on Mueller-Hinton agar (Oxoid). The inoculum turbidity of the isolates was compared against the McFarland 0.5 turbidity standard. *Salmonella*

Typhi isolates were inoculated evenly on Mueller-Hinton Agar plates using the spread plate technique. The plates were then impregnated with antimicrobial sensitivity discs using sterile forceps and then gently pressed down onto the agar. Plates were then incubated at 37°C for 18 hours. The following OxoidTM antibiotic disks were used; amoxicillin-clavulanate (AMC 20:10 µg), nalidixic acid (NA 30µg), ciprofloxacin (CIP 5µg), ampicillin (AMP 10µg), ceftazidime (CAZ 30µg), cefotaxime (CTX 30µg), ceftriaxone (CRO 30µg), sulfamethoxazole-trimethoprim (SXT 1.25/23.57µg), chloramphenicol (CHL 30µg), and tetracycline (TET 30µg)., cefpodoxime (CPD 10µg) kanamycin (K 30µg) azithromycin (AZT 15µg). For each *S.*Typhi isolate, two plates with antibiotics were used, labeled as Plate A and B. Plate A targeted Extended Spectrum Beta Lactamases (ESBL) production. The arrangement of antibiotics was as follows, penicillins (AMP), 3rd generation cephalosporin (CRO, CAZ, CTX, CPD), inhibitor at the middle (AMC). In plate B CIP, NA (targeting fluoroquinolones resistance), GEN, KAN, (targeting aminoglycosides resistances), CHL, SXT, and TET were also used because they are the recommended first-line drugs for the treatment of typhoid. Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used for quality control of media quality and disc potency. CLSI guidelines were used to interpret zones of inhibition into susceptible, intermediate, and resistant (AST 2022.n.d.). The isolates exhibiting resistance to chloramphenicol, ampicillin, and sulfamethoxazole-trimethoprim were classified as MDR (Marchello et al., 2020).

3.12. Polymerase Chain Reaction

Out of the 54 *S.*Typhi isolates, twenty MDR isolates that showed resistance to ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim were further examined for the corresponding resistance genes using the conventional polymerase chain reaction. DNA was extracted using the boiling method. Briefly, a pea-sized amount of freshly cultured isolates was placed in 500µl PCR water (Invitrogen) in an Eppendorf tube. The mixture was then heated in a heating block at 95℃ for 12 minutes, followed by centrifugation at 14000rpm for 5 minutes to obtain the supernatant. 200 µl of the supernatant was used as the template. The following resistance genes were tested; beta-lactamase genes (*blaCTX*-

M, bla$_{TEM-1B}$, bla$_{SHV}$) *qnrS,* and *qnrA* for plasmid-mediated quinolones resistance. PCR water was used as a negative control. Amplification was performed in 0.2ml PCR tubes on a thermocycler.

Amplification conditions were as follows, initial denaturation at 95℃ for 5 minutes, 95℃ for 30 seconds variable annealing temperature for 30 seconds, 72℃ for 2 minutes 30 seconds and a final extension at 72℃ for 7 minutes. Annealing temperatures differed for the different genes as shown in **Table 3.1.**

Table 3.1: Primer Sequences for ESBLs and Quinolone Resistance Used in this Study.

Primer	Sequence	Annealing Temp℃	Expected Bps
bla$_{CTX-M}$	5'-SCSATGTGCAGYACCAGTAA-3' 5'-CCGCRATATGRTTGGTGGTG-3'	60℃	593
bla$_{TEM-1B}$	5'-TCGGGGAAATGTGCGCG-3' 5'-TCGGGGAAATGTGCGCG-3'	50℃	851
bla$_{SHV}$	5'-AGCCGCTTGAGCAAATTAAAC-3' 5'-ATCCCGCAGATAAATCACCAC-3'	50℃	650
qnrA	5'-TTCAGCAAGAGGATTTCTCA-3' 5'-GGCAGCACTATTACTCCCAA-3'	55℃	657
qnrS	5'-ACGACATTCGTCAACTGCAA-3' 5'-TTAATTGGCACCCTGTAGGC-3'	54℃	417

3.13. Gel Electrophoresis Protocol

For the analysis of DNA fragments, agarose gel electrophoresis was performed using a 1.5% agarose gel prepared in 1XTBE (Tris-Borate-EDTA) buffer. A 1.5% agarose gel was prepared by dissolving 1.5 g of agarose in 100 mL of 1XTBE buffer. The agarose was melted by heating in a microwave until completely dissolved. The solution was then

cooled to approximately 60°C before pouring into a gel casting tray with a comb to create wells. The gel was allowed to solidify at room temperature for approximately 30 minutes. DNA samples were mixed with a loading dye and loaded into the wells of the solidified agarose gel. A DNA ladder (molecular weight marker) was also included in one well for size reference. The gel was submerged in a tank filled with TBE buffer. Electrophoresis was carried out at 100 volts for approximately 1 hour or until the dye front had migrated an appropriate distance through the gel. After electrophoresis, the gel was stained with an appropriate DNA stain (e.g., ethidium bromide or SYBR Green) for visualization under ultraviolet (UV) light. DNA bands were visualized and documented using a gel documentation system. The size of the DNA fragments was estimated by comparing them to the DNA ladder, and the results were analyzed for further interpretation.

3.14. Whole Genome Sequencing

3.14.1. DNA Extraction

All 54 archived *S*.Typhi isolates stored in glycerol at -80°C were sub-cultured and revived for DNA extraction. DNA isolation was done using the GenElute bacterial DNA kit (Sigma-Aldrich), and the concentrations were measured using Nanodrop (Thermo Fisher Scientific) and stored at - 80℃. The DNA was shipped according to the Kenya Medical Research Institute material transfer agreement guidelines (MTA) to the National Institute of Infectious Diseases (NIID), Japan, for whole genome sequencing.

The DNA concentration was first measured using the Qubit 4.0 fluorometer (Thermo Fisher Scientific). Following the manufacturer's instructions, a genomic library was prepared using the QIAseq FX DNA Library kit (Qiagen). The steps involved in library preparation are detailed below.

3.14.2. Fragmentation

The prepared master mix was added to PCR tubes, and the DNA was subsequently fragmented using a thermocycler set with a lid temperature of 70℃.

3.14.3. Ligation

Briefly, 2.5µl of DNA adapter and ligation master mix was added to PCR tubes while tracking the barcodes from each adapter well used. This was followed by incubation at 20℃ for 15 minutes in a thermocycler with the lid open.

3.14.4. Amplification of the Library

After adapter ligation cleanup, 13.25µl of the prepared master mix was added to PCR tubes. This was followed by programming the thermocycler with the following conditions for six cycles for library amplification: 98℃ for 2 minutes, 98℃ for 20 seconds, 60℃ for 30 seconds, 72℃ for 30 seconds, 72℃ for 1 minute and 4℃. The concentrations of the amplified Fx library were measured using Qubit and pooled. To assess the quality of the library, BluePippin (Sage Science) was used to size-select the DNA. This was followed by doing qPCR (Light Cycler, Roche) to measure the quantity of the DNA library pools. The purified libraries were sequenced on the Illumina Miseq platform, next-generation sequencing technology.

3.15. Genomic Sequence Analysis

The resultant paired-end reads from Illumina were assembled using SPAdes software (version 3.15), generating a draft genome for each sample. CheckM (version 1.1.6) was used to assess the quality and check for contamination of the draft genome.

The assembled genomes were analyzed using online bioinformatics pipelines like the Center for Genomic Epidemiology (CGE) of the Technical University of Denmark http://www.genomicepidemiology.org/services/. Species identification was done using KmerFinder (version 3.2) https://cge.food.dtu.dk/services/KmerFinder/ (Larsen et al., 2014)(Hasman et al., 2014) (Clausen et al., 2018) determination of the MLST profile was performed using MLST v2.0 https://cge.food.dtu.dk/services/MLST/ (Larsen et al., 2012). Identification of resistance genes was done using ResFinder 4.4.3 https://cge.food.dtu.dk/services/ResFinder/ (Bortolaia et al., 2020). *Salmonella* serovars

were predicted using the SeqSero2 v1.2 https://cge.food.dtu.dk/services/SeqSero/ (Zhang et al., 2019). Plasmid presence was investigated using PlasmidFinderf version 2.1 https://cge.food.dtu.dk/services/PlasmidFinder/ (Camacho et al.,2009; Carattoli et al., 2014)

In addition, raw paired-end reads (FastQ format) were also analyzed using Enterobase (Zhou et al., 2020), a web-based platform that enables visualization of genetic variation within enteric bacteria. The raw sequencing data was uploaded on Enterobase https://enterobase.warwick.ac.uk/ for subsequent analysis. Serotype prediction was performed using SeqSero and SISTR1 (Yoshida et al., 2016). To analyze the phylogeny and genetic relatedness of the isolates, the Core-genome MLST tool, which incorporates 3002 genes, was used utilizing the CgMLST2 + HierCC V1 scheme. Phylogenetic and genetic relatedness was visualized using a GrapeTree-generated minimum spanning tree using the 'MSTree V2 algorithm' tool found within the database (Zhou et al., 2018).

The clusters were formed by collapsing branches by setting the value of the branches to 5, indicating that all isolates with ≤5 allelic differences were grouped, creating a single circular node. Genotyping of the isolates (as called by single nucleotide polymorphism) was performed using the GenoTyphi Scheme (Wong et al., 2016) at the Pathogenwatch platform https://pathogen.watch/. We further investigated the phylogenetic relatedness and diversity of our *S*.Typhi from the three clusters against other strains in the Enterobase data. This was done by searching for the nearest matches with a cut-off criterion of up to 20 allele differences.

3.16. Data Analysis

The data obtained was entered into MS Excel. The isolation rate was determined by dividing typhoid-positive cases by the total number of study participants and expressed as a percentage. Frequenciesand proportions of categorical data were analyzed using SPSS version 26. The zones of inhibitionby disc diffusion were measured in millimeters and interpretation was done according to Clinical and Laboratory Standard Institute (CLSI)

guidelines (AST 2022.n.d.). WHONET was used to generate the graph depicting the distribution of resistance using disk diffusion zone diameters (WHONET, 2023).

3.17. Ethical Consideration

Ethical approval for the study was obtained from the Scientific Ethical Review Committee (SERU) of Kenya Medical Research Institute (No. KEMRI/SERU/CMR/P00156/4198). Written informed consent was obtained from adults participating in this study. For children, written informed consent was obtained from the parents/guardians and verbal assent for older children 13-17 years was recorded. Unique identifiers in place of names were used to ensure that the participants' anonymity was upheld. All the gadgets where data was stored were password protected and access to the data was strictly given to authorized staff.

3.18. Intellectual Property Rights (IPR)

All IPR-related issues were handled per the KEMRI guidelines.

3.19. Biosafety Measures

Protective gloves and laboratory coats were worn when handling specimens. Work surfaces were decontaminated periodically during processing using 70% ethanol. The contaminated material after use e.g. Petri dishes and swabs were collected in red biohazard bags for disposal to the incinerator following KEMRI biosafety guidelines.

3.20. Dissemination of Findings

Findings from this study were shared with the health facilities for clinical care purposes. The data collected in this study was used to write a manuscript for publication in a relevant journal.

CHAPTER FOUR

RESULTS

4.1. Identification of *S*.Typhi Isolates

The identification of *S*.Typhi isolates was confirmed through a series of microbiological tests as summarized in **Table 4.1** including API 20E, where all isolates showed the expected profiles.

Table 4.1 Summary of Test Results for Identification of *S*.Typhi

Test/Method	Expected Results for *S*.Typhi	Results for Isolates (n=54)
Culturing		
MacConkey Agar	Pale colonies	Pale colonies (n=54)
XLD Agar	Brick red colonies with black centers	Brick red colonies with black centers (n=54)
Biochemical Tests		
Simmon's Citrate	No color change (citrate negative)	No color change (n=54)
Urea	No color change/yellow (urease negative)	No color change/yellow (n=54)
Triple Sugar Iron (TSI)	Red slant, yellow butt with H_2S production	Red slant, yellow butt with slight H_2S (n=54)
Sulphur Indole Motility (SIM)	Motile, no indole production	Motile, no indole production (n=54)
Serological Tests		
Poly O	Presence of slide agglutination	Positive (n=54)
O-9	Presence of slide agglutination	Positive (n=54)
Vi	Presence of slide agglutination	Positive (n=54)
d	Presence of slide agglutination	Positive (n=54)

4.1.1. API 20E Results

The API 20E panel confirmed the identification of the isolates as *S.*Typhi as shown in **Table 4.2**, with all isolates showing the expected profiles for this organism.

Table 4.2: API 20E Biochemical Test Results for *Salmonella* Typhi Identification

Test	***S.*Typhi**
ONPG (Ortho- nitro- phenyl-galactoside)	--
ADH (Arginine dihydrolase.)	--
LDC (Lysine decarboxylase.)	+
ODC (Ornithine decarboxylase)	--
CIT (Citrate.)	--
H2S (Hydrogen sulphide.)	+
URE (Urea.)	--
TDA (Tryptophane deaminase.)	--
IND (Indole.)	--
VP (Voges proskauer.)	+
GEL (Gelatin.)	--
GLU (Glucose.)	+
MAN (Mannitol.)	--
INO (Innositol.)	--
SOR (Sorbitol.)	+
RHA (Rhabinose.)	--
SAC (Sucrose.)	--
MEL (Melbiose.)	+
AMY (Amygdalin.)	--
ARA (Arabinose.)	--

4.2. Social Demographics Characteristics and Isolation Rate of *S.*Typhi

This study recruited a total of 1,014 participants presenting with typhoid-like symptoms across four study sites over one year, from 2021 to 2022. Initially, the sample size was calculated using Fisher's formula, with a 2% margin of error, which estimated 368 participants. This calculation aimed to provide a sufficient sample size to estimate the prevalence of *S.*Typhi with reasonable accuracy. However, after reviewing the study's objectives and considering the potential for variability, we decided to increase the sample size to 1,014 participants as a result of sampling for one year to enhance the precision of our findings. Moreover, given the public health importance of typhoid fever in the Mukuru settlement and the resources available for this study, it was both feasible and ethically justifiable to recruit more participants. This decision ensured that our results would have the greatest possible impact on guiding future public health policies regarding typhoid control and prevention in similar settings.

The majority of participants were from the Medical Missionaries of Mary (MMM) clinic, which accounted for 415 participants (41%). The gender distribution was nearly equal, with 516 females (51%) and 498 males (49%) participating in the study. Out of the 1,014 participants, *S.*Typhi was isolated from 54 individuals by serotyping, resulting in an overall isolation rate of 5%. Among the positive cases, females had a slightly higher isolation rate, with 29 out of 54 cases (54%) compared to males, who accounted for 25 cases (46%) as shown in **Table 4.3**. In terms of the study site, the highest number of *S.*Typhi isolates came from MMM, with 31 out of 54 cases (57%) being identified at this site. Additionally, the study identified three asymptomatic carriers, representing 0.3% of the total participant pool. These carriers were contacts of confirmed cases and were identified through stool sample testing. The age of participants with positive *S.*Typhi isolates ranged from 8 months to 45 years, with an average age of 19 years. The highest isolation rate was observed in the 21-30 age group, which accounted for 24 out of 54 cases (44%)

Table 4.3: Demographic Characteristics of Study Participants and Isolation

Social demographic characteristics		Typhoid status Negative *n*=960	Positive *n*=54	Total=1014
Gender	Female	487 (51%)	29 (54%)	516 (51%)
	Male	473 (49%)	25 (46%)	498 (49%)
Age_group	0-10	577 (60%)	19 (35%)	596 (58.7%)
	11-20	88 (9%)	3 (6%)	91 (8.9%)
	21-30	125 (13%)	24 (44%)	149 (23.5%)
	31-40	102 (11%)	5 (9%)	107 (10.5%)
	41-50	45 (4.7%)	3 (6%)	48 (4.7%)
	51-60	16 (1.7%)	0 (0%)	16 (1.4%)
	61-70	5 (0.5%)	0 (0%)	5 (0.4%)
	71-80	2 (0.2%)	0 (0%)	2 (0.2%)
Specimen_type	Blood	0 (0%)	16 (30%)	16
	Stool	0 (0%)	38 (70%)	38
Recruitment_facility	MCC	216 (23%)	10 (19%)	226 (22%)
	MLK	142 (15%)	11 (20%)	153 (15%)
	MMM	384 (40%)	31 (57%)	415 (41%)
	MR	218 (23%)	2 (4%)	220 (22%)
Participant_type	0ut-patient	907 (94%)	51 (94%)	958 (94.5%)
	Contact	51 (5.3%)	3 (6%)	54 (5.3%)
	Emergencyroom	1 (0.1%)	0 (0%)	1 (0.01%)
	In-patient	1 (0.1%)	0 (0%)	1 (0.01%)
Carrier status			3 (0.3%)	3(0.3%)

Table 4.2 legend: MCC (Municipal City Council Clinic), MLK (Mama Lucy Kibaki Hospital), MMM (Medical Missionaries of Mary), MR (Mukuru Kwa Reuben)

4.3. Antimicrobial Susceptibility Patterns

The overall prevalence for the Multi-Drug Resistant (MDR) phenotype was 37% (20/54). In addition, resistance to chloramphenicol was 39% (21/54) and 46% (25/54) both for ampicillin andsulfamethoxazole-trimethoprim. Moreover, the most common resistance phenotype was nalidixicacid where more than half of the isolates were resistant at 52% (28/54).

A statistical comparison between the resistance to nalidixic acid and chloramphenicol using Fisher's Exact Test indicated that the difference in resistance rates was not statistically significant ($p = 0.246$). This suggests that while the prevalence of resistance to nalidixic acid is higher, it may not represent a significantly different pattern of resistance compared to chloramphenicol within this sample.

A high proportion of the isolates showed reduced susceptibility towards ciprofloxacin which is the drug of choice for the treatment of typhoid fever at 43% (23/54). All the isolates were susceptible to the aminoglycoside gentamicin but resistance to kanamycin was observed at 2% (1/54) as shown in **Table 4.4.** Interestingly, all the *S*.Typhi isolates analyzed were fully susceptible to the 3rd generation of cephalosporins used in this study i.e., ceftriaxone, cefotaxime, ceftazidime and cefpodoxime.

Table 4.4: Percentage of Antimicrobial Resistance Among the 54 *S*.Typhi Isolates

Antimicrobial	Susceptible	Resistant
	Number (%)	Number (%)
Ampicilin	29(54)	25 (46)
Ceftadizime	54 (100)	0
Ceftriaxone	54 (100)	0
Cefpodoxime	54 (100)	0
Cefotaxime	54 (100)	0
Amoxicillin clavulanate	53 (98)	1 (2)
Sulfamethoxazole-trimethoprim	29 (54)	25 (46)
Ciprofloxacin	31 (57)	23 (43)
Nalidixic acid	26 (48)	28 (52)
Azithromycin	49 (91)	5 (9)
Gentamicin	54 (100)	0
Kanamycin	53 (98)	1 (2)
Tetracycline	52 (96)	2 (4)
Chloramphenicol	33 (61)	21 (39)

In addition, resistance to amoxicillin-clavulanate was recorded at 2% (1/54). Resistance to tetracycline and azithromycin was 4% and 9%, respectively as shown in Table 4.4 Two out of three of the asymptomatic individuals harbored *S*.Typhi resistant to nalidixic acid and ciprofloxacin while one carrier harbored the *S*.Typhi MDR phenotype.

Table 4.5 depicts resistance levels among isolates from different study sites. Medical Missionaries of Mary exhibited the highest resistance rate towards nalidixic acid at 75%, in contrast to lower rates observed at other sites. This elevated resistance percentage is partly attributable to the higher number of isolates obtained from this site. This figure underscores significant geographic variability in resistance patterns and indicates a need for localized intervention strategies to address elevated resistance in specific areas.

Table 4.6 illustrates antimicrobial resistance profiles stratified by age group. Resistance to ciprofloxacin, which is the current first-line treatment for typhoid fever in Kenya, was most prevalent in the 19-30 age group at 52%, whereas it was lowest in the 41-50 age group at 4%. The observed age-specific resistance patterns are likely influenced by the differential number of isolates collected across age groups. This figure highlights the necessity of incorporating age-specific resistance data into treatment protocols and surveillance strategies to optimize therapeutic efficacy and monitor resistance trends effectively.

The resistance towards the first-line drugs; chloramphenicol, ampicillin and sulfamethoxazole-trimethoprim was high in Medical Missionaries of Mary ranging from 50% to 62% and was lowest in Mama Lucy Kibaki hospital ranging from 1-2% at 62%. The percentage resistance to ciprofloxacin which is currently the recommended drug for the treatment of typhoid in Kenya was highest in Medical Missionaries of Mary at 73% and lowest in Mukuru Kwa Reuben and Mama Lucy Kibaki Hospital at 4%.

Table 4.5: Antimicrobial Resistance According to Sampling Site

Study site	AMP	CAZ	CTX	CPD	CRO	SXT	AMC	CIP	NA	AZM	CN	K	TCY	CHL
Mukuru Kwa Reuben	2 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (10%)
Medical Missionaries of Mary	14 (56%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	15 (60%)	0 (0%)	17 (73%)	21 (75%)	2 (40%)	0 (0%)	0 (0%)	1 (50%)	13 (62%)
Mama Lucy Kibaki Hospital	2 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	1 (4%)	1 (4%)	1 (20%)	0 (0%)	0 (0%)	1 (50%)	1 (5%)
Municipal City County Clinic	7 (28%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (24%)	1 (100%)	4 (17%)	5 (19%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)	5 (24%)

Table 4.6: Antimicrobial Resistance According to Age Group

Age_group	AMP	CAZ	CTX	CPD	CRO	AMC	CIP	NA	AZM	CN	K	TCY	CHL	SXT
<5	4 (19%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	2 (9%)	3 (11%)	3 (60%)	0 (0%)	0 (0%)	1 (50%)	2 (10%)	4 (16%)
6-18	5 (23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (9%)	5 (18%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (24%)	5 (20%)
19-30	12 (57%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	12 (52%)	15 (54%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	11 (52%)	12 (48%)
31-40	3 (14%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (22%)	4 (14%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	2 (10%)	3 (12%)
41-50	1 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (9%)	1 (4%)	1 (20%)	0 (0%)	1 (100%)	0 (0%)	1 (5%)	1 (4%)

KEY: TCY-tetracycline, CHL-chloramphenicol, AZM-azithromycin, SXT- sulfamethoxazole- trimethoprim, CIP-ciprofloxacin, NAL-nalidixic acid, KAN-kanamycin, GEN-gentamicin, CPD-cefpodoxime, CTX-cefotaxime, CRO-ceftriaxone, CAZ-ceftazidime, AMC-amoxicillin clavulanate, AMP-ampicillin.

4.4. Detection of Resistance Genes

A total of twenty isolates exhibiting the MDR phenotype were screened for *bla*$_{TEM-1B}$, *bla*$_{CTX-M}$, *bla*$_{SHV}$, *qnrA*, and *qnrS* genes. Of all the five genes, the beta-lactamase gene *bla*$_{TEM-1}$ was positive and found present in 95% (19/20) of the MDR isolates, as shown in Figure 4.1

Figure 4.1: Gel Electrophoresis for *bla*$_{tem-1B}$ 851 bp Positive (from S2-S20) MDR Isolates. Abbreviations; M-molecular ladder (100-5000bp), S1-S20- MDR *S.*Typhi isolates. NC-NegativeControl (PCR water); PC-Positive Control (known positive control strains). *Numbers at the toprepresent DNA numbers of the isolate.

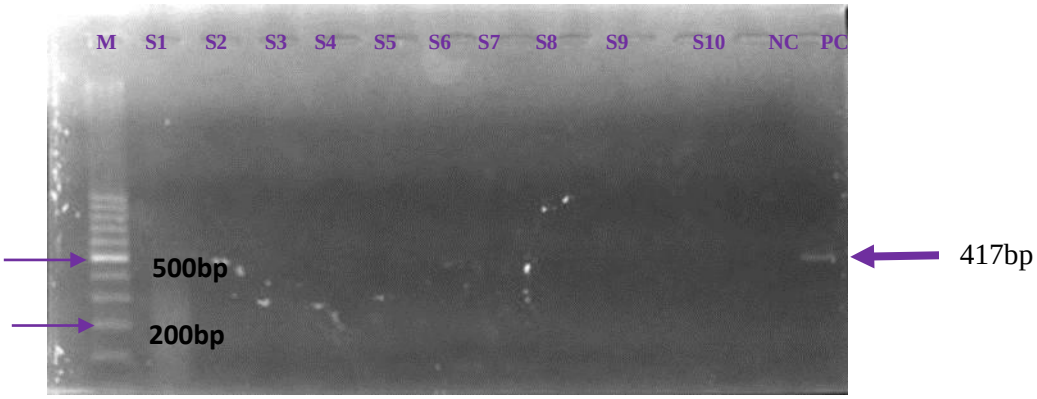

Figure 4.2: Gel Electrophoresis for *qnrS* 417 bp Negative from Selected *S*.Typhi Isolates. Abbreviations; M-molecular ladder (100-5000bp), S1-S10 *S*.Typhi isolates. NC-NegativeControl (PCR water); PC-Positive Control (known positive control strains).

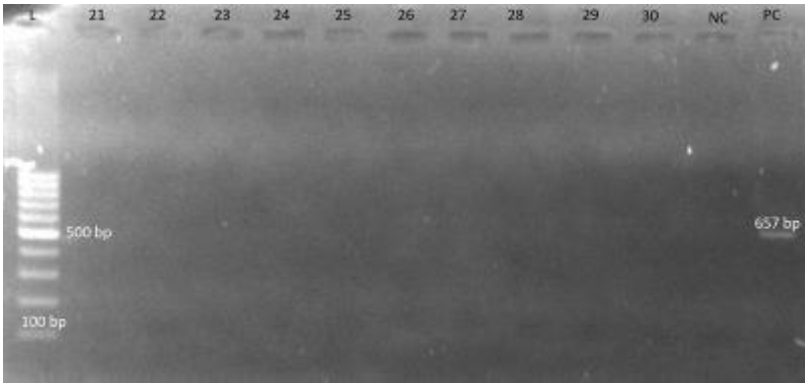

Figure 4.3: Gel Electrophoresis for *qnrA* 657 bp Negative from Selected *S*.Typhi Isolates. Abbreviations; L-molecular ladder (100-5000bp), 21-30 *S*.Typhi isolates. NC-Negative Control (PCR water); PC-Positive Control (known positive control strains).

4.5. Cluster Identification-Phylogenetic Relatedness of Isolates

In total, 54 presumed *Salmonella* Typhi DNA samples were sequenced, but 10 (18%) failed quality control (QC) and could not be assembled. From the 44 (81%) that were assembled, 35 (79%) were confirmed to be *S.*Typhi by WGS, while the remaining 9 (20%) belonged to other serovars.

Core genome MLST data analysis showed varied genetic diversity and clustering among isolates. Three major clusters were identified among 35 *S.*Typhi isolates, as shown in Figure 4.4. The cluster definition for this study was ≥5 isolates that are closely related to cgMLST and defined by a distinct genotype. The five-allele difference threshold is indicative of a high probability of epidemiological relatedness.

Cluster 1 had the highest number of isolates, comprising 15 cases and 1 isolate from a carrier. In total, 69% (11/16) of the isolates were from MMM, 19% (3/16) from MCC, and 13% (2/16) from MLK and MR. All isolates in this cluster depicted the same AMR profile as shown in Table 4.7 and 50% (8/16) of the strains in this cluster were isolated from blood.

Cluster 2 included six isolates, as shown in Figure 1, with five cases and one from a carrier. All the strains were sourced from Mama Lucy Kibaki Hospital. Most of the isolates were from stool at 83% (5/6) and 17% (1/6) from blood. The six isolates in this cluster all had a mutation in *gyrB* (S464F) gene while only one isolate harbored a plasmid. Cluster 3 had five isolates, including a single carrier. All the isolates were from Medical Missionaries of Mary and showed varied AMR profiles.

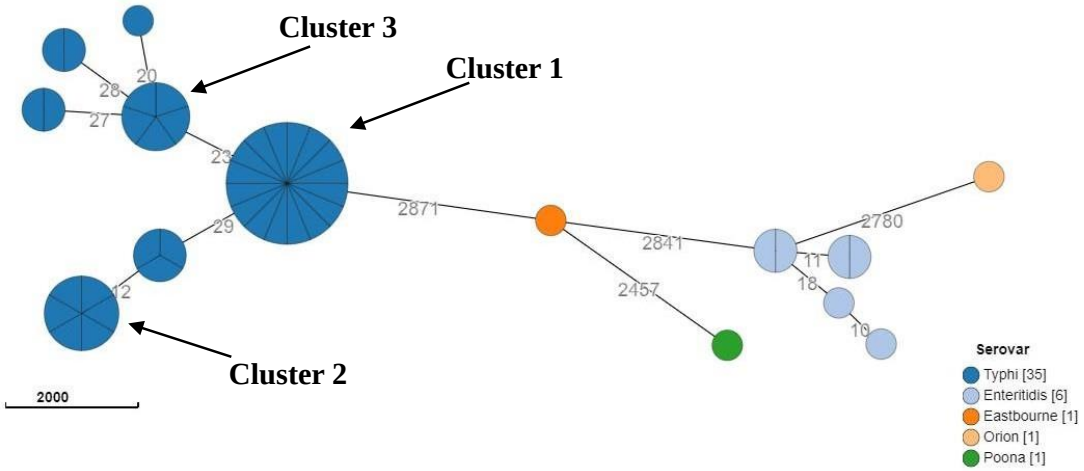

Figure 4.4: Minimum Spanning Tree Drawn Using cgMLST Data from 54 Presumed *S.*Typhi Isolatedfrom Mukuru settlement, Nairobi Kenya 2021-2022. The circular nodes represent a number of isolates with identical cgMLST profiles. Nodes are colored by serovar type. Isolates showing ≤5 allelic differences are collapsed together to form a single node. The number values between adjacent nodes indicate the number of allele differences between connecting nodes or isolate(s).

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/ GenBank under the following BioProjects and accession numbers: BioProject PRJNA1064142: Accession number: JAYWIQ000000000. The version described in this paper is version JAYWIQ010000000.

https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1064142.BioProjectPRJNA1065890:Accession numbers: JAYXJA000000000 to JAYXKH000000000. The version described in this paper is version JAYXJA010000000. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1065890.

4.6. Genotypic Diversity and Phylogeographical Relatedness

All the 35 confirmed *S.*Typhi isolates were of the sequence type 1 identified by MLST profiling. The 35 strains were highly diverse and classified into sub-lineages I and II, three East African sub- groups EA1 to 3 were observed. Sub lineage 4.3.1.2 EA3 was found at high frequency at 46% (16/35), followed by 4.3.1.2 EA2 at 28% (10/35) and 4.3.1.1 EA1

at 27% (9/35). To determine the phylogenetic relatedness of our clusters and other strains in the Enterobase database, the cgMLST hierarchical cluster tool at the EnteroBase platform was used. At HC50 (Hierarchical clustering) - isolates clustered at 50 allele differences, all the isolates showed HC50:202, an Enterobase assignment indicative of the haplotype 58.

Further analysis at the HC2 level provided more sensitive discrimination of the isolates. For cluster one, at the HC2 level, all isolates were assigned as HC2:73749, as shown in Table 4.7. The nearest matches were isolates from the U.K. (2019), Kenya (2018), U.S. (2017), Tanzania (2010), India (2011) and Rwanda (2013). For cluster 2, isolates were assigned HC2:364463 (n=6), HC2:363947(n=2), and HC2:366515 (n=1). Close matches were found to be from Kenya, Tanzania (2012), India (2011), Malawi (2009), South Africa (2012) and Malawi (2009). Cluster 3 isolates were grouped under HC2:364082 (n=6), HC2:363947(n=2) and HC2:364465 (n=1), HC2:366515 (n=2), HC2:155256 (n=1) and HC2:155253 (n=1). Close matches were found to be from Kenya, Tanzania (2008-2009), Zambia (2011), Malawi (2010), South Africa (2012) and Vietnam (1996)

Table 4.7: Genotypic Diversity and cgMLST Hierarchical Clustering of the Strains

Cluster No.	**Mlst**	**cgMLST HC2 no.***	**CgMLST HC50 no.***	**Genotype†**
1	1	73749	202	4.3.1.2 EA3
2	1	364463;363947 366515	202	4.3.1.1 EA1
3	1	364082;363947;364465 366515;155256;155253	202	4.3.1.2 EA2

*As per assignment at Enterobase (https://enterobase.warwick.ac.uk/species/index/senterica)

†As per assignment at Pathogenwatch (https://pathogen.watch/)As per assignment at the Center for Genomic Epidemiology, TDU(https://cge.food.dtu.dk/services/MLST/)

CgMLST, core genome Multi Locus Sequence Typing; QRDR, Quinolone Resistance DeterminingRegion

4.7. Antimicrobial Resistance Determinants from Whole Genome Sequencing Data

In this study, the following genes associated with antimicrobial resistance were detected; beta- lactam-*TEM1B* at 57% (ampicillin resistance), *dfrA7* at 57%, *sul2*; *sul1* at 20%, *sul1* at 37% (resistance to trimethoprim and sulfonamides), *catA1* at 57% (chloramphenicol resistance) and *aph(6)-Id at 37%* (streptomycin resistance). These acquired resistance genes were mostly likely carried on the plasmid IncQ1, which was found to be present in 57% (20/35) of the *S*.Typhi isolates.Sixteen isolates of genotype 4.3.1.2 EA3 all showed the presence of the plasmid, while three isolates of genotype 4.3.1.2 EA2 had the plasmid, and one isolate of 4.3.1.1 EA1.

Resistance towards quinolones/fluoroquinolones was mediated by chromosomal mutations in the QRDR Quinolone resistance-determining region of DNA gyrase. Mutations were found in the following codons: *gyrA* (S83Y) in 49% of the isolates and *gyrB* (S464Y) in 43%. No mutations were identified in the *parC* and *parE* genes. Notably, no 3rd generation cephalosporins resistancegene determinants were found in ResFinder 4.3.3 pipelines at the time of analysis. These results are in line with the phenotypic screening of resistance which observed no resistance towards 3rd generation cephalosporin drugs.

The 3 clusters differed in their patterns of conferring resistance, with cluster 1 isolates having thehighest number of R-genes, as shown in **Table 4.**

Table 4.8: Main AMR Gene Distribution Detected Among Isolates Associated with the Three Clusters

Cluster	Isolates	Beta-lactamas	Fluoroquinolones	Phenicols	Sulphonamides	Aminoglycosides	Trimethoprim	Plasmid Type
1	16	*bla_{TEM-1B}*	*gyrA (S83Y)*	*catA1*	*sul2, sul1, sul2 sul1*	*aac(6')-Iaa, aph(6)-Id(strB), aph(3'')-Ib (strA)*	*dfrA7*	IncQ1
2	6	*bla_{TEM-1B}*	*gyrB (S464F) ,*	*catA1*	*sul2 sul1*	*aac(6')-Iaa, aph(3'')-Ib (strA)*	*dfrA7*	IncQ1
3	5	*bla_{TEM-1B}*	*gyrA (S83Y) , gyrB (S464F)*	*catA1*	*sul2 sul1*	*aac(6')-Iaa, aph(3'')-Ib (strA)*	*dfrA7*	IncQ1

The table presents the resistance genes identified for various antimicrobial classes, including beta-lactams, fluoroquinolones, phenicols, sulphonamides, aminoglycosides, and trimethoprim. It also highlights mutations in the Quinolone Resistance Determining Region (QRDR) and the presence of specific plasmid types (IncQ1) is noted.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. Discussion

In the current study, the initial isolation of *S.*Typhi was recorded at 5%, with 54 *S.*Typhi isolates obtained from 1,014 patients. However, Whole Genome Sequencing (WGS) confirmed that only 35 of the 44 isolates subjected to sequencing were indeed *S.*Typhi. Therefore, the confirmed isolation rate based on WGS results is lower than initially reported, giving approximately 3.5%. The isolation rate of 3.5% found in this study is comparable to the 3.2% reported by Kariuki et al. (2021) indicating the endemicity of typhoid in the area. Of the 54 *S.*Typhi isolated in this study, three were from asymptomatic individuals giving an isolation rate of 0.2% from carriers in contrast to Kariuki*et al* (2019) who reported a carriage rate of 1% (Kavai et al., 2018). This variation in results could be attributed to the differences in the study participants' inclusion criteria.

The isolation rate from stool was high at 70% (38/54) compared to 30% (16/54) from blood. A similar variation was observed by Kariuki *et al.* (2021) who reported an isolation rate of 58% fromstool samples and 48% from blood (Kariuki et al., 2021). The low isolation rate from blood is mainly due to insentivity of blood culture technique, with only 50-55% detection rate at best (Mogasale et al., 2016).

In this current study, the highest isolation rate at 44% (24/54) was from the age group 21-30. In addition, it was also observed in the above-mentioned study that *S.*Typhi was more prevalent in patients over five years of age (Kariuki et al., 2021). The difference in isolation rate is evidence that the prevalence of *S.*Typhi varies within populations over time. The isolation of *S.*Typhi strains resistant to at least three different classes of antimicrobials—ampicillin, sulfamethoxazole-trimethoprim, and chloramphenicol—indicates that MDR strains continue to pose a health challenge in our community. Our study found an MDR prevalence of 37%, which is lower than the 55.5% prevalence

reported by Kavai et al. (2018) in Mukuru. This observed decline could be attributed to several factors. Improved antimicrobial stewardship and the implementation of policies on prudent antimicrobial use may have contributed to this reduction. Additionally, changes in antimicrobial use patterns over time could play a role. The reduction in MDR prevalence might also be linked to enhanced public health measures, such as improved sanitation and hygiene practices, which can reduce the transmission of *S.*Typhi. Furthermore, ongoing efforts in the National Action Plan to combat and prevent Antimicrobial Resistance (AMR) might have had an impact. These findings are consistent with Tack et al. (2019), who reported an MDR prevalence of 38.3% in the Democratic Republic of Congo, suggesting that similar factors might be influencing MDR trends across different regions.

Ciprofloxacin is the antimicrobial of choice for the treatment of typhoid fever caused by MDR *S.*Typhi (Yousafzai et al., 2019). Recommendations to use ciprofloxacin/ofloxacin over the yearshave resulted in the rise of resistance (Tack et al., 2019). We recorded resistance and decreased ciprofloxacin susceptibility to ciprofloxacin and nalidixic acid at 43% and 52%, respectively. These results are comparable to Mutai et al., *2019* who reported high resistance to ciprofloxacin at69% in different settings. Typhoid infection by strains that are resistant to nalidixic acid has beenlinked to the causation of prolonged illness and hepatomegaly (Kadhiravan et al., 2005). Various studies have highlighted a strong correlation between *S.*Typhi strains resistant to nalidixic acid/fluoroquinolones and poor clinical outcomes (Rahman et al., 2014). This implies that for successful treatment, other antimicrobial alternatives such as azithromycin and 3rd generation cephalosporin should be considered.

For cases of complicated typhoid fever, third-generation cephalosporins are the drugs of choice (Johnson & Mylona, 2018); our isolates were fully susceptible to this class of antimicrobials. However, these findings contrast those of Kavai *et al,* (2018) study, who reported less than 5% resistance towards third-generation cephalosporins (Kavai et al., 2018). Similarly, Tack *et al.* (2019) reported a 0.2% resistance towards 3rd generation cephalosporins in a study done in DRC (Tack et al., 2019). The variation in resistance

levels could be attributed to them not being the first-line treatment for typhoid fever in the Mukuru settlement. Because no resistance was observed inour study phenotypically, the 3rd generation cephalosporins, specifically ceftriaxone, can be effective in the treatment of typhoid fever in our endemic settings. Full susceptibility implies that *S*.Typhi isolates in this study did not harbor extended-spectrum beta-lactamase genes (ESBL) that could otherwise render the antimicrobial ineffective. This was confirmed by the absence of the ESBL $bla_{CTX\text{-}M}$ gene by conventional PCR.This study reports a high prevalence of the $bla_{TEM\text{-}1B}$ gene at 95% (19/20 MDR isolates). These results are comparable with Kavai *et al* 2018, who reported an 80% prevalence of the $bla_{TEM\text{-}1B}$ gene from *S*.Typhi isolated in selected Nairobi clinics (Kavai et al., 2018). Temporal variation when the studies were done could account for the differences in the prevalence. The $bla_{TEM\text{-}1B}$ gene confers resistance to ampicillin and is associated with the emergence of ESBL-producing bacteria that are resistant to advanced cephalosporins (Devanga et al., 2016). The high prevalence of thegene implies that the strains are evolving to carry genes that make them survive in the presence ofdifferent classes of antimicrobials hence causing treatment failure that translates to therapeutic challenges with the available antibiotics for the treatment of typhoid.

S.Typhi resistance patterns are evolving at different rates with diverse phenotypic characteristics in various endemic areas (Tack et al., 2019). Typhoid in the African region is known to be drivenby the MDR *S*.Typhi. These strains harbor resistance genes in the incH1 plasmid such as *catA, sul1, sul2, dfrA,* $bla_{TEM\text{-}1B}$*, strA, strB, tetA, tetB, tetC, and tetD* (Id et al., 2018). These genes contribute to resistance to chloramphenicol, ampicillin sulfamethoxazole-trimethoprim, and tetracycline drugs (Johnson et al.,2018)

It has been reported that chromosomal mutation in the *gyrA* gene is the most common source of reduced ciprofloxacin susceptibility in Africa (Acheampong et al., 2019). Ciprofloxacin is designed to target DNA gyrase specifically GyrA, a protein that is essential in bacterial DNA replication (Chau et al., 2007). Mutation on a single nucleotide at either codon position 83 or 87 of the gene (*gyrA*) encoding for the GyrA protein leads

to resistance to nalidixic acid which translates to reduced susceptibility to fluoroquinolones like ciprofloxacin (Id et al., 2018). It hasbeen discovered that resistance to nalidixic acid is a marker for fluoroquinolone resistance becausemutations on the *gyrA* gene eventually evolve to cause resistance (Chau et al., 2007). This study screened for the plasmid-mediated resistance genes; *qnrB* and *qnr*S. None of these genes were found present among the twenty MDR isolates. This shows that the resistance observedin our present isolates could be caused by a mutation in the chromosomally located *gyrA* gene. However, all the 54 isolates will be subjected to whole genome sequencing which will be able to provide comprehensive data of the resistance genes found in the *S.*Typhi strains.

Findings in this study show the current isolation rate of *S.*Typhi from an informal settlement including the trends in antimicrobial resistance across four sites. Comprehensive approaches to mitigate typhoid burden include improvements in hygiene and vaccination strategies which requireidentification of high-risk populations through continuous surveillance programs.

All the 35 *S.*Typhi isolates confirmed by WGS in this study were found to be of Sequence Type (ST) 1. This finding is comparable to studies done in Kenya and Zambia (Kariuki et al., 2021) (Yamba et al., 2022). The dominance of ST1 in endemic regions has been attributed to its ability to escape innate immunity, persisting in human carriers and expression of virulence genes (Yap etal., 2016). A previous study using a case-control design identified close phylogenetic relationshipsbetween *S.*Typhi from cases and carriers (Kariuki et al., 2021). *S.*Typhi isolates from the three carriers in this study were distributed in each of the major clusters. Because clusters represent highly genetically similar isolates, this observation implies that these carriers could be a source ofinfection in the informal settlement, contributing to the endemicity of typhoid in the area (Gopinathet al., 2012) as previously studied in Zambia and South Africa (Yamba et al., 2022) (Smith et al., 2023). *Salmonella* clusters indicate a common infection or transmission source (Smith et al., 2023).

Sub-lineages I and II of the 4.3.1 genotype were observed in this study, including the

subgroups EA1, EA2, and EA3. However, 4.3.1.2 EA3 was found at high frequency at 46% (16/35), contrary to Kariuki *et al*., (*2021)* who reported 4.3.1.2 EA2 as the most dominant (Kariuki et al., 2021). This variation could result from the smaller sample size of 35 *S.*Typhi isolates used in this study compared to the previous study that analyzed 240 *S.*Typhi. Our findings contrast that of Smith *et al 2023* who identified genotype 4.3.1.1EA1 in South Africa as the most prevalent, implying thatgenotypic diversity differs with geographical locations (Smith et al., 2023). H58 is known to originate from South Asia and later was disseminated in different parts of the world. Previous studies have shown that both sub-lineages I and II are well established in East and Southern African countries, and the presence of very close clustering of the isolates indicates transmission events and regional outbreaks (Wong et al., 2015). A recent study has observed that lineage I strain descendants form a single monophyletic clade while lineage II has two clades. All consist of *S.*Typhi from East African countries, hence the annotation EA1, EA2, and EA3 (Kariuki et al., 2021).

Furthermore, all three East African lineages harbored mutations for decreased ciprofloxacin susceptibility (DCS). This study identified a single *gyrA* mutation and *gyrB* mutation in the quinolone-resistant determining region (QRDR) of the *Salmonella* Typhi strains. In *gyrA* mutation(S83Y) occurring in 49% (17/35) of the isolates analyzed, the amino acid serine was substituted with tyrosine at codon 83. For *gyrB* mutations, the amino acid serine was substituted forphenylalanine at codon 464 (S464Y), and this was identified in 43% (15/35) of the isolates. These findings are similar to Wong *et al.* 2015 who reported the same gene mutations in the QRDR region which led to decreased ciprofloxacin susceptibility (Wong et al., 2015).

The *gyrA* gene mutation in this study was high compared to *gyrB.* This agrees with findings fromAcheampong *et al* 2019., who reported a high frequency of g*yrA* mutations compared to other topoisomerase genes (Acheampong et al., 2019). In Africa, it has been documented that the prevalent mutation known to account for DCS in *S.*Typhi isolates is found in the *gyrA* gene (Tadesse et al., 2018). These observations likely reflect the therapeutic use of fluoroquinolones totreat typhoid caused by multidrug-resistant

(MDR) *S.*Typhi in Mukuru settlement. In addition, nomutations were observed in *parC* or *parE* genes in this study, consistent with previous studies (Kariuki et al., 2021). Fluoroquinolones target the DNA gyrase subunits (*gyrA* and *gyrB*) and topoisomerase components (*parC* and *parE*). Mutations in these genes can decrease the susceptibility to fluoroquinolones and occur more frequently in H58 isolates than in other *S.*Typhi haplotypes (Wong et al., 2015). In addition, the most common mutation observed in these strains is at the codon position 83 encoding p.Ser83Tyr.

The gene $bla_{TEM\text{-}1B}$, which codes for resistance to ampicillin, was among the common resistance genes observed at 57% (20/54), similar to a study in Zambia (Yamba et al., 2022). In addition, most of the resistance determinants for phenicols and folate pathway antagonists identified in thisstudy were similar to those in previous studies (Das et al., 2017). Twenty isolates were found to have the IncQ1 plasmid, which was mainly associated with the MDR strains. The presence of theIncQ1 plasmid in this study was associated with *catA1, $bla_{TEM\text{-}1B}$, sul2, sul1, and dfrA7* genes, implying that these AMR genes were acquired. This finding is similar to a study done in South Africa, which identified the IncQ1 plasmid from genotype 4.3.1 *S.*Typhi isolates (Smith et al., 2023). The IncQ is an incompatibility group non-conjugative but mobilizable plasmid found in a wide range of bacteria responsible for spreading antimicrobial resistance genes and increasing multidrug-resistant bacteria (Oliva et al., 2017).

5.2. Study Limitations

Determination of the true prevalence of typhoid carriers requires long-term follow-up of asymptomatic individuals. This study could not accomplish that because it utilized a cross-sectional study design which could have affected the accuracy of prevalence estimates. Thus, thisdesign was not sufficient to understand typhoid fever trends in our settings. During the screening of resistance genes, only available primers at the time of analysis were used; hence other relevantresistance genes could not be observed. This limitation could be a potential area for future researchto explore other resistance genes or conduct more comprehensive genomic analyses to better understand the genetic basis of

resistance of the isolates.

5.3. Conclusion

1. The study found a 3.5% isolation rate of *S.*Typhi among cases and carriers in Mukuru informal settlement between 2021-2022. This suggests the persistence of *S.*Typhi in this densely populated area, indicating ongoing endemicity. Additionally, the isolation of *S.*Typhi from carriers highlights their role in transmitting the disease within the community.
2. The study revealed high resistance levels among the *S.*Typhi isolates, with 37% classified as multidrug-resistant (MDR). Resistance to nalidixic acid was particularly high at 52%, and reduced susceptibility to ciprofloxacin was noted at 43%. These findings underscore the need for alternative treatment options, such as 3rd generation cephalosporins, to improve clinical outcomes. However, the potential emergence of resistance to these alternatives necessitates continuous surveillance and stringent use of antimicrobials.
3. Key resistance genes, including $bla_{TEM\text{-}1B}$, *catA1*, *sul1*, *sul2*, and *dfrA7*, were identified among the MDR isolates. Additionally, single mutations in the quinolone resistance-determining regions of the *gyrA* (S83Y) and *gyrB* (S464F) genes were detected, contributing to the resistance profiles observed. The presence of these genes and mutations underscores the ongoing challenge of treating typhoid fever in Mukuru, highlighting the need for updated treatment guidelines and public health interventions aimed at reducing the spread of resistant strains. This comprehensive understanding of genetic resistance mechanisms is crucial for developing effective strategies to combat MDR *S.*Typhi.
4. The study identified a genetically diverse population of *S.*Typhi in Mukuru, with the 4.3.1.2 EA3 genotype being the most prevalent. The close phylogenetic relatedness between case and carrier isolates indicates that asymptomatic carriers are likely a significant source of ongoing infections. This genetic diversity and the presence of AMR determinants emphasize the importance of targeted public health strategies, including improved sanitation, clean water access, and continuous

epidemiological monitoring to curb the spread of typhoid fever in this community.

5.4. Recommendation

1. Given the identification of *S*.Typhi in asymptomatic carriers, there is a need to prioritize routine screening and targeted treatment of carriers within high-risk areas like Mukuru. This approach is critical to interrupting the silent transmission cycle and reducing the prevalence of typhoid fever in such communities.
2. Surveillance and identification of MDR Hotspots- Regular and systematic surveillance of *S*.Typhi isolates should be conducted to identify hotspots of multidrug-resistant (MDR) strains. There is need to focus on monitoring resistance patterns and promptly initiating interventions, such as updating treatment guidelines and optimizing antibiotic use in these regions.
3. Mapping resistance Genes: The study identified specific resistance genes that contribute to the MDR phenotype. There is a need to map these resistance genes across different regions to understand their spread and to develop strategies that target these genetic determinants. Researchers and public health officials should collaborate to create a national database of resistance genes to facilitate this mapping effort.
4. The genetic diversity observed among *S*.Typhi isolates, particularly the prevalence of genotype 4.3.1.2 EA3, indicates the need for a nationwide genotyping initiative. Such efforts will help in understanding the transmission dynamics and evolutionary trends of *S*.Typhi across Kenya. This data can inform targeted public health responses, including vaccination strategies and the development of localized interventions.

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APPENDICES

Appendix I: Informed Consent Form for Carriers

***Study title*: Typhoidal salmonella disease, carriage, diversity and antimicrobial resistantgenes in Mukuru informal settlement, Nairobi Kenya.**

Principal Investigator: Purity Kasiano

Co-Investigator: Prof. Sam Kariuki **Organization**: Kenya Medical Research Institute

Explanation of the purposes of the research

You are being asked to take part in a medical research study being performed by the Kenya Medical Research Institute (KEMRI), Centre for Microbiology Research. It is very important that you understand the following general principles that apply to all participants in our studies:

1)Your participation is entirely voluntary;
2)You may withdraw from participation in this study or any part of this study at any time, with no penalty, harm, or loss of access to treatment and care;
3)After you read about the study please ask any questions that will allow you to understand the study more clearly.

What is typhoid disease?

Typhoid fever is a disease cause by a germ. It is usually characterized by acute onset of fever, headache, abdominal pain, diarrhoea/constipation, weakness, and cough and sometimes vomiting. This disease can be life threatening especially in children and people whose immune system is weak. Typhoid fever is only spread through contact with an infected person or an item contaminated by an infected person. Some people can carry the disease for a long time in their bodies without showing symptoms. Those that have the disease for a long time can also have gallstones in their gall bladders.

(i) Why do we want to conduct this research?

One of the objectives of this study is to identify healthy individuals who might be carriers of thebacteria that causes typhoid fever in Mukuru settlement. These seemingly healthy individuals, shed the bacteria in faeces and urine that reach food and water meant for human consumption. As long as they do not show symptoms or feel sick, treatment will not be sought after hence maintaining the source of typhoid fever in Mukuru.

If we isolate salmonella from your stool or bloodsample , we will do further laboratory tests inorder to know which is the best drug to use for treatment.

The knowledge accrued from this study will go a long way in identifying the types of such germsinfecting the population in order to identify possible sources of infections and provide data on appropriate modes of prevention and control of such diseases.

Samples collected will be tested in a laboratory in KEMRI in order to understand how the disease spreads in the community – no sample from you or any data containing your identity willbe revealed to anybody else outside of this study. We also wish to visit your home so that we can obtain further information through questionnaires that may be useful in this study.

(ii) What additional procedures will there be to help with research?

Samples will be taken once only, no other procedures will be done.

(iii) How many people will be involved in the study

We are expecting to obtain samples from 118 individuals.

(iv) Benefits

If you are found to be a carrier, through our laboratory tests, you will be recommended to a clinician to start treatment. Carriage of the bacteria has been known to cause gall bladder

cancerin the long run.

(v) Risks

The study carries no extra risk to participate in this study because these will be the routine procedures carried out in cases of blood infections or diarrhea. Taking these samples may causesome discomfort to you and we aim to minimize this by using most current stool collection techniques, including moistening the rectal swab. A qualified phlebotomist will be tasked with drawing blood. If there is any injury in the process of obtaining specimen for the purpose of thisstudy, medical care will be provided by the attending clinician at the cost of the project.

(vi) Who will read or hear about information collected from me?

The information collected about you in connection with this research will be stored using codesso that you will not be recognized. Coded information will be held on computers protected by passwords known to the research team only.

I confirm that I understand the information provided for the above study and have had the opportunity to ask questions. I understand that participation is voluntary and that I am free towithdraw my consent from the study at any time without giving reasons, without my medicalcare or legal rights being affected.

I agree, (Study Number) ----------, to take part in this study.

YES NO

Signature of the participant_____ Date _____

Name of the participant_____ Date _____

Specimen Storage/Sequencing

As part of effort to use modern techniques to develop new methods for disease prevention, suchas vaccines, we propose to perform advanced genetic studies on the bacteria causing disease at alaboratory abroad in collaboration with research partners working in this area and who have access to advanced genotyping and sequencing equipment. We request that you allow us to use your samples for further studies as well as export these bacteria to the expert laboratory at National Institute of Infectious Diseases, Japan (Nagasaki University collaboration) for further testing. The experiments will be done under the care of the Principal Investigator. Is it okay to send your sample to Japan?

Yes_____ No _____

Participant's signature.....

Do you have any questions that you would like me to answer now? If you would like to know more details about the research or have any issues that need to be discussed in the future you cancontact any of the following people at KEMRI, Centre for Microbiology Research, PO Box 43640-00100, Nairobi.

Purity Kasiano(Principal Investigator)-Mobile No:0712209928or Secretary, KEMRI/NationalEthical Review Committee, seru@kemri.org TEL: +254 020 272 2541 Ext 3333/3332 or

+254717 719 477

If the participant does not know how to read:

I have read/ been explained to the consent form and I have had the opportunity to ask questions. Iconfirm that I have given the consent without any force or coercion.

Name of witness: _____

Signature of witness:_____Date _____

_____

_____ Name of research assistant who performed this process: _____

_____

_____ Signature of the research staff who performed this process: _____

_____

Date: _____

Appendix II: Informed Consent Form for Carriers (Swahili Version)

FOMU YA IDHINI

Kichwa cha utafiti: Ugonjwa na Ubebaji wa homa ya matumbo na usugu wa dawa; aina tofautiza jeni, katika maeneo ya makazi ya Mukuru, Nairobi, Kenya.

Mchunguzi Mkuu: Purity Kasiano **Mpelelezi Mwenza**: Profesa Sam Kariuki **Shirika**: Taasisi ya Utafiti wa Tiba ya Kenya**Maelezo ya madhumuni ya utafiti**

Unaulizwa kushiriki katika utafiti wa matibabu unaofanywa na Taasisi ya Utafiti wa Tiba ya Kenya (KEMRI), Kituo cha Utafiti wa Microbiology. Ni muhimu sana uelewe kanuni zifuatazoza jumla ambazo zinatumika kwa washiriki wote katika masomo yetu:

1)Ushiriki wako ni wa hiari kabisa;
2)Unaweza kujiondoa katika kushiriki katika utafiti huu au sehemu yoyote ya utafiti huu wakatiwowote, bila adhabu, madhara, au kupoteza ufikiaji wa matibabu na utunzaji;
3)Baada ya kusoma juu ya utafiti tafadhali uliza maswali yoyote ambayo yatakuruhusu kuelewautafiti kwa uwazi zaidi.

Ugonjwa wa homa ya matumbo ni nini?

Homa ya matumbo ni ugonjwa unaosababishwa na vijidudu. Kawaida inajulikana na kuanza kwahoma kali, maumivu ya kichwa, maumivu ya tumbo, kuhara / kuvimbiwa, udhaifu, na kikohozi na wakati mwingine kutapika. Ugonjwa huu unaweza kutishia maisha hasa kwa watoto na watu ambao kinga yao ni dhaifu. Homa ya matumbo inaenea tu kupitia kuwasiliana na mtu aliyeambukizwa au kitu kilichochafuliwa na mtu aliyeambukizwa. Watu wengine wanaweza kubeba ugonjwa kwa muda mrefu katika miili yao bila kuonyesha dalili. Wale ambao wana ugonjwa kwa muda mrefu pia wanaweza kuwa na mawe ya nyongo katika nyongo zao.

(i) Kwa nini tunataka kufanya utafiti huu?

Moja ya malengo ya utafiti huu unatafuta kutambua watu wenye afya ambao wanaweza kuwa wabebaji wa bakteria ambao husababisha homa ya matumbo katika makazi ya Mukuru. Watu hawa wanaoonekana wenye afya, wanamwaga bakteria kwenye kinyesi na mkojo ambao hufikiachakula na maji yaliyokusudiwa matumizi ya binadamu. Ilimradi hawaonyeshi dalili au kuhisi kuugua, matibabu hayatafutwa baada ya hapo kudumisha chanzo cha homa ya matumbo katika Mukuru.

Ikiwa tutatenga salmonella kutoka kwa sampuli yako ya kinyesi au damu, tutafanya vipimo zaidivya maabara ili kujua ni dawa ipi bora kutumia kwa matibabu.

Ujuzi uliopatikana kutoka kwa utafiti huu utasaidia sana kubainisha aina za vijidudu hivyo vinavyoambukiza idadi ya watu ili kutambua vyanzo vya maambukizo na kutoa data juu ya njiazinazofaa za kuzuia na kudhibiti magonjwa kama hayo.

Sampuli zilizokusanywa zitajaribiwa katika maabara huko KEMRI ili kuelewa jinsi ugonjwa unavyoenea katika jamii - hakuna sampuli kutoka kwako au data yoyote iliyo na kitambulishochako itafunuliwa kwa mtu mwingine yeyote nje ya utafiti huu. Tunataka pia kutembelea nyumba yako ili tuweze kupata habari zaidi kupitia dodoso ambazo zinaweza kuwa muhimu katika utafiti huu.

ii) Kutakuwa na taratibu gani za ziada kusaidia utafiti huu?

Sampuli zitachukuliwa mara moja tu, hakuna taratibu zingine zitafanywa.

(iii) Ni watu wangapi watakaoshiriki katika utafiti huu

Tunatarajia kupata sampuli kutoka kwa watu 118.

(iv) Faida

Ikiwa utapatikana kama mbebaji, kupitia vipimo vyetu vya maabara, utashauriwa kutafuta

matibabu. Ubebaji wa bakteria umejulikana kusababisha saratani ya nyongo mwishowe.

(v) Hatari

Utafiti hauna hatari zaidi ya kushiriki katika utafiti huu kwa sababu hizi zitakuwa taratibu za kawaida zinazofanywa wakati wa maambukizo ya damu au kuhara. Kuchukua sampuli hizi kunaweza kukusababishia usumbufu na tunakusudia kupunguza hii kwa kutumia mbinu nyingiza ukusanyaji wa kinyesi, pamoja na kulainisha usufi wa rectal. Mtaalamu wa maabara aliyestahili atapewa jukumu la kuchora damu. Ikiwa kuna jeraha lolote katika mchakato wa kupata kielelezo kwa kusudi la utafiti huu, huduma ya matibabu itatolewa na kliniki anayehudhuria kwa gharama ya mradi huo.

(vi) Nani atasoma au kusikia habari inayokusanywa kutoka kwangu?

Habari iliyokusanywa juu yako kuhusiana na utafiti huu itahifadhiwa kwa kutumia nambari iliusitambulike. Habari iliyosimbwa itafanyika kwenye kompyuta zilizolindwa na nywila zinazojulikana na timu ya utafiti tu.

Ninathibitisha kuwa ninaelewa habari iliyotolewa kwa utafiti hapo juu na nimepata nafasi ya kuuliza maswali. Ninaelewa kuwa ushiriki ni wa hiari na kwamba niko huru kutoa idhini yangu kutoka kwa utafiti wakati wowote bila kutoa sababu, bila huduma yangu ya matibabu au haki zakisheria kuathiriwa.

Ninakubali, (Nambari ya Utafiti) ----------, kushiriki katika utafiti huu.

NDIO LA

Saini ya mshiriki_____Tarehe _____

Jina la mshiriki_____ Tarehe _

_____

Uhifadhi wa Sampuli

Kama juhudi za kutumia mbinu za kisasa kuunda mbinu mpya za kuzuia magonjwa, kama vile chanjo, tunapendekeza kufanya tafiti za kina za kijenetiki kwa bakteria wanaosababisha magonjwa katika maabara nje ya nchi kwa ushirikiano na washirika wa utafiti wanaofanya kazi katika eneo hili na ambao wanaweza kupata vifaa vya hali ya juu vya kujua jeni. Tunaomba uturuhusu kutumia sampuli zako kwa tafiti zaidi na pia kusafirisha bakteria hizi kwenye maabara ya wataalamu Katika Taasisi ya Kitaifa ya Magonjwa ya Kuambukiza, Japan kwa majaribio zaidi.

Majaribio yatafanywa chini ya uangalizi wa Mpelelezi Mkuu. Je, ni sawa kutuma sampuli yakoJapani

Ndio_____ la _____

Saini ya mshiriki .....

Una maswali yoyote ambayo ungependa nijibu sasa? Ikiwa ungependa kujua maelezo zaidi juuya utafiti huo au kuwa na maswala yoyote ambayo yanahitaji kujadiliwa katika siku zijazo unaweza kuwasiliana na yeyote kati ya watu wafuatao katika KEMRI, Kituo cha Utafiti waMicrobiology, SLP 43640-00100, Nairobi.

Purity Kasiano(Mchunguzi mkuu)-Nambari ya simu:0712209928, KEMRI / Kamati ya Kitaifa ya Mapitio ya Maadili, seru@kemri.org SIMU: +254 020 272 2541 Ext 3333/3332 au +254717719 477

Ikiwa mshiriki hajui kusoma:

Nimesoma / nimeelezewa fomu ya idhini na nimepata nafasi ya kuuliza maswali. Ninathibitishakuwa nimetoa idhini bila nguvu yoyote au kulazimishwa.

Jina la shahidi: _____

Saini ya shahidi: Tarehe _____

Jina la msaidizi wa utafiti ambaye alifanya mchakato huu:

Saini ya wafanyikazi wa utafiti ambao walifanya mchakato huu: _____ Tarehe: _____

Appendix III: Informed Consent Form for Children

Study title: Typhoidal salmonella disease and carriage, diversity and antimicrobial resistantgenes in Mukuru informal settlement, Nairobi Kenya.

Principal Investigator: Purity KasianoCo-Investigator: Prof. Sam Kariuki

Organization: Kenya Medical Research Institute Explanation of the purposes of the research

You are being asked to take part in a medical research study being performed by the Kenya Medical Research Institute (KEMRI), Centre for Microbiology Research. It is very important that you understand the following general principles that apply to all participants in our studies:

1)Your participation is entirely voluntary;
2)You may withdraw from participation in this study or any part of this study at any time, with no penalty, harm, or loss of access to treatment and care;
3)After you read about the study please ask any questions that will allow you to understand the study more clearly.

Your child is being asked to participate in this study because he/she has presented to this clinic with symptoms similar to those of typhoid disease, and lives within Mukuru informal settlement.

What is typhoid disease?

Typhoid fever is a disease cause by a germ. It is usually characterized by acute onset of fever, headache, abdominal pain, diarrhoea/constipation, weakness, and cough and sometimes vomiting. Typhoid fever is only spread through contact with an infected person or an item contaminated by an infected person.

This disease can be life threatening especially in children and people whose immune system is weak. They are usually spread to humans by animal products such as poultry, beef, fish, eggs, and dairy products. At times, however, other foods such as fruits, vegetables, and bakery products have caused outbreaks, most often when contaminated by contact with an animal product. The bacteria can also be spread by drinking contaminated water, as well as through contact with infected pets. Typhoid fever is only spread through contact with an infected person or an item contaminated by an infected person. Some people can carry the disease for a long timein their bodies without showing symptoms. Those that have the disease for a long time can also have gall stones in their gall bladders.

(i) Why do we want to conduct this research?We aim to obtain information about a disease that is common in Kenya, which may cause severetyphoid disease in blood with or without diarrhoea that may require admission to hospital. This disease is caused by a germ called Salmonella, which may spread through handling food with unwashed hands, contamination with dirt from animals and drinking contaminated water. If we isolate salmonella from your child, we will do further laboratory tests in order to know which is the best drug to use for treating him/her

If we isolate salmonella bacteria from your child, we will also send him/her to a health facility where he/she will have an ultrasound scan done to establish if he/she has gall stones, where thebacteria is known to hide.

The knowledge accrued from this study will go a long way in identifying the types of such germsinfecting the population in order to identify possible sources of infections and provide data on appropriate modes of prevention and control of such diseases.

Stool samples collected will be tested in a laboratory in KEMRI in order to understand how thedisease spreads in the community – no samples from your child or any data containing your identity will be revealed to anybody else outside of this study. We also wish to visit your homeso that we can obtain further information that may be useful in

finding out the likely source of this disease and the various means by which the disease may be spreading within your community.

(ii) Your child's part in the research

Your child will be investigated for bacteria that cause blood infection and/or diarrhoea. We ask that you consent to your child being investigated for these diseases. The process will involve taking a stool and blood sample for laboratory testing. Taking these samples may cause discomfort to your child and we aim to minimize this by using moist stool collection techniques, including moistening the rectal swab. If he/she positive for salmonella bacteria, we will refer youto take him/ her to a health facility to have an abdominal ultrasound done to find out if he/she hasgall stones where these bacteria are known to hide. This will not cause any discomfort at all but the child may have a cold feeling when the gel is applied on his/her abdomen. All the tests will be offered free of charge by the project, so you will not be required to pay any money. All the results will be availed to the doctor treating your child.

Results from these tests will be useful for informing doctors in the clinic and community healthcare workers about how best to treat the disease and ways to prevent the disease spreadingin the community. As part of effort to use modern techniques to develop new methods for disease prevention, such as vaccines, we propose to perform advanced studies on the germ causing disease at a laboratory abroad in collaboration with research partners working in this area and who have access to advanced scientific equipment. We request that you allow us to export these germs to the expert laboratory for further testing. The tests will be done under the care of the Principal Investigator.

(iii) What additional procedures will there be to help with research?

Samples will be taken on the day your child will be presented to the clinic having clinical symptoms of typhoid. After 14 days of treatment, another sample will be taken to check if thetreatment being offered is effective.

(iv) How many people will be involved in the study and how will they be selected?

We are expecting to obtain salmonella positive samples from about 118 individuals. We willexamine patients as they come in to the clinic after they or their parents/ guardians agree to participate in this study.

(v) Benefits

If your child has salmonella bacteria that we discover through our laboratory tests, your child will be treated with appropriate medicines according to the hospital guidelines and you and yourchild also offered post counselling and health education on how to prevent infections that may happen due to poor sanitation and handling of foods and water in the home. After the study we will be able to establish a database on the best drugs to use for blood poisoning infections and diarrhea affecting the populations and how the bacteria may be finding their way into the community. These data will be available to caregivers and the Ministry of Health for the generalimprovement of treatment of these infections in the population.

(vi) Risks

The study carries no extra risk to your child or cost to you to participate in this study because these will be the routine procedures carried out in cases of blood infections or diarrhea. Taking these samples may cause your child some discomfort and we aim to minimize this by using mostcurrent stool collection techniques, including moistening the rectal swab. A qualified phlebotomist will be tasked with drawing blood and collection of stool specimen. If there is anyinjury in the process of obtaining specimen for the purpose of this study, medical care will be provided by the attending clinician at the cost of the project.

(vii) What if I change my mind about my child participating in this research?

If you agree to enrol your child in this research and later change your mind you are free

to with draw him/her from the study at any time. You or your child will not be discriminated against inany way in the future if you either do not agree to participate or later change your mind.

(viii) Who will read or hear about information collected from me/ my child?

The information collected about you or your child in connection with this research will be storedusing codes so that you will not be recognised. Coded information will be held on computers protected by passwords known to the research team only.

Do you have any questions that you would like me to answer now? If you would like to know more details about the research or have any issues that need to be discussed in the future you cancontact any of the following people at KEMRI, Centre for Microbiology Research, PO Box 43640-00100, Nairobi.Purity Kasiano(Principal Investigator)-Mobile No:0712209928 or Secretary, KEMRI/National Ethical Review Committee, seru@kemri.org TEL: +254 020 272 2541 Ext 3333/3332 or+254717 719 477

I confirm that I understand the information provided for the above study and have had the opportunity to ask questions. I understand that participation is voluntary and that I am free to withdraw my child from the study at any time without giving reasons, without my medical careor legal rights being affected.

I agree, (Study Number) ----------, to take part in this study.

YES NO

Signature of the parent/guardian _____ Date _____

Or Thumb print:

Name of the parent/guardian t_____ Date _____

Name of witness _____ Treatment Consent

Date _____If your child has typhoid fever, he/she can be offered treatment. The treatments will be paid forby this project and you will not be asked for any money. Is it okay for your child to receive treatment?

Yes_____ No _____

Parent's/Guardian's signature_____

Specimen Storage/Sequencing

As part of effort to use modern techniques to develop new methods for disease prevention, suchas vaccines, we propose to perform advanced genetic studies on the bacteria causing disease at alaboratory abroad in collaboration with research partners working in this area and who have access to advanced genotyping and sequencing equipment. We request that you allow us to use your samples for further studies as well as export these bacteria to the expert laboratory at National Institute of Infectious Diseases, Japan (Nagasaki University collaboration) for further testing. The experiments will be done under the care of the Principal Investigator. Is it okay to send your sample to Japan?

Yes_____ No _____

_____ parent/guardian signature

..... OFFICIAL STAMP

If the parent/guardian does not know how to read:

I have read/ been explained to the consent form and I have had the opportunity to ask questions. Iconfirm that I have given the consent without any force or coercion.

Name of witness: _____

Signature of witness:_____Date _

_____

Name of research assistant who performed this process: _

Signature of the research staff who performed this process: _

Date: _____

Appendix IV: Swahili Version for Consent Seeking Form for Children

Kichwa cha masomo: Ugonjwa na Ubebaji wa homa ya matumbo na usugu wa dawa; aina tofautiza jeni, katika maeneo ya makazi ya Mukuru, Nairobi, Kenya.

Mchunguzi Mkuu: Purity Kasiano Mpelelezi Mwenza: Prof Sam Kariuki Shirika: Taasisi ya Utafiti wa Tiba ya KenyaMaelezo ya madhumuni ya utafiti

Unaulizwa kushiriki katika utafiti wa matibabu unaofanywa na Taasisi ya Utafiti wa Tiba ya Kenya (KEMRI), Kituo cha Utafiti wa Microbiology. Ni muhimu sana uelewe kanuni zifuatazoza jumla ambazo zinatumika kwa washiriki wote katika masomo yetu:

1)Ushiriki wako ni wa hiari kabisa;
2)Unaweza kujiondoa katika kushiriki katika utafiti huu au sehemu yoyote ya utafiti huu wakatiwowote, bila adhabu, madhara, au kupoteza ufikiaji wa matibabu na utunzaji;
3)Baada ya kusoma juu ya utafiti tafadhali uliza maswali yoyote ambayo yatakuruhusu kuelewautafiti kwa uwazi zaidi.

Mtoto wako anaulizwa kushiriki katika utafiti huu kwa sababu amewasilisha kliniki hii na dalilizinazofanana na zile za ugonjwa wa typhoid, na anaishi katika makazi duni ya Mukuru.

Ugonjwa wa homa ya matumbo ni nini?

Homa ya matumbo ni ugonjwa unaosababishwa na mdudu. Kawaida inajulikana na kuanza kwahoma kali, maumivu ya kichwa, maumivu ya tumbo, kuharisha / kuvimbiwa, udhaifu, na kikohozi na wakati mwingine kutapika. Homa ya matumbo inaenea tu kupitia kuwasiliana na mtu aliyeambukizwa au kitu kilichochafuliwa na mtu aliyeambukizwa.

Ugonjwa huu unaweza kutishia maisha hasa kwa watoto na watu ambao kinga yao ni dhaifu. Kawaida huenezwa kwa wanadamu na bidhaa za wanyama kama kuku, nyama ya nyama, samaki, mayai, na bidhaa za maziwa. Wakati mwingine, hata hivyo, vyakula

vingine kama matunda, mboga mboga, na bidhaa za mikate vimesababisha milipuko, mara nyingi ikichafuliwana kuwasiliana na bidhaa ya wanyama. Bakteria pia inaweza kuenea kwa kunywa maji machafu, na pia kwa kuwasiliana na wanyama wa kipenzi walioambukizwa. Homa ya matumbo inaenea tu kupitia kuwasiliana na mtu aliyeambukizwa au kitu kilichochafuliwa na mtu aliyeambukizwa.

Watu wengine wanaweza kubeba ugonjwa kwa muda mrefu katika miili yao bila kuonyesha dalili. Wale ambao wana ugonjwa kwa muda mrefu pia wanaweza kuwa na mawe ya nyongokatika nyongo zao

Kwa nini tunataka kufanya utafiti huu?

Tunakusudia kupata habari juu ya ugonjwa ambao ni wa kawaida nchini Kenya, ambao unawezakusababisha ugonjwa mkali wa typhoid katika damu na au bila kuhara ambayo inaweza kuhitaji kulazwa hospitalini. Ugonjwa huu unasababishwa na vijidudu vinavyoitwa Salmonella, ambavyo vinaweza kuenea kwa kushughulikia chakula kwa mikono ambayo havijaoshwa, kuchafuliwa na uchafu kutoka kwa wanyama na kunywa maji machafu. Ikiwa tutatenga salmonella kutoka kwa mtoto wako, tutafanya vipimo zaidi vya maabara ili kujua ni dawa ipi bora kutumia kwa kumtibu

Ikiwa tutatenga bakteria ya salmonella kutoka kwa mtoto wako, tutampeleka pia kwenye kituocha afya ambapo atafanyiwa uchunguzi wa ultrasound ili kubaini ikiwa ana mawe ya nyongo, ambapo bakteria inajulikana kujificha.

Ujuzi uliopatikana kutoka kwa utafiti huu utasaidia sana kubainisha aina za vijidudu hivyo vinavyoambukiza idadi ya watu ili kutambua vyanzo vya maambukizo na kutoa data juu ya njiazinazofaa za kuzuia na kudhibiti magonjwa kama hayo.

Sampuli za kinyesi zilizokusanywa zitajaribiwa katika maabara huko KEMRI ili kuelewa jinsi ugonjwa unavyoenea katika jamii - hakuna sampuli kutoka kwa mtoto wako au data yoyote iliyona kitambulisho chako itafunuliwa kwa mtu mwingine yeyote nje ya utafiti huu. Tunapenda pia kutembelea nyumba yako ili tuweze kupata habari zaidi ambayo

inaweza kuwa muhimu katika kutafuta chanzo cha ugonjwa huu na njia anuwai ambazo ugonjwa unaweza kusambaa ndani ya jamii yako.

(i) Sehemu ya mtoto wako katika utafiti

Mtoto wako atachunguzwa kwa bakteria ambao husababisha maambukizo ya damu na / au kuhara. Tunakuomba ukubali mtoto wako achunguzwe kwa magonjwa haya. Mchakato huo utahusisha kuchukua sampuli ya kinyesi na damu kwa upimaji wa maabara. Kuchukua sampuli hizi kunaweza kukusababishia usumbufu na tunakusudia kupunguza hii kwa kutumia mbinu nyingi za ukusanyaji wa kinyesi, pamoja na kulainisha usufi wa rectal. Ikiwa ana vijidudu vya salmonella, tutakupeleka kwenye kituo cha afya ili kufanyiwa uchunguzi wa tumbo ili kujua ikiwa ana mawe ya nyongo ambapo bakteria hawa wanajulikana kujificha. Hii haitasababisha usumbufu wowote lakini mtoto anaweza kuwa na hisia baridi wakati gel inapowekwa kwenye tumbo lake. Vipimo vyote vitatolewa bure na mradi, kwa hivyo hautahitajika kulipa pesa yoyote.Matokeo yote yatapatikana kwa daktari anayemtibu mtoto wako.

Matokeo ya vipimo hivi yatakuwa muhimu kwa kuwajulisha madaktari katika zahanati na wafanyikazi wa huduma za afya ya jamii juu ya njia bora ya kutibu ugonjwa na njia za kuzuia ugonjwa kuenea katika jamii. Kama sehemu ya juhudi za kutumia mbinu za kisasa kukuza njiampya za kuzuia magonjwa, kama vile chanjo, tunapendekeza kufanya tafiti za juu juu ya ugonjwa unaosababisha magonjwa kwenye maabara nje ya nchi kwa kushirikiana na washirikawa utafiti wanaofanya kazi katika eneo hili na ambao wana uwezo vifaa vya kisayansi.

Tunaomba uturuhusu kusafirisha viini hivi kwa maabara ya wataalam kwa upimaji zaidi. Uchunguzi utafanywa chini ya uangalizi wa Mchunguzi Mkuu.

(ii) Je! kutakuwa na taratibu gani za ziada kusaidia utafiti?

(iii) Sampuli zitachukuliwa siku ambayo mtoto wako atawasilishwa kliniki akiwa na dalili za klinikiza typhoid. Baada ya siku 14 za matibabu, sampuli nyingine itachukuliwa

kuangalia ikiwa matibabu yanayotolewa ni bora.

(iv) Je! ni watu wangapi watakaoshiriki katika utafiti huu na watachaguliwa vipi?

Tunatarajia kupata sampuli chanya za salmonella kutoka kwa watu wapatao 118. Tutachunguza wagonjwa wanapoingia kliniki baada ya wao au wazazi / walezi wao kukubali kushiriki katika utafiti huu.

(v) Faida

Ikiwa mtoto wako ana bakteria ya salmonella ambayo tunagundua kupitia vipimo vyetu vya maabara, mtoto wako atatibiwa dawa zinazofaa kulingana na miongozo ya hospitali na wewe na mtoto wako pia mlitoa ushauri nasaha na elimu ya afya juu ya jinsi ya kuzuia maambukizo ambayo yanaweza kutokea kwa sababu ya hali mbaya. usafi wa mazingira na utunzaji wa vyakula na maji nyumbani. Baada ya utafiti tutaweza kuanzisha hifadhidata juu ya dawa bora za kutumia kwa maambukizo ya sumu ya damu na kuhara inayoathiri idadi ya watu na jinsi bakteria wanaweza kuwa wanaingia katika jamii. Takwimu hizi zitapatikana kwa walezi na Wizara ya Afya kwa uboreshaji wa jumla wa matibabu ya maambukizo haya kwa idadi ya watu.

(vi) Hatari

Utafiti hauna hatari zaidi kwa mtoto wako au gharama kwako kushiriki katika utafiti huu kwa sababu hizi zitakuwa taratibu za kawaida zinazofanywa wakati wa maambukizo ya damu au kuhara. Kuchukua sampuli hizi kunaweza kumsababishia mtoto wako usumbufu na tunakusudia kupunguza hii kwa kutumia mbinu nyingi za ukusanyaji wa kinyesi, pamoja na kulainisha usufiwa rectal. Mtaalam wa maabara aliyestahili atapewa jukumu la kutoa damu na ukusanyaji wa vielelezo vya kinyesi. Ikiwa kuna jeraha lolote katika mchakato wa kupata kielelezo kwa kusudila utafiti huu, huduma ya matibabu itatolewa na kliniki anayehudhuria kwa gharama ya mradi huo.

Ikiwa kuna jeraha lolote katika mchakato wa kupata kielelezo kwa kusudi la utafiti huu, hudumaya matibabu itatolewa na kliniki anayehudhuria kwa gharama ya mradi huo

(vii) Je! nikibadilisha mawazo yangu juu ya mtoto wangu kushiriki katika utafiti huu?

Ikiwa unakubali kuandikisha mtoto wako katika utafiti huu na baadaye ubadilishe mawazo yakouko huru kumvuta kutoka kwa masomo wakati wowote. Wewe au mtoto wako hautabaguliwa kwa njia yoyote siku zijazo ikiwa haukubali kushiriki au baadaye ubadilishe mawazo yako.

(viii) Nani atasoma au kusikia habari inayokusanywa kutoka kwangu / kwa mtoto wangu?

Habari iliyokusanywa kukuhusu wewe au mtoto wako kuhusiana na utafiti huu itahifadhiwa kwa kutumia nambari ili usitambulike. Habari iliyosimbwa itafanyika kwenye kompyuta zilizolindwana nywila zinazojulikana na timu ya utafiti tu.

Je! Una maswali yoyote ambayo ungependa nijibu sasa? Ikiwa ungependa kujua maelezo zaidijuu ya utafiti huo au kuwa na maswala yoyote ambayo yanahitaji kujadiliwa katika siku zijazo unaweza kuwasiliana na yeyote kati ya watu wafuatao katika KEMRI, Kituo cha Utafiti waMicrobiology, SLP 43640-00100, Nairobi.

Purity Kasiano (Mpelelezi Mkuu) -Namba ya Simu: 0712209928 au Katibu, KEMRI / Kamati yaKitaifa ya Mapitio ya Maadili, seru@kemri.org SIMU: +254 020 272 2541 Ext 3333/3332 au

+254717 719 477

Ninathibitisha kwamba ninaelewa habari iliyotolewa kwa utafiti hapo juu na nimepata nafasi yakuuliza maswali. Ninaelewa kuwa ushiriki ni wa hiari na kwamba niko huru kumtoa mtoto wangu kutoka kwa masomo wakati wowote bila kutoa sababu, bila huduma yangu ya matibabuau haki za kisheria kuathiriwa.

Ninakubali, (Nambari ya Utafiti) ----------, kushiriki katika utafiti huu.

NDIO LA

Saini ya mzazi / mlezi_____Tarehe _____ Au kuchapisha kidole gumba:

Jina la mzazi / mlezi t_____Tarehe _____ Jina la shahidi_____Tarehe _____

Idhini ya Matibabu

Ikiwa mtoto wako ana homa ya matumbo, anaweza kupatiwa matibabu. Matibabu yatalipwa namradi huu na hautaulizwa pesa yoyote. Je! Ni sawa kwa mtoto wako kupata matibabu?

Ndio la _____

Saini ya Mzazi / Mlezi_____

Uhifadhi wa Sampuli / Mpangilio

Kama juhudi za kutumia mbinu za kisasa kuunda mbinu mpya za kuzuia magonjwa, kama vile chanjo, tunapendekeza kufanya tafiti za kina za kijenetiki kwa bakteria wanaosababisha magonjwa katika maabara nje ya nchi kwa ushirikiano na washirika wa utafiti wanaofanya kazi katika eneo hili na ambao wanaweza kupata vifaa vya hali ya juu vya kujua jeni. Tunaomba uturuhusu kutumia sampuli zako kwa tafiti zaidi na pia kusafirisha bakteria hizi kwenye maabara ya wataalamu Katika Taasisi ya Kitaifa ya Magonjwa ya Kuambukiza, Japan kwa majaribio zaidi.

Majaribio yatafanywa chini ya uangalizi wa Mpelelezi Mkuu. Je, ni sawa kutuma sampuli yakoJapani

Ndio la _____

_____Saini ya mzazi / mlezi

..... KIWANGO CHA RASMI

Ikiwa mzazi / mlezi hajui kusoma:

Nimesoma / nimeelezewa fomu ya idhini na nimepata nafasi ya kuuliza maswali. Ninathibitishakuwa nimetoa idhini bila nguvu yoyote au kulazimishwa.

Jina la shahidi: _____

Saini ya shahidi: Tarehe _____

Jina la msaidizi wa utafiti ambaye alifanya mchakato huu:

Saini ya wafanyikazi wa utafiti ambao walifanya mchakato huu: _____ Tarehe: _

_____

Appendix V: Informed Consent for Patients/Cases.

***Study title*: Typhoidal salmonella disease and carriage, diversity and antimicrobial resistantgenes in Mukuru informal settlement, Nairobi Kenya.**

Principal Investigator: Purity Kasiano

Co-Investigator: Prof. Sam Kariuki **Organization**: Kenya Medical Research Institute

Explanation of the purposes of the research

You are being asked to take part in a medical research study being performed by the Kenya Medical Research Institute (KEMRI), Centre for Microbiology Research. It is very important that you understand the following general principles that apply to all participants in our studies:

1)Your participation is entirely voluntary;
2)You may withdraw from participation in this study or any part of this study at any time, with no penalty, harm, or loss of access to treatment and care;
3)After you read about the study please ask any questions that will allow you to understand the study more clearly.

(i) What is the purpose of this research?

We aim to obtain information about a disease that is common in children below the age 5 years and young school-going children less than 16 years of age, which may cause severe bloodstream or diarrhea illness that may require admission to hospital. This disease is caused by a germ called *Salmonella,* which may spread through handling food with unwashed hands, contamination with dirt from animals and drinking contaminated water. If we isolate salmonella from your fecal sample, we will do further laboratory tests in order to know which is the best drug to use for treatment.

The knowledge accrued from this study will go a long way in identifying the types of such

germsinfecting you in order to identify possible sources of infections and provide data on appropriate modes of prevention and control of such diseases. Bacteria isolated from you will be tested in a laboratory in KEMRI in order to understand how the disease spreads in the community.

No sample data from you or any data containing your identity will be revealed to anybody else outside of this study. We also wish to visit your home so that we can obtain further information that may be useful in investigating the likely source of this disease and the various means by whichthe disease may be spreading within your community.

(ii) Your part in the research

You will be investigated for bacteria that cause blood infection and/or diarrhoea. We ask that youconsent to being investigated for these diseases. The process will involve taking a blood and stoolsample or rectal swab for laboratory testing once you present with clinical symptoms of typhoid at the clinic. After 14 days following treatment, we will take another stool sample for investigationto find out if the treatment offered is effective.

Results from these tests will be useful for informing doctors in the clinic and community healthcareworkers about how best to treat the disease and ways to prevent the disease spreading in the community.

As part of effort to use modern techniques to develop new methods for disease prevention, such as vaccines, we propose to perform advanced genetic studies on the germ causing disease ILRI incollaboration with research partners working in this area and who have access to advanced genotyping and sequencing equipment. We request that you allow us to do so.. The experiments will be done under the care of the Principal Investigator.

.

(iv) How many people will be involved in the study

We are expecting to obtain samples from about 118 individuals.

Risks

The study carries no extra risk to you because these will be the routine procedures carried out in cases of blood infections or diarrhea. Taking these samples may cause you some discomfort and we aim to minimize this by using most current stool collection techniques, including moistening the rectal swab. A qualified phlebotomist will be tasked with drawing blood and collection of stoolspecimen. If there is any injury in the process of obtaining specimen for the purpose of this study, medical care will be provided by the attending clinician at the cost of the project

(v) What will happen after the study?

If you are found with the illness that we discover through our laboratory tests, you will be treatedwith appropriate medicines according to the hospital guidelines and also offered post counsellingand health education on how to prevent infections that may happen due to poor sanitation and handling of foods and water in the home. After the study we will be able to establish a database on the best drugs to use for blood poisoning infections and diarrhea affecting people in your area and how the germ may be finding their way into the community.

These data will be available to caregivers and the Ministry of Health for the general improvementof treatment of these infections in children.

(vi) What if I change my mind about helping with this research?

If you agree to help with this research and later change your mind you are free to with draw fromthe study at any time. You will not be discriminated against in any way in the future if you eitherdo not agree to participate or later change your mind.

(vii) Who will read or hear about information collected from you?

The information collected from you will help will be stored using codes so that you cannot be recognized. Coded information will be held on computers protected by passwords known to the research team only.

Do you have any questions that you would like me to answer now? If you would like to know more details about the research or have any issues that need to be discussed in the future you can contact any of the following people at KEMRI, Centre for Microbiology Research, PO Box 43640-00100, Nairobi.Purity Kasiano – Mobile Phone No:0712209928 or Secretary, KEMRI/National Ethical Review Committee, PO Box 54840-00200, Nairobi, TEL: +245-20-2722541

I confirm that I understand the information provided for the above study and have had the opportunity to ask questions. I understand that participation is voluntary and that I am free to withdraw from the study at any time without giving reasons, without my medical care or legalrights being affected.

I agree to_____(Study Number)_____, to take part in this study.YES NO

Signature of the participant_____Date _____

Or Thumb print:

Name of the participant _____ Date _____

Appendix VI: Swahili Version of Patient/Case Seeking Form

Kichwa cha somo: Ugonjwa na Ubebaji wa homa ya matumbo na usugu wa dawa katika kaya;aina tofauti za jeni, katika maeneo ya makazi ya Mukuru, Nairobi, Kenya.

Mchunguzi Mkuu: Purity Kasiano Mpelelezi Mwenza: Profesa Sam Kariuki Shirika: Taasisi ya Utafiti wa Tiba ya KenyaMaelezo ya madhumuni ya utafiti

Unaulizwa kushiriki katika utafiti wa matibabu unaofanywa na Taasisi ya Utafiti wa Tiba ya Kenya (KEMRI), Kituo cha Utafiti wa Microbiology. Ni muhimu sana uelewe kanuni zifuatazoza jumla ambazo zinatumika kwa washiriki wote katika masomo yetu:

1)Ushiriki wako ni wa hiari kabisa;
2)Unaweza kujiondoa katika kushiriki katika utafiti huu au sehemu yoyote ya utafiti huu wakatiwowote, bila adhabu, madhara, au kupoteza ufikiaji wa matibabu na utunzaji;
3)Baada ya kusoma juu ya utafiti tafadhali uliza maswali yoyote ambayo yatakuruhusu kuelewautafiti kwa uwazi zaidi.

(i) Ni nini kusudi la utafiti huu?

Tunakusudia kupata habari juu ya ugonjwa ambao ni wa kawaida kwa watoto walio chini ya umri wa miaka 5 na watoto wadogo wanaokwenda shule chini ya umri wa miaka 16, ambayo inaweza kusababisha ugonjwa mkali wa damu au kuhara ambao unaweza kuhitaji kulazwa hospitalini. Ugonjwa huu unasababishwa na vijidudu vinavyoitwa Salmonella, ambavyo vinaweza kuenea kwa kushughulikia chakula kwa mikono ambayo havijaoshwa, kuchafuliwa nauchafu kutoka kwa wanyama na kunywa maji machafu. Ikiwa tutatenga salmonella kutoka kwa sampuli yako ya kinyesi, tutafanya vipimo zaidi vya maabara ili kujua ni dawa ipi bora kutumiakwa matibabu.

Ujuzi uliopatikana kutoka kwa utafiti huu utasaidia sana kubaini aina za vijidudu hivyo vinavyokuambukiza ili kubaini vyanzo vya maambukizo na kutoa data juu ya njia

zinazofaa za kuzuia na kudhibiti magonjwa kama haya. Bakteria waliotengwa na wewe watajaribiwa katikamaabara huko KEMRI ili kuelewa jinsi ugonjwa unavyoenea katika jamii.

Hakuna data ya mfano kutoka kwako au data yoyote iliyo na kitambulisho chako itafunuliwa kwa mtu mwingine yeyote nje ya utafiti huu. Tunataka pia kutembelea nyumba yako ili tuweze kupata habari zaidi ambayo inaweza kuwa muhimu katika kuchunguza chanzo kinachowezekanacha ugonjwa huu na njia anuwai ambazo ugonjwa unaweza kusambaa ndani ya jamii yako.

(ii) Sehemu yako katika utafiti

Utachunguzwa kwa bakteria ambao husababisha maambukizo ya damu na / au kuhara. Tunakuomba ukubali kuchunguzwa kwa magonjwa haya. Utaratibu huo utahusisha kuchukua sampuli ya kinyesi au usufi wa rectal na damu kwa upimaji wa maabara mara tu utakapowasilisha dalili za kliniki za typhoid kwenye kliniki. Baada ya siku 14 kufuatia matibabu,tutachukua sampuli nyingine ya kinyesi kwa uchunguzi ili kujua ikiwa tiba inayotolewa ni nzuri.

Matokeo ya vipimo hivi yatakuwa muhimu kwa kuwajulisha madaktari katika zahanati na wafanyikazi wa huduma za afya ya jamii juu ya njia bora ya kutibu ugonjwa na njia za kuzuiaugonjwa kuenea katika jamii.

Kama sehemu ya juhudi za kutumia mbinu za kisasa kukuza njia mpya za kuzuia magonjwa, kama vile chanjo, tunapendekeza kufanya tafiti za maumbile juu ya ugonjwa unaosababisha ugonjwa wa ILRI kwa kushirikiana na washirika wa utafiti wanaofanya kazi katika eneo hili na ambao wana ufikiaji wa hali ya juu na vifaa vya mpangilio. Tunaomba uturuhusu kufanya hivyo. Majaribio yatafanywa chini ya uangalizi wa Mchunguzi Mkuu.Hakuna taratibu zingine zitakazofanyika kwako.

*(iv)Ni watu wangapi watakaoshiriki katika utafiti huu*Tunatarajia kupata sampuli kutoka kwa watu 118. *Hatari*

Utafiti hauna hatari zaidi kwako kwa sababu hizi zitakuwa taratibu za kawaida zinazofanywa wakati wa maambukizo ya damu au kuhara. Kuchukua sampuli hizi kunaweza kukusababishia usumbufu na tunakusudia kupunguza hii kwa kutumia mbinu za sasa za ukusanyaji wa kinyesi,pamoja na kulainisha usufi wa rectal. Mtaalamu wa maabara aliyestahili atapewa jukumu la kutoa damu na ukusanyaji wa vielelezo vya kinyesi. Ikiwa kuna jeraha lolote katika mchakatowa kupata kielelezo kwa kusudi la utafiti huu, huduma ya matibabu itatolewa na kliniki anayehudhuria kwa gharama ya mradi huo

(v)Ni nini kitatokea baada ya utafiti?

Ikiwa utapatikana na ugonjwa ambao tunagundua kupitia vipimo vyetu vya maabara, utatibiwa na dawa zinazofaa kulingana na miongozo ya hospitali na pia utapewa ushauri nasaha na elimu ya afya juu ya jinsi ya kuzuia maambukizo ambayo yanaweza kutokea kwa sababu ya usafi duniwa mazingira na utunzaji wa vyakula na maji nyumbani. Baada ya utafiti tutaweza kuanzisha hifadhidata juu ya dawa bora za kutumia kwa maambukizo ya sumu ya damu na kuhara inayoathiri watu katika eneo lako na jinsi virusi vinaweza kuwa vikiingia katika jamii.

Takwimu hizi zitapatikana kwa walezi na Wizara ya Afya kwa uboreshaji wa jumla wa matibabuya maambukizo haya kwa watoto.

(vi)Nikibadilisha maoni yangu kuhusu kusaidia katika utafiti huu?

Ikiwa unakubali kusaidia na utafiti huu na baadaye ubadilishe mawazo yako uko huru kutekakutoka kwa utafiti wakati wowote. Hautabaguliwa kwa njia yoyote siku zijazo ikiwa labda haukubali kushiriki au baadaye ubadilishe mawazo yako.

(vii)Nani atasoma au kusikia habari inayokusanywa kutoka kwako?

Habari iliyokusanywa kutoka kwako itasaidia kuhifadhiwa kwa kutumia nambari ili usiwezekutambuliwa. Habari iliyosimbwa itafanyika kwenye kompyuta zilizolindwa na nywila zinazojulikana na timu ya utafiti tu.

Una maswali yoyote ambayo ungependa nijibu sasa? Ikiwa ungependa kujua maelezo zaidi juuya utafiti huo au kuwa na maswala yoyote ambayo yanahitaji kujadiliwa katika siku zijazo unaweza kuwasiliana na yeyote kati ya watu wafuatao katika KEMRI, Kituo cha Utafiti wa Microbiology, SLP 43640-00100, Nairobi. Purity Kasiano - Nambari ya simu ya rununu: 0712209928 au Katibu, KEMRI / Kamati yaKitaifa ya Ukaguzi wa Maadili, PO Box 54840-00200, Nairobi, TEL: + 245-20-2722541

Ninathibitisha kwamba ninaelewa habari iliyotolewa kwa utafiti hapo juu na nimepata nafasi yakuuliza maswali. Ninaelewa kuwa ushiriki ni wa hiari na kwamba niko huru kujiondoa kwenye utafiti wakati wowote bila kutoa sababu, bila huduma yangu ya matibabu au haki za kisheria kuathiriwa.

Ninakubali_____(Nambari ya Utafiti)_____, kushiriki katika utafiti huu.

NDIO LA

Saini ya mshiriki_____Tarehe _____

Au kuchapisha kidole gumba:

Jina la mshiriki_____Tarehe _____

Appendix VII: Assent Form for Children

Study title: Typhoidal salmonella disease and carriage, diversity and antimicrobial resistant genesin Mukuru informal settlement, Nairobi Kenya.

Principal Investigator: Purity KasianoCo-Investigator: Prof. Sam Kariuki

Organization: Kenya Medical Research Institute

Explanation of the purposes of the research

You are being asked to take part in a medical research study being performed by the Kenya Medical Research Institute (KEMRI), Centre for Microbiology Research. It is very important that you understand the following general principles that apply to all participants in our studies:

1)Your participation is entirely voluntary;
2)You may withdraw from participation in this study or any part of this study at any time, with no penalty, harm, or loss of access to treatment and care;
3)After you read about the study please ask any questions that will allow you to understand the study more clearly.

(i) What is the purpose of this research?

We aim to obtain information about a disease that is common in children below the age 5 years and young school-going children less than 16 years of age, which may cause severe bloodstream or diarrhea illness that may require admission to hospital. This disease is caused by a germ called *Salmonella,* which may spread through handling food with unwashed hands, contamination with dirt from animals and drinking contaminated water. If we isolate *Salmonella* from your fecal sample, we will do further laboratory tests in order to know which is the best drug to use for treatment.

The knowledge obtained from this study will go a long way in identifying the types of such germs infecting you in order to identify possible sources of infections and provide data on appropriate modes of prevention and control of such diseases. Bacteria isolated from you will betested in a laboratory in KEMRI in order to understand how the disease spreads in the community.

No sample data from you or any data containing your identity will be revealed to anybody else outside of this study. We also wish to visit your home so that we can obtain further informationthat may be useful in investigating the likely source of this disease and the various means by which the disease may be spreading within your community.

(ii) Your part in the research

You will be investigated for the germ that causes blood infection and/or diarrhea. We ask that you allow us to being investigate you for these diseases. The process will involve taking a blood

and stool sample or rectal swab and blood for laboratory testing once you present with clinical symptoms of typhoid at the clinic. After 14 days following treatment, we will take another stoolsample for investigation to find out if the treatment offered is effective.

Results from these tests will be useful for informing doctors in the clinic and community healthcare workers about how best to treat the disease and ways to prevent the disease spreadingin the community.

As part of effort to use modern techniques to develop new methods for disease prevention, such as vaccines, we propose to perform advanced genetic studies on the germ causing disease ILRI incollaboration with research partners working in this area and who have access to advanced genotyping and sequencing equipment. We request that you allow us to do so.. The experiments will be done under the care of the Principal Investigator.

(iii) What additional procedures will there be for children who help with research?

No other procedures will be done on you.

(iv) How many people will be involved in the study

We are expecting to obtain samples from about 118 individuals including young children.
Risks

The study carries no extra risk to you because these will be the routine procedures carried out incases of blood infections or diarrhea. Taking these samples may cause you some discomfort andwe aim to minimize this by using most current stool collection techniques, including moistening the rectal swab. A qualified phlebotomist will be tasked with drawing blood and collection of stool specimen. If there is any injury in the process of obtaining specimen for the purpose of thisstudy, medical care will be provided by the attending clinician at the cost of the project.

(v) What will happen after the study?

If you are found with the illness that we discover through our laboratory tests, you will be treatedwith appropriate medicines according to the hospital guidelines and also offered post counseling and health education on how to prevent infections that may happen due to poor sanitation and handling of foods and water in the home. After the study we will be able to establish a database on the best drugs to use for blood poisoning infections and diarrhea affecting people in your areaand how the germ may be finding their way into the community.

These data will be available to caregivers and the Ministry of Health for the general improvement of treatment of these infections in children.

(vi) What if I change my mind about helping with this research?

If you agree to help with this research and later change your mind you are free to with draw fromthe study at any time. You will not be discriminated against in any way in the

future if you eitherdo not agree to participate or later change your mind.

(vii) Who will read or hear about information collected from you?

The information collected from you will help will be stored using codes so that you cannot be recognized. Coded information will be held on computers protected by passwords known to theresearch team only.

Do you have any questions that you would like me to answer now? If you would like to know more details about the research or have any issues that need to be discussed in the future you cancontact any of the following people at KEMRI, Centre for Microbiology Research, PO Box 43640-00100, Nairobi. Purity Kasiano – Mobile Phone No:0712209928 or Secretary, KEMRI/National Ethical Review Committee, PO Box 54840-00200, Nairobi, TEL: +245-20-2722541

I confirm that I understand the information provided for the above study and have had the opportunity to ask questions. I understand that participation is voluntary and that I am free to withdraw from the study at any time without giving reasons, without my medical care or legalrights being affected.

I agree to_____(Study Number)_____, to take part in this study.YES NO

Signature of the participant_____Date _____ Or Thumb print:

Name of the participant _____ Date _____

Appendix VIII: Swahili Version of Assent Seeking Form

Kichwa cha somo: Ugonjwa na Ubebaji wa homa ya matumbo na usugu wa dawa katika kaya;aina tofauti za jeni, katika maeneo ya makazi ya Mukuru, Nairobi, Kenya.

Mchunguzi Mkuu: Purity Kasiano Mpelelezi Mwenza: Profesa Sam Kariuki Shirika: Taasisi ya Utafiti wa Tiba ya KenyaMaelezo ya madhumuni ya utafiti

Unaulizwa kushiriki katika utafiti wa matibabu unaofanywa na Taasisi ya Utafiti wa Tiba ya Kenya (KEMRI), Kituo cha Utafiti wa Microbiology. Ni muhimu sana uelewe kanuni zifuatazoza jumla ambazo zinatumika kwa washiriki wote katika masomo yetu:

1)Ushiriki wako ni wa hiari kabisa;
2)Unaweza kujiondoa katika kushiriki katika utafiti huu au sehemu yoyote ya utafiti huu wakatiwowote, bila adhabu, madhara, au kupoteza ufikiaji wa matibabu na utunzaji;
3)Baada ya kusoma juu ya utafiti tafadhali uliza maswali yoyote ambayo yatakuruhusu kuelewautafiti kwa uwazi zaidi.

(i) Ni nini kusudi la utafiti huu?

Tunakusudia kupata habari juu ya ugonjwa ambao ni wa kawaida kwa watoto walio chini ya umri wa miaka 5 na watoto wadogo wanaokwenda shule chini ya umri wa miaka 16, ambayo inaweza kusababisha ugonjwa mkali wa damu au kuhara ambao unaweza kuhitaji kulazwa hospitalini. Ugonjwa huu unasababishwa na vijidudu vinavyoitwa Salmonella, ambavyo vinaweza kuenea kupitia kushughulikia chakula kwa mikono ambayo havijaoshwa, kuchafuliwana uchafu kutoka kwa wanyama na kunywa maji machafu. Ikiwa tutatenganisha Salmonella na sampuli yako ya kinyesi, tutafanya vipimo zaidi vya maabara ili kujua ni dawa ipi bora kutumiakwa matibabu.

Ujuzi uliopatikana kutoka kwa utafiti huu utasaidia sana kubainisha aina za vijidudu hivyo vinavyokuambukiza ili kubaini vyanzo vya maambukizo na kutoa data juu ya njia

zinazofaa zakuzuia na kudhibiti magonjwa kama hayo. Bakteria waliotengwa na wewe watajaribiwa katikamaabara huko KEMRI ili kuelewa jinsi ugonjwa unavyoenea katika jamii.

Hakuna data ya mfano kutoka kwako au data yoyote iliyo na kitambulisho chako itafunuliwa kwa mtu mwingine yeyote nje ya utafiti huu. Tunataka pia kutembelea nyumba yako ili tuweze kupata habari zaidi ambayo inaweza kuwa muhimu katika kuchunguza chanzo kinachowezekanacha ugonjwa huu na njia anuwai ambazo ugonjwa unaweza kusambaa ndani ya jamii yako.

(ii) Sehemu yako katika utafiti

Utachunguzwa kwa bakteria ambao husababisha maambukizo ya damu na / au kuhara. Tunaomba uturuhusu tukuchunguze magonjwa haya. Utaratibu huu utahusisha kuchukua sampuli ya kinyesi au usufi wa rectal na damu kwa upimaji wa maabara mara tu utakapowasilisha daliliza kliniki za typhoid kwenye kliniki. Baada ya siku 14 kufuatia matibabu, tutachukua sampuli nyingine ya kinyesi kwa uchunguzi ili kujua ikiwa tiba inayotolewa ni nzuri.

Matokeo ya vipimo hivi yatakuwa muhimu kwa kuwajulisha madaktari katika zahanati na wafanyikazi wa huduma za afya ya jamii juu ya njia bora ya kutibu ugonjwa na njia za kuzuiaugonjwa kuenea katika jamii.

Kama sehemu ya juhudi za kutumia mbinu za kisasa kukuza njia mpya za kuzuia magonjwa, kama vile chanjo, tunapendekeza kufanya tafiti za maumbile juu ya ugonjwa unaosababisha ugonjwa wa ILRI kwa kushirikiana na washirika wa utafiti wanaofanya kazi katika eneo hili na ambao wana ufikiaji wa hali ya juu na vifaa vya mpangilio. Tunaomba uturuhusu kufanya hivyo Majaribio yatafanywa chini ya uangalizi wa Mchunguzi Mkuu.

(iii) Hatari

Utafiti hauna hatari zaidi kwako kwa sababu hizi zitakuwa taratibu za kawaida zinazofanywa wakati wa maambukizo ya damu au kuhara. Kuchukua sampuli hizi kunaweza kukusababishia usumbufu na tunakusudia kupunguza hii kwa kutumia mbinu za sasa za ukusanyaji wa kinyesi,pamoja na kulainisha usufi wa rectal. Mtaalamu wa maabara aliyestahili atapewa jukumu la kutoa damu na ukusanyaji wa vielelezo vya kinyesi. Ikiwa kuna jeraha lolote katika mchakato wa kupata kielelezo kwa kusudi la utafiti huu, huduma ya matibabu itatolewa na kliniki anayehudhuria kwa gharama ya mradi huo

(iv) Ni watu wangapi watakaoshiriki katika utafiti

Tunatarajia kupata sampuli kutoka kwa watu wapatao 118 pamoja na watoto wadogo.

(v) Ni nini kitatokea baada ya utafiti?

Ikiwa utapatikana na ugonjwa ambao tunagundua kupitia vipimo vyetu vya maabara, utatibiwa na dawa zinazofaa kulingana na miongozo ya hospitali na pia utapewa ushauri nasaha na elimu ya afya juu ya jinsi ya kuzuia maambukizo ambayo yanaweza kutokea kwa sababu ya usafi duniwa mazingira na utunzaji wa vyakula na maji nyumbani. Baada ya utafiti tutaweza kuanzisha hifadhidata juu ya dawa bora za kutumia kwa maambukizo ya sumu ya damu na kuhara inayoathiri watu katika eneo lako na jinsi virusi vinaweza kuwa vikiingia katika jamii.Takwimu hizi zitapatikana kwa walezi na Wizara ya Afya kwa uboreshaji wa jumla wa matibabuya maambukizo haya kwa watoto.

(vi) Je! nikibadilisha maoni yangu kuhusu kusaidia katika utafiti huu?

Ikiwa unakubali kusaidia na utafiti huu na baadaye ubadilishe mawazo yako uko huru kutekakutoka kwa utafiti wakati wowote. Hautabaguliwa kwa njia yoyote siku zijazo ikiwa labda haukubali kushiriki au baadaye ubadilishe mawazo yako.

(vii) Nani atasoma au kusikia habari inayokusanywa kutoka kwako?

Habari iliyokusanywa kutoka kwako itasaidia kuhifadhiwa kwa kutumia nambari ili usiwezekutambuliwa. Habari iliyosimbwa itafanyika kwenye kompyuta zilizolindwa na nywila zinazojulikana na timu ya utafiti tu.

Je! Una maswali yoyote ambayo ungependa nijibu sasa? Ikiwa ungependa kujua maelezo zaidijuu ya utafiti huo au kuwa na maswala yoyote ambayo yanahitaji kujadiliwa katika siku zijazounaweza kuwasiliana na yeyote kati ya watu wafuatao katika KEMRI, Kituo cha Utafiti wa Microbiology, SLP 43640-00100, Nairobi.

Purity Kasiano - Nambari ya simu ya rununu: 0712209928 au Katibu, KEMRI / Kamati yaKitaifa ya Kupitia Maadili, PO Box 54840-00200, Nairobi, TEL: + 245-20-2722541

Ninathibitisha kwamba ninaelewa habari iliyotolewa kwa utafiti hapo juu na nimepata nafasi yakuuliza maswali. Ninaelewa kuwa ushiriki ni wa hiari na kwamba niko huru kujiondoa kwenye utafiti wakati wowote bila kutoa sababu, bila huduma yangu ya matibabu au haki za kisheria kuathiriwa.

Ninakubali_____(Nambari ya Utafiti)_____, kushiriki katika utafiti huu.NDIO LA

Saini ya mshiriki_____Tarehe _____ Au kuchapisha kidole gumba:

Jina la mshiriki_____Tarehe _____

CITI PROGRAM

Completion Date 15-Dec-2020
Expiration Date 15-Dec-2021
Record ID 39972498

This is to certify that:

Purity Kasiano

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Biomedical Research - Basic/Refresher
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KEMRI/RES/7/3/1 **July 09, 2021**

TO: **KASIANO N. PURITY,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DEPUTY DIRECTOR, CMR,**
NAIROBI.

Forwarded 13/7/2021

Dear Madam,

RE: **KEMRI/SERU/CMR/P00156/4198 *(RESUBMISSION II OF INITIAL SUBMISSION)*: TYPHOIDAL SALMONELLA HOUSEHOLD CARRIAGE, DIVERSITY AND ANTIMICROBIAL RESISTANT GENES IN MUKURU INFORMAL SETTLEMENT, NAIROBI KENYA.**

Reference is made to the letter dated July 06, 2021. The KEMRI Scientific and Ethics Review Unit (SERU) Secretariat acknowledges receipt of the revised study documents on July 07, 2021.

This is to inform you that the Committee notes that the issues during the 310th Committee C meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **April 29, 2021,** have been adequately addressed.

Consequently, the study is **granted approval** for implementation effective this day, **July 09, 2021** for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **July 08, 2022.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **May 27, 2022.**

Please note that only approved documents including (informed consents, study instruments, Material Transfer Agreement) will be used. You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and please, inform SERU when the study is completed or discontinued.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours faithfully,

PROF. CHARLES OBONYO,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

PLOS ONE

RESEARCH ARTICLE

Typhoidal salmonella disease in Mukuru informal settlement, Nairobi Kenya; carriage,diversity, and antimicrobial resistant genes

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Abstract

Introduction

Multiple studies have shown that typhoid fever is endemic in developing countries characterized by poor hygiene. A unique way of *Salmonella* Typhi (*S*.Typhi) pathogenicity is establishing a persistent, usually asymptomatic carrier state in some infected individuals who excrete large numbers of bacteria in faeces. This study aimed to determine the isolation rate of *S*.Typhi from blood and stool samples among cases and asymptomatic individuals in the Mukuru informal settlement and identify antibiotic resistance patterns within the same population.

Materials and methods

We recruited 1014 outpatient participants presenting with typhoid-like symptoms in selected health centres in Nairobi, Kenya. Bacterial isolation was done on Xylose Lysine Deoxycholate agar (XLD) and Mac Conkey agar (Oxoid), followed by standard biochemical tests. Identification was done using API20E, and *S*.Typhi was confirmed by serotyping using polyvalent antisera 0–9 and monovalent antisera d. The Kirby-Bauer disc diffusion method was used to test the antimicrobial susceptibility of *S*.Typhi isolates, while Multi-Drug Resistant (MDR) strains were characterized using conventional PCR.

Results

Of 1014 participants, 54 (5%) tested positive for *S*.Typhi. Thirty-eight (70%) of the *S*.Typhi isolated were from stool samples, while sixteen (30%) were from blood. Three (0.2%) of the isolates were from asymptomatic carriers. Of the 54 *S*.Typhi isolates, 20 (37%) were MDR. Resistance to ciprofloxacin and nalidixic acid was 43% and 52%, respectively. Resistance to amoxicillin-clavulanic acid (a beta-lactam inhibitor) was 2%. The Bla_{TEM-1} gene was present in 19/20 (95%) MDR isolates.

Competing interests: The authors have declared that no competing interests exist.

Conclusion

MDR *S*.Typhi is prevalent in Mukuru Informal settlement. The sharp increase in nalidixic acid resistance is an indication of reduced susceptibility to fluoroquinolones, which are currently the recommended drugs for the treatment of typhoid fever. This study highlights the need for effective antimicrobial stewardship and routine surveillance of antimicrobial resistance (AMR) to inform policy on the prevention and control of MDR Typhoid disease.

Introduction

Salmonella enterica serovar Typhi (*S*.Typhi) is a Gram-negative bacillus in the Enterobacteriaceae family and is predominantly a human pathogen [1]. The pathogen is responsible for causing typhoid fever a systemic illness characterized by prolonged fever, headache, dry cough, and alteration of bowel habits [2]. Other common symptoms of the illness include fatigue, high fever ($>$ 39˚C), vomiting, coughing, and rapid pulse [3]. This disease poses a significant global health threat, with 5.6 billion people worldwide at risk of infection [4].

Living in densely populated areas, with poor sanitation and lack of safe drinking water, predisposes people living within urban slums in Africa and Asia to typhoid fever [5]. An incidence of 520 per 100,000 person-years of observation was found in children 8 years and below in Kenya's largest slum, Kibera [6]. Mukuru settlement is one of the largest and most overcrowded urban slums in Nairobi, Kenya, with a population of approximately 250,000 [7]. As an informal settlement, it is characterized by poor infrastructure, sanitation, and a lack of safe water [8]. In addition, the patients can easily access antimicrobials over the counter perpetuating the emergence and spread of antimicrobial resistance [9].

Even after successful treatment, approximately 1–6% of typhoid infections become carriers [10]. The long-term persistence of *S*.Typhi in carriers explains why typhoid fever remains endemic in regions of the world with poor-quality drinking water and limited sewage treatment. *S*.Typhi dissemination occurs through the ingestion of food or water contaminated with faeces from typhoid patients and human carriers [11]. Acute infections and asymptomatic carriage caused by *S*.Typhi can be treated using effective antibiotics such as fluoroquinolones, however, the selection and spread of a highly resistant *S*.Typhi clade prevents such measures in areas with limited resources and irrational use of antibiotics [4]. The rise and spread of Multi-Drug Resistant (MDR) *S*.Typhi, strains resistant to 3 classes of antimicrobials: ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim, is a major public health concern because it increases patients' hospital stays, it is costly to treat and increases the chance of mortality [12]. Over the years, because of the steady rise in antimicrobial resistance, the majority of typhoid cases in Kenya have been MDR coupled with reduced susceptibility to fluoroquinolones. Fluoroquinolones are the recommended drugs for the treatment of MDR typhoid in Kenya but the indiscriminate use of fluoroquinolones has led to a rapid increase in strains that are resistant to the antimicrobial. To prevent clinical treatment failure, it is critical to know the current trend of antimicrobial resistance in an endemic setting of typhoid fever like the Mukuru settlement.

For instance, Kavai *et al*., (2018) reported a Multi-Drug Resistance (MDR) prevalence of 55.5% of *S*.Typhi isolated from outpatient clinical samples from Mukuru villages in Nairobi [9]. Regular monitoring of the emergence of MDR *S*.Typhi and the dissemination of AMR-associated genes is crucial to gather essential information for making informed decisions on antibiotic usage [13]. It is therefore essential to investigate the typhoid carriage and circulating

MDR strains in Mukuru. Our study aimed to determine the isolation rate of *S*.Typhi from patients and asymptomatic individuals and the MDR prevalence in Mukuru Informal settlement.

Materials and methods

Study design and site

A cross-sectional study design was used to determine the isolation rate of *S*.Typhi among cases and asymptomatic individuals in the Mukuru settlement and to identify and characterize antibiotic resistance genes within the same population. The study participants were recruited from three clinics in Mukuru informal settlement: Medical Missionaries of Mary (MMM), Municipal County Council Clinic (MCC), Mukuru Kwa Reuben Clinic (MR), and one inpatient facility, Mama Lucy Kibaki Hospital.

Recruitment and laboratory procedures

Recruitment. Study participants were recruited from 12th July 2021 to 5th July 2022. These were outpatients who visited the four health facilities and presented with typhoid-like symptoms such as headache, diarrhoea/constipation, fatigue, and a temperature $>$ 37.5˚C. After evaluation of the patient's symptoms and inclusion criteria, the attending clinicians filled out the patients' case report forms. The patients were then introduced to the study and made to understand its objectives by the research team. Patients who were willing to participate were taken through the informed consent or assent forms in their preferred language i.e., English or Kiswahili, and those who gave their consent were given a unique study identifier that was filled in on questionnaires and the same ID used on sample collection tubes. For every confirmed *S*. Typhi case, at least one contact of the patient living in the same household was requested to give a stool sample to check for carriage. Before sample collection, the contact was taken through the same ethical considerations described above and if found to be a typhoid carrier, the individual was treated.

Sample collection. The field research team guided the participants on how to use stool cups or rectal swabs. For children, their guardians were provided with rectal swabs and instructed on how to use them correctly. Rectal swabs were collected and dipped into Cary Blair media (Oxoid, Basingstoke, UK). Aseptically, blood samples were drawn from the arm by venipuncture and transferred directly into the blood culture bottle by qualified phlebotomists. 1-3ml and 8-10ml of blood were collected for children and adults respectively. Following the collection of the samples, a unique study identification number/barcode was labeled on the sample. Blood was transported at room temperature while stool/rectal swabs were transported at 4–8˚C in cooler boxes to the CMR-KEMRI laboratory within 6 hours of collection.

Isolation and identification. Upon being received in the laboratory, rectal swabs were placed in selenite faecal enrichment broth and incubated at 37 ˚C overnight. From the enriched selenite F broth, streaking was done onto Xylose Lysine Deoxycholate agar (XLD) agar and Mac Conkey agar (Oxoid). Incubation was done at 37˚C for 24 hours. *S*.Typhi isolates were initially identified using distinguishing colony morphology characteristics such as pale-yellow colonies on MAC and brick red with black centres on XLD. Non-duplicate colonies from standard biochemical tests such as citrate, indole, urease, and Triple Sugar Iron (TSI) were examined with API20E (Biomeriux) and confirmed with serology. Serotyping tests were done using the slide agglutination technique using polyvalent antisera O- 9, and monovalent anti-sera d (Murex Diagnostics, Dartford, UK) [9].

Blood culture bottles were incubated in a BACTEC 9050 Culture System (Becton Dickinson, USA) at 37˚C for up to seven days. A positive culture bottle with the reference strain was

used to validate the results. Samples flagged as negative by the BACTEC were discarded while those flagged as positive were cultured onto MacConkey agar, Blood agar, and Chocolate agar. Isolates showing growth characteristics of *S*.Typhi were subcultured onto Mueller Hinton (Oxoid, Basingstoke, UK) for the growth of single discrete colonies.

Antibiotic susceptibility of isolates. The Kirby-Bauer disc diffusion technique was used in testing the antimicrobial susceptibility of *S*.Typhi isolates on Mueller-Hinton agar (Oxoid). The inoculum turbidity of the isolates was compared against the McFarland 0.5 turbidity standard. *S*.Typhi isolates were inoculated evenly on Mueller-Hinton Agar plates using the spread plate technique. The plates were then impregnated with antimicrobial sensitivity discs using sterile forceps and then gently pressed down onto the agar. Plates were then incubated at 37˚C for 18 hours.

The following Oxoid™ antibiotic disks were used; amoxicillin-clavulanate (AMC 20:10 μg), nalidixic acid (NA 30μg), ciprofloxacin (CIP 5μg), ampicillin (AMP 10μg), ceftazidime (CAZ 30μg), cefotaxime (CTX 30μg), ceftriaxone (CRO 30μg), sulfamethoxazole-trimethoprim (SXT 1.25/23.57μg), chloramphenicol (CHL 30μg), and tetracycline (TET 30μg)., cefpodoxime (CPD 10μg) kanamycin (K 30μg) azithromycin (AZT 15μg).

For each *S*.Typhi isolate, two plates with antibiotics were used, labeled as Plate A and B. Plate A targeted Extended Spectrum Beta Lactamases (ESBL) production. The arrangement of antibiotics was as follows, penicillins (AMP), 3rd generation cephalosporin (CRO, CAZ, CTX, CPD), inhibitor at the middle (AMC). In plate B CIP, NA (targeting fluoroquinolones resistance), GEN, KAN, (targeting aminoglycosides resistances), CHL, SXT, and TET were also used because they are the recommended first-line drugs for the treatment of typhoid. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used for quality control of media quality and disc potency. Clinical and Laboratory Standard Institute (CLSI) guidelines were used to interpret zones of inhibition into susceptible, intermediate, and resistant. The isolates exhibiting resistance to chloramphenicol, ampicillin, and sulfamethoxazole-trimethoprim were classified as MDR [14].

Polymerase chain reaction. Out of the 54 *S*.Typhi isolates, twenty MDR isolates that showed resistance to ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim were further examined for the corresponding resistance genes using the conventional polymerase chain reaction. DNA was extracted using the boiling method. Briefly, a pea-sized amount of freshly cultured isolates was placed in 500μl PCR water (Invitrogen) in an Eppendorf tube. The mixture was then heated in a heating block at 95˚C for 12 minutes, followed by centrifugation at 14000rpm for 5 minutes to obtain the supernatant. 200 μl of the supernatant was used as the template. The following resistance genes were tested; beta-lactamase genes ($bla_{CTX\text{-}M}$, $bla_{TEM\text{-}1B}$, bla_{SHV}) *qnrS*, and *qnrA* for plasmid-mediated quinolones resistance. The Qiagen PCR kit (Qiagen) was used for amplification. The reaction mixture for PCR consisted of 12μl PCR water, 12 μl master mix (DNA polymerase, dNTPs, $MgCL_2$, and buffer), 1μl of forward and 1μl reverse primer, and 1μl DNA template. PCR water was used as a negative control. Amplification was performed using published primers as summarised in **Table 1** in 0.2ml PCR tubes on a thermocycler.

Amplification conditions were as follows, initial denaturation at 95˚C for 5 minutes, 95˚C for 30 seconds variable annealing temperature for 30 seconds, 72˚C for 2 minutes 30 seconds, and a final extension at 72˚C for 7 minutes. Annealing temperatures differed for the different genes as shown in **Table 1.**

Table 1. Shows primer sequences for ESBLs and quinolone resistance used in this study.

Target gene	Primer name	Primer sequence	Annealing T˚C	Expected Bps	Reference
bla_{CTX-M}	CTX-M-F	5^0-ATGTGCAGYACCAGTAARGTKATGGC-3^0	60˚C	593	[15]
	CTX-M-R	5^0-TGGGTRAARTARGTSACCAGAAYCAGCGG-3^0			
bla_{TEM-1B}	TEM-F	5′-TCGGGGAAATGTGCGCG-3′	50˚C	851	[15]
	TEM-R	5′-TCGGGGAAATGTGCGCG-3′			
bla_{SHV}	SHV-F	5^0- TTCGCCTGTGTATTATCTCCCTG- 3^0	50˚C	854	[15]
	SHV-R	5^0- TTAGCGTTGCCAGTGYTCG- 3^0			
qnrA	QNRA-F	5′-AGAGGATTTCTCACGCCAGG-3′	55˚C	580	[16]
	QNRA-R	5′-TGCCAGGCACAGATCTTGAC-3′			
qnrS	QNRS-F	5′-GCAAGTTCATTGAACAGGGT-3′	54˚C	428	[16]
	QNRS-R	5′-TCTAAACCGTCGAGTTCGGCG-3′			

https://doi.org/10.1371/journal.pone.0298635.t001

Ethical consideration

Ethical approval for the study was obtained from the Scientific Ethical Review Committee (SERU) of Kenya Medical Research Institute (No. KEMRI/SERU/CMR/P00156/4198). Written informed consent was obtained from adults participating in this study. For children, written informed consent was obtained from the parents/guardians, and verbal assent for older children 13–17 years was recorded. Unique identifiers in place of names were used to ensure that the participants' anonymity was upheld. All the gadgets where data was stored were password protected and access to the data was strictly given to authorized staff.

Data analysis

Data obtained in this study was entered into MS Excel. The isolation rate was determined by dividing typhoid-positive cases by the total number of study participants and expressed as a percentage. Frequencies and proportions of categorical data were analyzed using SPSS version 26. The zones of inhibition by disc diffusion were measured in millimeters and interpretation was done according to Clinical and Laboratory Standard Institute (CLSI) guidelines. WHONET was used to generate the graph depicting the distribution of resistance using disk diffusion zone diameters.

Results

Social demographics characteristics and isolation rate of typhoid

A total of 1014 participants with typhoid-like symptoms were recruited within one year from 2021 from four study sites. Of the 1014, the highest number of participants at 415 (41%) were from Medical Missionaries of Mary (MMM). In total, 516 (51%) were females while 498 (49%) were males. The overall *S*.Typhi isolation rate from the participants was 5% (54) with women having a higher isolation rate at 54% (29) compared to men at 46% (25) as shown in **Table 2**. Three (0.2%) of the 54 isolates were from asymptomatic carriers. These were contacts of positive cases who were requested to give a stool sample to check for carriage. In addition, the highest isolation rate was from MMM at 57% (31). The average age of the typhoid cases was 19 years with the oldest being 45 years and the youngest 8 months. The age group with the highest isolation rate from these findings was 21–30 at 44% (24).

Antimicrobial susceptibility patterns

The overall prevalence for the MDR phenotype was 37% (20/54). In addition, resistance to chloramphenicol was 39% (21/54) and 46% (25/54) both for ampicillin and sulfamethoxazole-

Table 2. Social demographic characteristics of study participants.

Social demographic characteristics		Typhoid status		
		Negative *n* = 960	Positive *n* = 54	Total = 1014
Gender	Female	487 (51%)	29 (54%)	516 (51%)
	Male	473 (49%)	25 (46%)	498 (49%)
Age_group	0–10	577 (60%)	19 (35%)	596 (58.7%)
	11–20	88 (9%)	3 (6%)	91 (8.9%)
	21–30	125 (13%)	24 (44%)	149 (23.5%)
	31–40	102 (11%)	5 (9%)	107 (10.5%)
	41–50	45 (4.7%)	3 (6%)	48 (4.7%)
	51–60	16 (1.7%)	0 (0%)	16 (1.4%)
	61–70	5 (0.5%)	0 (0%)	5 (0.4%)
	71–80	2 (0.2%)	0 (0%)	2 (0.2%)
Specimen_type	Blood	0 (0%)	16 (30%)	16
	Stool	0 (0%)	38 (70%)	38
Recruitment_facility	MCC	216 (23%)	10 (19%)	226 (22%)
	MLK	142 (15%)	11 (20%)	153 (15%)
	MMM	384 (40%)	31 (57%)	415 (41%)
	MR	218 (23%)	2 (4%)	220 (22%)
Participant_type	0ut-patient	907 (94%)	51 (94%)	958 (94.5%)
	Contact	51 (5.3%)	3 (6%)	54 (5.3%)
	Emergency room	1 (0.1%)	0 (0%)	1 (0.01%)
	In-patient	1 (0.1%)	0 (0%)	1 (0.01%)

https://doi.org/10.1371/journal.pone.0298635.t002

trimethoprim. Moreover, the most common resistance phenotype was nalidixic acid where more than half of the isolates were resistant at 52% (28/54). A high proportion of the isolates showed reduced susceptibility towards ciprofloxacin which is the drug of choice for treating typhoid fever at 43% (23/54). All the isolates were susceptible to the aminoglycoside gentamicin but resistance to kanamycin was observed at 2% (1/54) as shown in **Table 3.** Interestingly, all the *S*.Typhi isolates analyzed were fully susceptible to the 3rd generation of cephalosporins used in this study i.e., ceftriaxone, cefotaxime, ceftazidime and cefpodoxime.

In addition, resistance to amoxicillin-clavulanate was recorded at 2% (1/54). Resistance to tetracycline and azithromycin was 4% and 9%, respectively as shown in **Fig 1**. Two out of three asymptomatic individuals harbored *S*.Typhi resistant to nalidixic acid and ciprofloxacin while one carrier harboured the *S*.Typhi MDR phenotype.

The resistance towards nalidixic acid was the highest across all the recruitment sites, ranging from 4 to 75%, as shown in **Table 4.** The resistance towards the first-line drugs; chloramphenicol, ampicillin, and sulfamethoxazole-trimethoprim was high in Medical Missionaries of Mary ranging from 50% to 62%, and was lowest in Mama Lucy Kibaki hospital ranging from 1–2% at 62%. The percentage resistance to ciprofloxacin which is currently the recommended drug for the treatment of typhoid in Kenya was highest in Medical Missionaries of Mary at 73% and lowest in Mukuru Kwa Reuben and Mama Lucy Kibaki Hospital at 4%.

PCR for selected resistance genes

A total of twenty isolates exhibiting the MDR phenotype were screened for $bla_{TEM\text{-}1B}$, $bla_{CTX\text{-}M}$, bla_{SHV}, *qnrB*, and *qnrS* genes. Of all the five genes, the beta-lactamase gene $bla_{TEM\text{-}1}$ was positive and found present in 95% (19/20) of the MDR isolates, as shown in **Fig 2.**

Table 3. Percentage of antimicrobial resistance among the 54 *S*.Typhi isolates.

Antimicrobial	Susceptible	Resistant
	Number (%)	Number (%)
Ampicilin	29(54)	25 (46)
Ceftadizime	54 (100)	0
Ceftriaxone	54 (100)	0
Cefpodoxime	54 (100)	0
Cefotaxime	54 (100)	0
Amoxicillin clavulanate	53 (98)	1 (2)
Sulfamethoxazole-trimethoprim	29 (54)	25 (46)
Ciprofloxacin	31 (57)	23 (43)
Nalidixic acid	26 (48)	28 (52)
Azithromycin	49 (91)	5 (9)
Gentamicin	54 (100)	0
Kanamycin	53 (98)	1 (2)
Tetracycline	52 (96)	2 (4)
Chloramphenicol	33 (61)	21 (39)

https://doi.org/10.1371/journal.pone.0298635.t003

Discussion

In the current study, the overall isolation rate of *S*.Typhi was 5%, (54 *S*.Typhi isolated from 1014 patients). This isolation is higher when compared to another study by Kariuki *et al* (2021) which found an isolation rate of 3.2% [17]. This difference could be a result of the inclusion criteria where participants less than 16 years were sampled, but for this study, participants

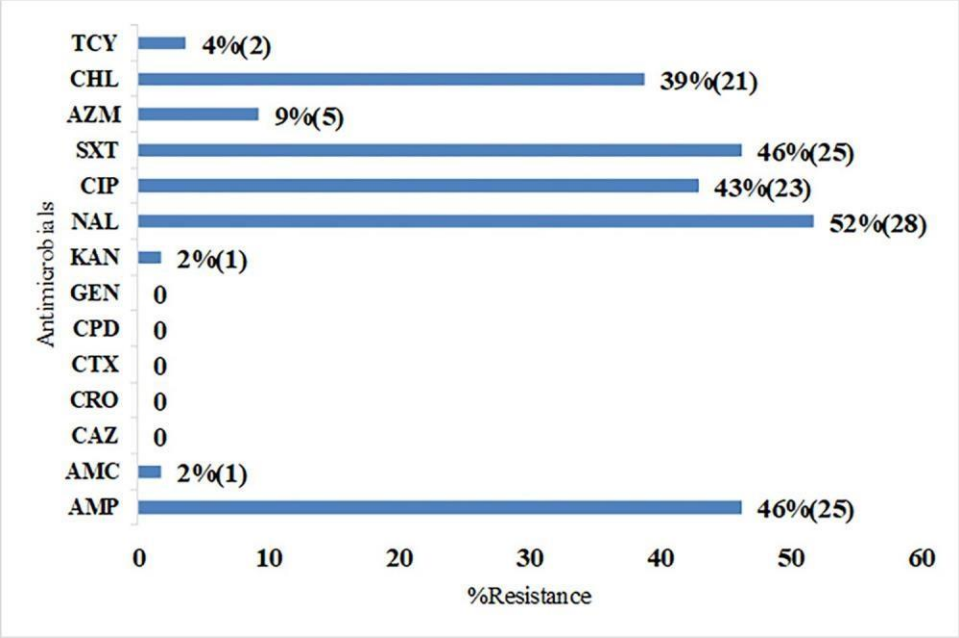

Fig 1. Antimicrobial resistance patterns (%) of isolated *S*.Typhi strains. KEY: Numbers in brackets represent the total number of resistant isolates for each drug. TCY-tetracycline, CHL-chloramphenicol, AZM-azithromycin, SXT-sulfamethoxazole-trimethoprim, CIP-ciprofloxacin, NAL-nalidixic acid, KAN-kanamycin, GEN-gentamicin, CPD-cefpodoxime, CTX-cefotaxime, CRO-ceftriaxone, CAZ-ceftazidime, AMC-amoxicillin clavulanate, AMP-ampicillin.

https://doi.org/10.1371/journal.pone.0298635.g001

Table 4. Antimicrobial resistance according to sampling site.

Study site	AMP	CAZ	CTX	CPD	CRO	SXT	AMC	CIP	NA	AZM	CN	K	TCY	CHL
Mukuru Kwa Reuben	2 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (10%)
Medical Missionaries of Mary	14 (56%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	15 (60%)	0 (0%)	17 (73%)	21 (75%)	2 (40%)	0 (0%)	0 (0%)	1 (50%)	13 (62%)
Mama Lucy Kibaki Hospital	2 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	1 (4%)	1 (4%)	1 (20%)	0 (0%)	0 (0%)	1 (50%)	1 (5%)
Municipal City County Clinic	7 (28%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (24%)	1 (100%)	4 (17%)	5 (19%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)	5 (24%)

KEY: TCY-tetracycline, CHL-chloramphenicol, AZM-azithromycin, SXT- sulfamethoxazole-trimethoprim, CIP-ciprofloxacin, NAL-nalidixic acid, KAN-kanamycin, GEN-gentamicin, CPD-cefpodoxime, CTX-cefotaxime, CRO-ceftriaxone, CAZ-ceftazidime, AMC-amoxicillin clavulanate, AMP-ampicillin.

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were recruited from 0–80 years. Of the 54 *S*.Typhi isolated in this study, three were from asymptomatic individuals giving an isolation rate of 0.2% from carriers in contrast to Kariuki *et al* (2019) who reported a carriage rate of 1% [9]. This variation in results could be attributed to the differences in the study participants' inclusion criteria.

The isolation rate from stool was high at 70% (38/54) compared to 30% (16/54) from blood. A similar variation was observed by Kariuki *et al*. (2021) who reported an isolation rate of 58% from stool samples and 48% from blood [18]. The variation in the isolation rate between blood and stool specimens in this study could be attributed to the pathogenesis of the bacterium during the development of the disease. Ingested bacteria reach the small intestine where, through microfold (M) cells of Peyer's patches, the bacteria migrate to the mesenteric lymph nodes, multiply, and are released into the bloodstream for the first time during infection[3,19]. *S*. Typhi disseminates causing transient primary bacteremia. Blood culture is the gold standard for detecting typhoid fever however its sensitivity decreases with the progression of the illness [20] In untreated cases, maximum blood culture yield is attained at the onset of typhoid fever during the first week of infection [19]. We assume that sample collection in our study could have been done when the participants were at the late stage of the disease.

In this current study, the highest isolation rate at 44% (24/54) was from the age group 21–30. In addition, it was also observed in the above-mentioned study that *S*.Typhi was more prevalent in patients over five years of age [17]. The difference in isolation rate is evidence that the prevalence of *S*.Typhi varies within populations over time.

The isolation of *S*.Typhi strains resistant to at least three different classes of antimicrobials; ampicillin, sulfamethoxazole-trimethoprim, and chloramphenicol, is evidence that the MDR

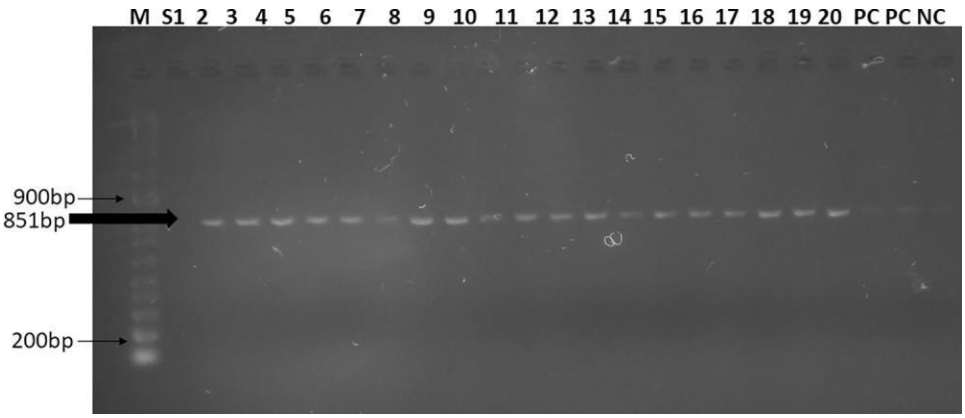

Fig 2. Gel electrophoresis of bla$_{TEM-1B}$ positive MDR isolates. Abbreviations; M-molecular ladder (100-5000bp), NC-negative control, PC-positive control, S1-S20- MDR S.Typhi samples.

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strains still pose a health challenge in our community. MDR strains have been implicated in causing typhoid outbreaks [4]. We found an MDR prevalence of 37%. These findings are lower than Kavai *et al* 2018, who reported a prevalence of 55.5% in the Mukuru informal settlement [9]. The decrease could be because of the active vaccination strategies that have been deployed in such high-risk populations. Also, the decline could be attributed to different patterns of antimicrobial use or improved enactment of policies on prudent use of antimicrobials over the years during the implementation of the National Action Plan to combat and prevent Antimicrobial Resistance (AMR). In addition, our study findings are similar to Tack *et al*. 2019 who found an MDR prevalence of 38.3% in the Democratic Republic of Congo (DRC) [20].

Ciprofloxacin is the antimicrobial of choice for the treatment of typhoid fever caused by MDR *S*.Typhi [21]. Recommendation to use ciprofloxacin/ofloxacin over the years have resulted in the rise of resistance [20]. We recorded resistance to ciprofloxacin and nalidixic acid at 43% and 52%, respectively. These results are comparable to Mutai *et al*, *2019* who reported high resistance to ciprofloxacin at 69% in different settings. Typhoid infection by strains that are resistant to nalidixic acid has been linked to the causation of prolonged illness and hepatomegaly [22]. Various studies have highlighted a strong correlation between *S*.Typhi strains resistant to nalidixic acid/fluoroquinolones and poor clinical outcomes [23]. This implies that for successful treatment, other antimicrobial alternatives such as azithromycin and 3rd generation cephalosporin should be considered.

For cases of complicated typhoid fever, third-generation cephalosporins are the drugs of choice [24]; our isolates were fully susceptible to this class of antimicrobials. However, these findings contrast those of Kavai *et al*, (2018) study, who reported less than 5% resistance towards third-generation cephalosporins [9]. Similarly, Tack *et al*. (2019) reported a 0.2% resistance towards 3rd generation cephalosporins in a study done in DRC [20]. The variation in resistance levels could be attributed to them not being the first-line treatment for typhoid fever in the Mukuru settlement. Because no resistance was observed in our study phenotypically, the 3rd generation cephalosporins, specifically ceftriaxone, can be effective in the treatment of typhoid fever in our endemic settings. Full susceptibility implies that *S*.Typhi isolates in this study did not harbour extended-spectrum beta-lactamase genes (ESBL) that could otherwise render the antimicrobial ineffective. This was confirmed by the absence of the ESBL $bla_{CTX\text{-}M}$ gene by conventional PCR.

This study reports a high prevalence of the $bla_{TEM\text{-}1B}$ gene at 95% (19/20 MDR isolates). These results are comparable with Kavai *et al* 2018, who reported an 80% prevalence of the $bla_{TEM\text{-}1B}$ gene from *S*.Typhi isolated in selected Nairobi clinics [9]. Temporal variation when the studies were done could account for the differences in the prevalence. The $bla_{TEM\text{-}1B}$ gene confers resistance to ampicillin and is associated with the emergence of ESBL-producing bacteria that are resistant to advanced cephalosporins [25]. The high prevalence of the gene implies that the strains are evolving to carry genes that make them survive in the presence of different classes of antimicrobials hence causing treatment failure that translates to therapeutic challenges with the available antibiotics for the treatment of typhoid.

S.Typhi resistance patterns are evolving at different rates with diverse phenotypic characteristics in various endemic areas [21]. Typhoid in the African region is known to be driven by the MDR *S*.Typhi. These strains harbor resistance genes in the incH1 plasmid such as *catA*, *sul1*, *sul2*, *dfrA*, $bla_{TEM\text{-}1B}$, *strA*, *strB*, *tetA*, *tetB*, *tetC, and tetD* (4). These genes contribute to resistance to chloramphenicol, ampicillin sulfamethoxazole-trimethoprim, and tetracycline drugs [25].

It has been reported that chromosomal mutation in the *gyr*A gene is the most common source of reduced ciprofloxacin susceptibility in Africa [26]. Ciprofloxacin is designed to target DNA gyrase specifically GyrA, a protein that is essential in bacterial DNA replication [27].

Mutation on a single nucleotide at either codon position 83 or 87 of the gene (*gyrA*) encoding for the GyrA protein leads to resistance to nalidixic acid which translates to reduced susceptibility to fluoroquinolones like ciprofloxacin [4]. It has been discovered that resistance to nalidixic acid is a marker for fluoroquinolone resistance because mutations on the *gyrA* gene eventually evolve to cause resistance [27].

This study screened for the plasmid-mediated resistance genes; *qnrB* and *qnr*S. None of these genes were found present among the twenty MDR isolates. This shows that the resistance observed in our present isolates could be caused by a mutation in the chromosomally located *gyrA* gene. However, all the 54 isolates will be subjected to whole genome sequencing which will be able to provide comprehensive data of the resistance genes found in the *S*.Typhi strains.

Findings in this study show the current isolation rate of *S*.Typhi from an informal settlement including the trends in antimicrobial resistance across four sites. Comprehensive approaches to mitigate typhoid burden include improvements in hygiene and vaccination strategies which require identification of high-risk populations through continuous surveillance programs.

Conclusion

MDR *S*.Typhi is prevalent in Mukuru Informal settlement posing a major public health threat to the population. The high resistance levels to nalidixic acid and reduced susceptibility to ciprofloxacin demonstrated in this study indicate that treatment of MDR *S*.Typhi by 3rd generation cephalosporins will have better clinical outcomes. However, overreliance on antimicrobials is likely to cause the emergence of resistance. This reinforces the importance of ongoing surveillance to identify high-risk populations which will allow the implementation of public health interventions and research efforts to combat the MDR *S*.Typhi threat and typhoid fever in general. Because humans are the only source of infection and transmission of *S*.Typhi is by the fecal-oral route through contaminated water or food, prevention measures need to include provision of clean water and sanitation improvements, as well as health education.

Study limitations

Determination of the true prevalence of typhoid carriers requires long-term follow-up of asymptomatic individuals. This study could not accomplish that because it utilized a cross-sectional study design which could have affected the accuracy of prevalence estimates. Thus, this design was not sufficient to understand typhoid fever trends in our settings. During the screening of resistance genes, only available primers at the time of analysis were used; hence other relevant resistance genes could not be observed. This limitation could be a potential area for future research to explore other resistance genes or conduct more comprehensive genomic analyses to better understand the genetic basis of resistance of the isolates.

Supporting information

S1 File.
(XLSX)

S2 File.
(XLSX)

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