SEROTYPING, ANTIMICROBIAL RESISTANCE PROFILING AND ASSESSMENT OF NON-TYPHOIDAL *SALMONELLA* RISK FACTORS IN CHILDREN AGED 5 YEARS AND BELOW IN SELECTED STUDY SITES IN MUKURU INFORMAL SETTLEMENT

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DECLARATION

This thesis is wholly original with no submissions for degrees at any other universities.

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DEDICATION

I humbly dedicate this thesis to my beloved late parents, who have been a constant source of inspiration and support throughout my academic journey. My father, Mr. George Odityo, instilled in me a strong work ethic and a passion for learning, while my mother, Mrs. Prisca Nyanguka, was my greatest inspiration, motivating me to work diligently and persevere in my studies. Their unwavering love and encouragement continues to guide me, and I am deeply grateful for the enduring impact they had on my life and my research.

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LIST OF ABBREVIATIONS

KEMRI	Kenya Medical Research Institute
RKI	The Robert Koch Institute
INTS	Invasive Non-Typhoidal *Salmonella*
AMR	Antimicrobial resistance
MDR	Multidrug-resistant
PCR	Polymerase Chain Reaction
SSA	Sub-Saharan Africa
ESBL	Extended-spectrum beta-lactamase
PYO	Person's years of observation
API 20E	Analytical Profile Indexing 20E
SERU	Scientific and Ethics Review Unit
STM	*Salmonella* Typhimurium
WGS	Whole genome sequencing
NGS	Next Generation Sequencing
cOR	Crude Odds Ratio
aOR	Adjusted Odds Ratio
ND	Not determined
CI	Confidence Interval

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ABSTRACT

Non-Typhoidal *Salmonella* (NTS) presents a pressing global health challenge, notably in low- and middle-income countries. In Kenya, NTS contributes to a substantial proportion of bacteremia cases in both children and adults, particularly affecting children under five in urban informal settlements. Simultaneously, the emergence of antimicrobial resistance (AMR) among non-Typhoidal *Salmonella* strains is a concerning public health issue. This study aimed to investigate factors associated with NTS disease and characterize antimicrobial-resistant non-Typhoidal *Salmonella* in children under five in Nairobi's Mukuru informal settlement. Participants with fever ≥ 38° C with or without diarrhea were enrolled from four outpatient healthcare facilities. Fecal and blood samples underwent culture, serotyping, antimicrobial sensitivity testing and PCR. Out of 3,071 participants 43 tested positive for NTS, with *Salmonella* Enteritidis and Typhimurium isolates accounting for 1.4% of the cases. Notably, some isolates exhibited resistance to first-line antibiotics, including ampicillin and sulfamethoxazole-trimethoprim, with resistance proportions of 9.3 %(4/43). The highest level of resistance was identified in *Salmonella* Typhimurium, with proportions of 16.7% for trimethoprim-sulfamethoxazole resistance and 22.2% for ampicillin resistance. Furthermore, 8% of *Salmonella* Enteritidis and 16.7% of *Salmonella* Typhimurium isolates demonstrated resistance to azithromycin. Although nalidixic acid resistance was noted in 8% of *Salmonella* Enteritidis isolates, these strains remained susceptible to ciprofloxacin, albeit with the potential for decreased susceptibility. Importantly, most non-Typhoidal *Salmonella* isolates remained susceptible to recommended third-generation cephalosporins, particularly ceftriaxone and cefotaxime. Molecular analysis of 11 isolates displaying phenotypic resistance to one or more antibiotic classes revealed the presence of the bla_{TEM} gene responsible for β-lactam resistance in 3 out of the 11 isolates, representing 27.3%. Interestingly, all of these bla_{TEM}-positive isolates were *Salmonella* Typhimurium. Demographically, the study indicated that infection rates were highest in children aged 12 to 24 months (2.1%) and in male children (1.7%). Nonetheless, statistical

analysis found no significant associations between NTS occurrence and gender, age, or contact with animals. Using drums and other open containers for water storage potentially predicted infection with NTS (2.0%; OR = 1.95, p = 0.040). Additionally, the proportion of children infected with *Salmonella* Enteritidis and Typhimurium was higher in households that did not treat water before drinking (1.8%) compared to those who treated their water (1.0%; OR = 0.85, p = 0.106). Lastly, having malignancy as a comorbidity was predictive of contracting NTS in children (33.3%; OR = 39.10, p = 0.003). This study highlights the importance of improving WASH infrastructure to reduce risk factors associated with transmission of non-Typhoidal *Salmonella* in the community. In the short-to medium-term, there is need for introduction of vaccine in the prevention and control of NTS. It also emphasizes the importance of using antibiotics prudently and continually monitoring the antimicrobial resistance of non-Typhoidal *Salmonella*.

CHAPTER ONE

1.1 Introduction

In sub-Saharan Africa, non-Typhoidal *Salmonella* (NTS) is the most common cause of bacteraemia (Feasey et al., 2012). The disease is often self-limiting gastroenteritis; however, the infection can become invasive (Stanaway et al., 2019). *Salmonella enterica* serovars Typhimurium and Enteritidis are the principal agents for invasive non-Typhoidal *Salmonella* infection. (Kariuki et al., 2015). In African sub-Saharan region young children (175–388 cases per 100,000) and adults who are HIV-positive (2000–7500 cases per 100,000) have a high prevalence of NTS bacteraemia (Profeta et al., 1985). Malnutrition in children and co-infection with malaria could also be to blame (Brent et al., 2006). Globally, it is estimated that > 93 million cases of non-Typhoidal *Salmonella* disease occur annually (Tichenor & Sridhar, 2019).

Over the past 20 years, several African countries have seen the emergence of multidrug-resistant NTS strains to antibiotics used as a first line of treatment, such as kanamycin, chloramphenicol, trimethoprim-sulfamethoxazole, and ampicillin. Previous reports of multi-drug resistance in NTS in Kenya and Malawi have made treatment and management options for the disease difficult (Bryce et al., 2005). One of the main causes of iNTS illness in Africa now is *S.* Typhimurium, sequence type 313, a unique evolutionary lineage. A recent study (Kariuki et al., 2020) showed isolation of sequence type 313 with both lineages 1 and 2. Lack of suitable diagnostic techniques to help control these multidrug-resistant bacteria is a major contributor to treatment failure and complications.

The most cases per year (1.9 million cases) are found in children who are 5 years old and below (Marks et al., 2015; Murray et al., 2012), with an associated case fatality of 20–25% (Feasey et al., 2015). Over 34% of Kenyans reside in urban areas with over 50% of this population residing in 5% of the country's residential area's comprising informal settlements. The informal settlements have inadequate access to clean water, electricity, solid waste management, sanitary amenities, and proper drainage (Olack et al., 2014). These

socioeconomic factors likely contribute to a high incidence of diarrheal diseases and mortality among children (Mutisya et al., 2011).

1.2 Statement of the Problem

Mukuru is an informal settlement in Nairobi characterized by poor infrastructure and sanitation, improper sewer disposal, and insufficient water supply and is a hotspot for NTS disease both invasive and non-invasive (Kariuki et al., 2019). These components generate an environment where enteric infections and other pathogens associated with sanitation can spread quickly through contaminated food and water (Olack et al., 2014). Children under 5 years are often infected by iNTS within the Mukuru informal settlement (Kariuki et al., 2006).

In Africa, the emergence of multi-drug resistance NTS has coincided with an increase in the prevalence of invasive NTS (Gordon et al., 2008). This poses major challenges in the ability to manage NTS infections, especially with the reduced susceptibility to recommended antibiotics for treatment. Therefore, it is crucial to conduct thorough surveillance to get information on the temporal variations in NTS serotype diversity and their antimicrobial sensitivity profiles.

Numerous studies have revealed that a significant amount of *Salmonella* transmission occurs as a result of contamination along the food chain, such as that caused by the slaughter of livestock, the gathering of vegetables as well as other food crops, and the cooking practices in homes (Zaidi et al., 2006). NTS disease epidemiology, mechanisms of transmission, circulating serotypes, genotypes and social economic risk factors are poorly understood in informal settlements.

1.3 Justification

Since NTS is endemic in Mukuru informal settlements, identifying the risk factors within this community was important for informing the setting up and putting into action targeted public health strategies to minimize disease prevalence as well as transmission. The data obtained also reveal whether the existing measures put in place to curb NTS disease in informal settlements are effective over time. This study also investigated the effectiveness of the recommended drugs of treatment for NTS including fluoroquinolones and Beta lactams. It's important to have data on antimicrobial resistance genotypes and phenotypes in order to inform clinical care, update treatment recommendations, and guide public health measures.

1.4 Research Questions

1. What are the serotypes and antimicrobial susceptibility profiles of non-Typhoidal *Salmonella?*
2. What are the risk factors associated with non-Typhoidal *Salmonella* infection in children aged 0-5 years in selected study sites in Mukuru slums?
3. What are the resistance genes (ESBLs) present in resistant non-Typhoidal *Salmonella* isolates?

1.5 General Objectives

To assess the risk factors associated with non-Typhoidal *Salmonella* infection and determination of the serotypes, antimicrobial resistance phenotypes and genotypes in children aged 0-5 years in selected study sites in Mukuru informal settlement.

1.6 Specific Objectives

1. To evaluate the serotypes and antimicrobial susceptibility profiles of non-Typhoidal *Salmonella*.
2. To assess the risk factors associated with non-Typhoidal *Salmonella* infection in children aged 0-5 years in selected study sites in Mukuru slums.
3. To identify ESBLs resistance genes present in resistant non-Typhoidal *Salmonella* isolates.

CHAPTER TWO: LITERATURE REVIEW

2.1 *Salmonella enterica* serovars

There are two species of *Salmonella, Salmonella enterica,* and *Salmonella bongori*, which are both members of the Enterobacteriaceae family. As an infrequent opportunist in humans, *S. bongori* is primarily found in cold-blooded species (Doolittle et al., 1996). According to biochemical, antigenic, and serological traits, *Salmonella enterica* is classified more into subspecies (I to VI) and serotypes or serovars. Over 2500 serotypes have been reported, with the *S. enterica* subspecies holding almost all of the serotypes known to be pathogenic to humans (Haeusler & Curtis, 2013). Well-known pathogens in subspecies I include *Salmonella* serotypes Typhimurium, Typhi, and Paratyphi, as well as *Salmonella* serotype Enteritidis. *Salmonella* is classified clinically as either Typhoidal (includes *S*. Typhi and *S*. Paratyphi) or non-Typhoidal *Salmonella* (*Salmonella* Typhimurium and *Salmonella* Enteritidis). In the majority of immunocompetent people in developed settings, non-Typhoidal *Salmonella* (NTS) serovars cause self-limiting enterocolitis (Acheson & Hohmann, 2001). However in sub-Saharan Africa, NTS often causes invasive disease in HIV-positive people and young children, defined by primary bacteraemia and frequently without signs of gastrointestinal infection (Raucher et al., 1983). With varied invasive virulence in humans, invasive NTS (iNTS) disease in humans can be caused by hundreds of NTS serovars (Jones et al., 2008). The most frequent cause of invasive non-Typhoidal *Salmonella* disease in African sub-Saharan region is the NTS serovars Typhimurium and Enteritidis, even though they are not highly invasive in industrialized settings.

2.2 The burden of NTS

Africa's Sub-Saharan area is where the iNTS disease is most prevalent. In 2010, 1.9 million cases of the iNTS disease were thought to have occurred out of an estimated 3.4 million cases worldwide (Deen et al., 2012). Host risk factors have a significant impact on the epidemiology of the iNTS infection in sub-Saharan Africa (Ao et al., 2015).There is a strong

association between the disease, malaria and malnutrition in children and infants (Tabu et al., 2012) along with adult HIV infection (Vugia et al., 1993). The incidence of iNTS and related mortality are likely to be influenced by the emergence of resistance to antibiotics across the continent (Gordon et al., 2010). In sub-Saharan Africa, the prevalence of iNTS is highest in children and young adults (Martin, 2012). Preventive measures, such as the development of a potential non-Typhoidal *Salmonella* vaccine, should mainly focus on these groups. Estimates of iNTS-related diseases and deaths around the globe, including the African sub-Saharan region, are probably underestimated (Feasey et al., 2012). Although it is crucial for finding cases and tracking iNTS trends, invasive bacterial illness surveillance is scarce in regions where disease is prevalent (Crump, 2012). Antimicrobials suitable for the treatment of iNTS must be included in guidelines for the empiric management of sepsis. Many strains of iNTS in sub-Saharan Africa are now resistant to the standard first-line medications, such as ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol (Reddy et al., 2010). *Salmonella* Typhimurium ST313 may have adapted to immunosuppressed people, notably those with HIV, in sub-Saharan Africa is where there is evidence that it has multi-drug resistance (Pulford et al., 2021a).Controlling iNTS will likely also require the prevention of and control of the host factors that make it likely to occur. People in their younger age groups and young adults are most at risk in groups with high HIV seroprevalence, while children under the age of five are most at risk for iNTS in regions with high malaria rates (James et al., 2018). Annually, there are 3.4 million cases of invasive non-Typhoidal *Salmonella* (iNTS) illness, ranging from 2.1 to 6.5 million cases, resulting in an overall incidence of 49 cases per 100,000 population, with a range of 30 to 94. Transmission of non-Typhoidal *Salmonella* disease has become a public health issue that NTS is becoming more common in industrialised countries (Weinberger & Keller, 2005). The Foodborne Diseases Active Surveillance Network (Food Net) in the US established that NTS infections were the most frequently reported condition (17.6 cases per 100,000 people), and the incidence has remained unchanged since the surveillance program was started in 1996(Crump et al., 2011). According to Food Net data from 1996- 2005, among foodborne bacterial pathogens, NTS is found to be the most common cause of death (39%) with the

highest mortality occurring in those over the age of 65 and the highest incidence occurring in children under the age of five (69.5 infections per 100,000 children) (Barton Behravesh et al., 2011). Inadequate food storage or insufficient preparation of food products were frequent factors associated with outbreaks, as well as coming into touch with contaminated people or animals (Lynch et al., 2006). Humans may contract non-Typhoidal *Salmonella* by contact with animals, drinking contaminated water, non-animal food products, and consumption of food animal products. The widespread production and distribution of food products spread pathogens rapidly throughout communities (Dione et al., 2011). In industrialized countries, farm animals serve as the primary reservoir for NTS, which is spread through their contaminated products (Dione et al., 2011). Public health officials face a challenge in controlling infection because non-Typhoidal *Salmonella* is naturally present in several wild animals as well as in chicken, caprine, ovine, swine, reptiles, amphibians, birds, domesticated rodents, dogs and cats (Dione et al., 2011). Attendance at daycare centers and contact with cats or reptiles were linked to childhood infections (Younus et al., 2007). Infants may be affected by NTS infections associated with pet transmission, which may cause invasive illness and severe issues (Swanson et al., 2007).

2.3 Epidemiology of non-Typhoidal *Salmonella* disease

Non-Typhoidal *Salmonella* is expected to result in 155 000 fatalities and 94 million cases of gastroenteritis per year (Majowicz et al., 2010). Approximately 80% of all human instances of food-borne illness are caused by *S.* Enteritidis and *S.* Typhimurium, according to SalmSurv (a network for monitoring food-borne illnesses supported by the World Health Organization). Non-Typhoidal *Salmonella* causes self-limiting enterocolitis in immunocompetent individuals from high-income countries; secondary bacteremia happens in up to 5% of patients, with attributed mortality of 1-5% (Crump et al., 2015a). However, immunocompromised individuals are more likely to develop primary NTS bacteremia, and their mortality is much higher (up to 21% in certain case series)(Dhanoa & Fatt, 2009). In contrast, NTS is now acknowledged as a significant contributor to severe febrile illness in low-income countries in both adults and children in studies of bacteremia in sub-Saharan Africa. NTS disease has a distinct bimodal age distribution, with the highest risk groups

being adults in their thirty's or forty's and infants aged 6 to 36 months (Feasey et al., 2012). More than 20% of iNTS cases in children and adults result in death, which is higher than that of high-income countries. Asia does not experience the high prevalence of NTS that exists in sub-Saharan Africa (Crump et al., 2015a). Ingestion of contaminated food items or coming into proximity with animals are ways in which NTS can be transmitted. Farm animals serve as the primary NTS reservoirs in industrialized nations, and large-scale food distribution and production increase tainting of the food supply chain, which accelerates the spread of NTS to local and international communities. Children can also contract NTS through contact with live poultry, feces from animals, and reptiles (Crump et al., 2015a).

2.4 Risk Factors for infection with non-Typhoidal salmonellosis

The informal settlements in Nairobi where this study is being conducted are notable for their high population density, poor sanitation, and unstable water supply. These components generate an environment where enteric infections and other pathogens associated with sanitation can spread quickly through contaminated food and water (Olack et al., 2014). Globally, approximately 90 million cases of diarrhea-related illnesses are thought to be caused annually by *Salmonella* spp., with 85% of those cases associated with foodborne sources (Chlebicz & Śliżewska, 2018). Numerous studies have revealed that a significant amount of *Salmonella* transmission occurs as a result of food chain contamination, such as that caused by slaughtering of cows, eating raw vegetables, plus other food crops collection, and the putting together of food in homes (Breiman et al., 2012). Outbreaks were highly correlated with crowded living conditions and poor sanitation, according to earlier research conducted in Africa and Asia (Breiman et al., 2012). Investigations from Bangladesh (Dewan et al., 2013)and Kenya (Mbae et al., 2020a), found that boys were statistically much more likely to have typhoid infection than girls. This showed that boys were more likely to consume tainted food and water away from the house due to their tendency for playing in slum areas with poor sanitation. In African children, iNTS disease risk factors are more complex, with HIV infection, malnutrition (Muthumbi et al., 2015), and malaria (Scott et al., 2011) all predisposing to disease. Anemia and sickle cell disease are two additional important host factors (Kariuki et al., 2020a). Malnutrition has a significant role in adult

infection in urban areas where iNTS is endemic, particularly where exposure to malaria is moderate (Verani et al., 2015). HIV infected individuals are also at higher risk of developing invasive NTS disease morbidity (Dhanoa & Fatt, 2009). *Salmonella* infections are frequently contracted by consuming contaminated food that is derived from animals, coming into contact with diseased animals, or being around contaminated environments (Patrick et al., 2010). Young children may have different sources of salmonellosis than older populations. Having a young child in a shopping cart adjacent to meat or poultry is one of the identified potential risk factors for salmonellosis in young children, exposure to reptiles, consuming powdered infant formula, attending a daycare facility with infected children, consuming eggs, undercooked ground beef, chicken or animal produce, family transmission of *Salmonella* and traveling abroad were also found to be potential risks (Marcus et al., 2007). These pediatric salmonellosis risk factors may differ based on lifestyle, food habits, and environmental conditions in different geographical regions. NTS isolates have been found in environmental sources such as soil and water, and they often survive for extended periods there (Baudart et al., 2000). There has also been evidence of variation of seasons by serovar, when compared to the months of fall (15.3%), winter (13.9%), and spring (23.6%), the number of Enteritidis cases was substantially higher in the summer (47.2%) (P-value = 0.0001) (Mukherjee et al., 2019).

2.5 Treatment and management of non-Typhoidal *Salmonella*

Efficient clinical management of non-Typhoidal *Salmonella* disease includes the provision of supportive care and treatment with antimicrobial drugs with enough anti-*Salmonella* action and penetration into tissues harboring the bacteria. Azithromycin, fluoroquinolones, trimethoprim-sulfamethoxazole and third-generation cephalosporins are among antibiotics that have activity against NTS. Despite showing potent in vitro activity, aminoglycosides are not advised due to their poor clinical efficacy (Haeusler & Curtis, 2013). Third-generation cephalosporins like ceftriaxone can penetrate cells, and their intracellular activity depends on the extracellular concentration (Chiu et al., 1999). Azithromycin and fluoroquinolones both concentrate in polymorphonuclear cells and macrophages (Parnham et al., 2014). When compared to plasma, azithromycin concentrations in phagocytes are at

least 200 times higher (Parnham et al., 2014). They work better in comparison to other antibiotics, like beta-lactams, at eliminating NTS that is intracellular, according to in vitro experiments (Chiu et al., 1999). Tigecycline and carbapenems are examples of broad-spectrum antibiotics with activity against multidrug-resistant NTS (Capoor et al., 2009). In vitro tests have shown that carbapenems are effective against *Salmonella* species, and high intracellular concentrations of meropenem have been reported in human macrophages (Cuffini et al., 1993). However, there is little experience clinically with carbapenems for treatment of infections caused by iNTS. There is a wide variety of management recommendations in the literature for NTS gastroenteritis and iNTS (Faulder et al., 2017). For older, otherwise healthy children, prior treatment with antibiotics before receiving results for NTS gastroenteritis is typically not advocated, but it is frequently indicated for newborns younger than 3 months of age and immunocompromised patients (Onwuezobe et al., 2012). Antibiotics should be given to children who have invasive diseases or suspected or confirmed NTS bacteremia. Following the resolution of diarrhea and vomiting, children having indicative gastroenteritis caused by NTS should not attend school or daycare before 24 hours end. Contacts without symptoms do not need to be excluded. Given the possibility of extended excretion and asymptomatic carriage, further stool testing is recommended.

2.6 Emergence of antimicrobial resistance and challenges in the treatment and management of NTS

Increasing AMR in NTS is a serious global issue. The effects of AMR are felt more in low and middle-income countries as alternative drugs for the successful treatment of life-threatening invasive illnesses are decreasing fast. This poses major challenges to treatment and management options. Recently Kenya reported emergence of MDR ST313 resistant to 3rd generation cephalosporins mediated by the extended-spectrum beta-lactamases (ESBL) (including ceftriaxone) (Kariuki & Onsare, 2015). Additionally, resistance to fluoroquinolones (both plasmid and chromosomally encoded) and, more recently, azithromycin (AZI) is becoming increasingly prevalent (Van Puyvelde et al., 2023). Kenya also saw the emergence of multi drug resistant *S*. Enteritidis ST11, that's linked to severe invasive illness (Kariuki et al., 2019a) and Malawi (Feasey et al., 2016) although ESBL-

producing strains have not yet been reported. Recently, *S.* Dublin ST10 has been connected to West African resistance, the two most common strains of NTS that are invasive and currently present in sub Saharan Africa are *S.* Typhimurium ST313 and *S.* Enteritidis ST11 (Kariuki et al., 2019a). These strains carry an incF plasmid (pSLT-BT) with the notorious *Tn21* transposon and a large number of resistance genes. The *gyrA* gene mutation has been linked to a lack of susceptibility to fluoroquinolones (Park et al., 2021). Multiple sub Saharan Africa countries, have now reported cases of the multidrug-resistant *S.* Typhimurium ST313 strain like Kenya (Kariuki et al., 2015a), Malawi(Ao et al., 2015), Central African Republic, Burkina Faso, Senegal, (Pulford et al., 2021b) DRC, (Falay et al., 2016) Nigeria, (Obaro et al., 2015)Ghana, (Aldrich et al., 2019) South Africa, and Mozambique, (Crump & Heyderman, 2015), where iNTS is endemic and causes septicemia without gastroenteritis.

It is extremely concerning that MDR rates are increasing in iNTS, and the problem is significantly worse in Sub Saharan Africa region where other working treatment options are losing their effectiveness. In Kenya, there have been findings of multi drug resistant iNTS at high levels (Gordon et al., 2008) and in other regions of Sub Saharan Africa, such as DRC (Vandenberg et al., 2010), Ghana (Aldrich et al., 2019) and Uganda (Bachou et al., 2006) posing a major challenge to available choices for severe disease management and treatment. High MDR ST313 carriage rates in Kenya's endemic areas are expected to act as a source for community-wide infection spread to vulnerable groups, creating significant challenges for treating and preventing iNTS infection (Kariuki et al., 2020b). Due to the lack of microbiology labs that can do culture in most settings, the gold standard test, treatment is frequently decided upon based solely on symptoms. Because of how similar symptoms are to those of other febrile illnesses like malaria, they are frequently worsened by or mistaken for such conditions. To enhance patient results and evaluate disease burden, there is a need to increase access to diagnostic services. Since rapid diagnostic methods are not yet available for the identification of NTS, the evolution of such tests will significantly aid in the research and medical care of NTS illness in African Sub-Saharan region. The various types of NTS serotypes makes it challenging to create new diagnostic techniques, although using

lipopolysaccharide antigens that are locally selected is likely to be useful (Crump & Heyderman, 2015).

2.7 Resistance to extended spectrum βeta lactams

Current recommendations for antimicrobial therapy in cases of enteric infections caused by non-Typhoidal *Salmonella* (NTS) involve prescribing third-generation cephalosporins for children and fluoroquinolones as an alternative treatment for adults facing infections resistant to third-generation cephalosporins. Nevertheless, the global issue arising from the widespread use of cephalosporins, leading to the emergence and dissemination of Extended-Spectrum Beta-Lactamase (ESBL)-producing NTS strains, poses significant and serious consequences (Sedrakyan et al., 2020). In Armenia and Georgia among 57 Multi-Drug Resistant (MDR) NTS strains, 52 were explored for ESBL resistance and 37 out of these 52 isolates (64.91%) displayed an ESBL-producing phenotype. All ESBL-producing isolates were identified as *S.* Typhimurium, indicating a high prevalence of ESBL-producers among this serovar (Sedrakyan et al., 2020). ESBL-producing isolates demonstrated resistance to ampicillin, ceftriaxone, and nalidixic acid (Van Puyvelde et al., 2023). In the case of non-Typhoidal *Salmonella*, resistance to cephalosporins is primarily attributed to the synthesis of extended-spectrum β-lactamases (ESBLs). The majority of ESBLs identified in NTS belong to the TEM and OXA β-lactamase families (Crump et al., 2015b). Additionally, other categories such as INT and CTX-M types have also been documented (Crump et al., 2011).

2.8 Laboratory diagnosis of non-Typhoidal *Salmonella*

Culture is the most common phenotypic method of detecting *Salmonella* spp. Media of choice is usually MacConkey media where it appears pale and medium colonies and does not ferment lactose. Xylose Lysine Deoxycholate Agar is also used where NTS appears brick red with or without black centers. A panel of several tests are used for biochemical testing. These tests are conducted using media including Triple Sugar Iron media, Simmon Citrate media, Sulphur Indole Motility media, and Urea media, or the commercially available kit and system for identification known as API 20E. Thereafter, serology testing is done. For NTS the antisera used include group B factor 4(1, 4) and group D1 factor 9 (1, 9) then phase

1 and phase 2, which include (I) and (1, 2) for *S.* Typhimurium and (gm) for *S* Enteritidis. The presence of agglutination with any of the commercial antisera confirms a positive reaction according to the Kauffmann-White classification scheme. Molecular methods such as Polymerase chain reaction are used for the detection of AMR-associated determinants as well as virulence genes. To determine the complete DNA sequence of an organism including AMR genes whole genome sequencing is performed.

CHAPTER THREE: METHODOLOGY

3.1 Study site

The study was conducted in Mukuru informal settlement. It is located 20 km east of Nairobi city, home to about 700,000 people and is divided into eight villages. This study focused on two of these villages, Mukuru Kwa Njenga and Mukuru Kwa Reuben, which have a combined population of approximately 150,000. Families stay in small corrugated iron huts that measure about 10 feet by 10 feet, and large families with 4-8 members are crowded into these tight living spaces.

It's characterized by limited access to clean water and proper sanitation facilities and poor waste disposal and water drainage. Samples were collected from patients who visited the four health facilities in the study sites namely; Reuben Health Centre, Medical Missionaries of Mary (Mukuru Health Centre), Mama Lucy Kibaki hospital (referral hospital) for patients who visited the three clinics in Mukuru, and Municipal County council hospital.

3.2 Study population

These were children under 5 years who visited any of the 4 health facilities in the study sites which comprised of, Reuben Health Centre, Medical Missionaries of Mary (Mukuru Health Centre), Mama Lucy Kibaki hospital (referral hospital), and Municipal County council, and fell within the inclusion criteria.

3.3 Study design

This was a cross-sectional study involving prospective sample collection from index patients (children under the age of 5 years presenting with fever (≥ 38° C) for more than 24 hours with or without diarrhea.

3.4 Determination of sample size

The sample size was determined using previous isolation rates within Mukuru informal settlement.

Fisher *et al.*, 2005

$N = Z^2 P (l-P)/d^2$

Where;

N = Minimal sample size:

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

P = In this case is 4% iNTS isolation rate (Kariuki et al., 2019)

d = degree of precision (0.8%)

$n = (1.96)^2 0.04(1-0.04)/ (0.008)^2 =$

n = **2305 participants**

3.5 Method of sampling

This study utilized purposive sampling using the following inclusion and exclusion criteria;

3.5.1 Inclusion criteria.

1. Children below the age of 5 years
2. Children presenting with fever (≥38° C axillary) for >24 h with or without diarrhea in any of the 4 health facilities in the study site.

3.5.2 Exclusion criteria

1. Children whose parents/ guardians did not consent.
2. Children with prior antibiotic treatment.
3. Children above 5 years of age.

3.6 Recruitment of participants

Eligible participants who fit the inclusion criteria were identified after screening by the clinicians in the health facilities. The purpose of the study was then explained to the parents/guardians of the participants before obtaining a signed consent.

3.7 Collection and transport of rectal/fecal swabs

Rectal/fecal swabs were collected using sterile cotton swabs and placed in Cary-Blair transport media from Oxoid, Basingstoke UK. They were later transported inside a cool box to the Centre for Microbiology Research-*Salmonella* and Antimicrobial Resistance Surveillance Unit 1 (CMR-SASU1) laboratory located at the Kenya Medical Research Institute where they were processed.

3.8 Collection and transport of blood specimen

Venipuncture was used to collect 3-5ml of blood from the recruited patient's arm and the blood was then aseptically transferred into Bactec blood culture media (BD, Franklin Lakes; New Jersey). The blood was afterwards transported with warm packs to the Centre for Microbiology Research-*Salmonella* and Antimicrobial Resistance Surveillance Unit 1 (CMR-SASU1) laboratory located at the Kenya Medical Research Institute for processing.

3.9 Sample processing

All media for bacteriological analysis was made two days in advance before sampling. *E. coli* 25922 and *Salmonella* ATCC Strains were used for quality control and quality assurance of the media. A sterility check of the media was done to ensure there was no contamination. The flow chart in Figure 1 illustrates how samples were processed in the laboratory

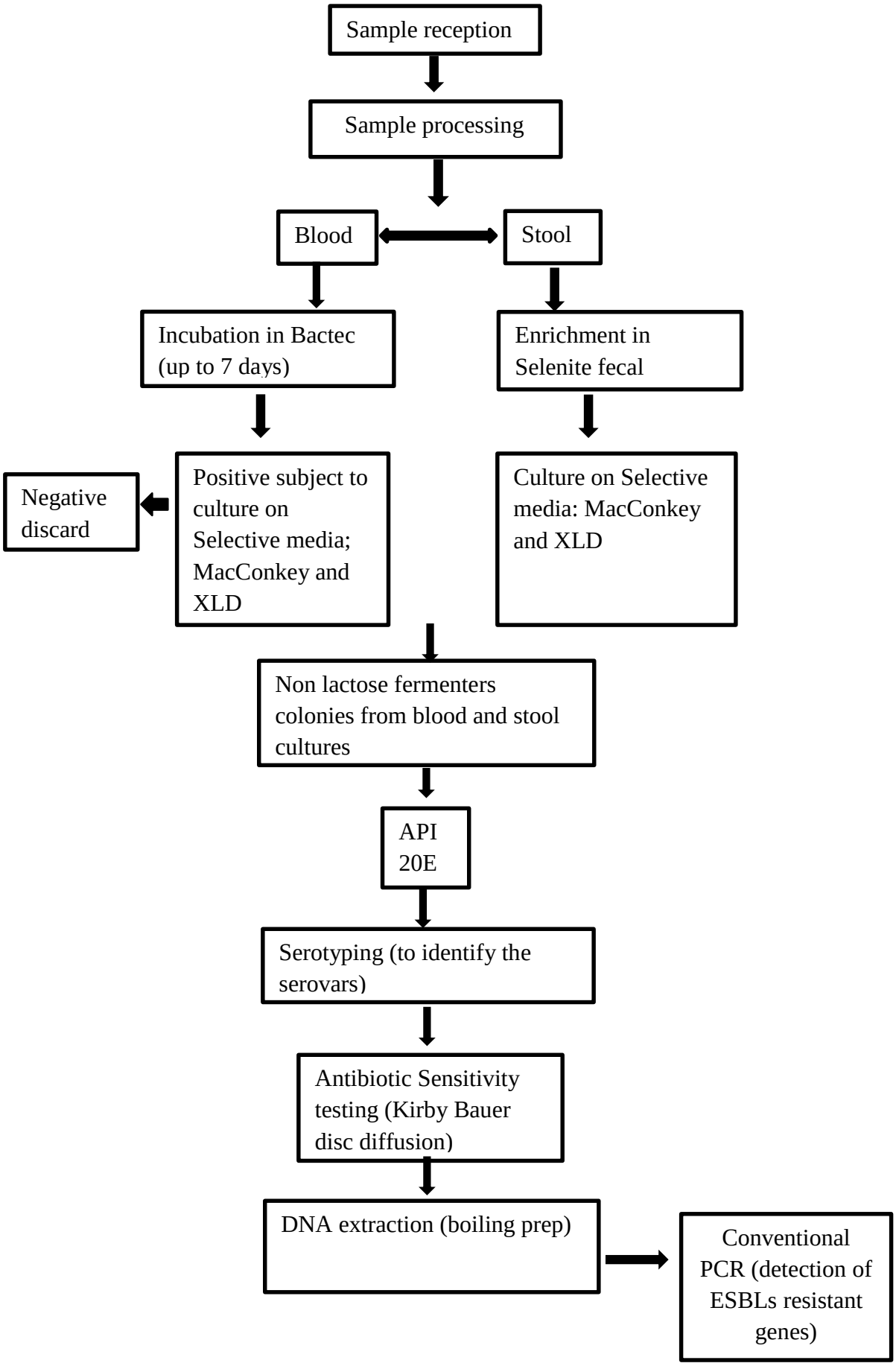


Figure 1: Flow chart illustrating sample processing

Objective 1: To determine the serotypes and antimicrobial susceptibility profiles of Nontyphoidal Salmonella infection among children under 5 years of age in Mukuru slums.

3.10 Serotyping

All *Salmonella* species were identified using biochemical test API 20E, their serotype was then determined by slide agglutination with commercial *Salmonella* antisera (Remel, Thermo Fisher Scientific, MA, USA) as categorized by the Kauffmann-White system. Polyvalent O antisera was used first followed by the monovalent antisera comprising of O9 and gm for *S.* Enteritidis and 04, I (monophasic); 1 and 2(diphasic) for *S.* Typhimurium. A distinct isolated bacterial colony was carefully selected from the Muller Hinton agar (MH, Oxoid, Basingstoke UK) plate, and was emulsified with one drop of normal saline that is sterile on a slide. A drop of the test antisera was added and swirled slightly and observed on a dark background for agglutination. Agglutination confirmed the serovars. Negative controls were also included for quality assurance.

3.11 Antibiotic Sensitivity Testing

Kirby–Bauer disc diffusion method was used to perform the antibiotic sensitivity testing. *E. coli* ATCC 25922 strain was utilized as the test quality control organism. Inoculums of the required turbidity standard (McFarland 0.5) were prepared. Sterile cotton swabs were used to swab the inoculum on the Mueller-Hinton agar plates forming uniform lawns of bacteria and then the plates were allowed to dry for 5 minutes. Fourteen antibiotic discs (Oxoid, UK) were then dispensed onto the agar plates using a disc dispenser each sample had 2 plates. The first plate had the following antibiotics-impregnated discs: ampicillin (AMP, 10 µg), 30 µg), ceftazidime (CAZ 30 µg), cefotaxime (CTX 30 µg), ceftriaxone (CRO, cefpodoxime (CPD 30 µg), sulfamethoxazole-trimethoprim (SXT 30 µg), and amoxicillin-clavulanate acid (AMC 30 µg) which was placed in the centre. The second plate comprised of nalidixic acid (NA 30 µg), azithromycin (AZM 30 µg), kanamycin (K 30 µg), tetracycline (TE 30 µg), gentamicin (CN 10 µg), ciprofloxacin (CIP 5 µg) and chloramphenicol (C 30 µg).

Sterilized forceps were used to press onto each disc on the agar. Thereafter, incubation of the plates at 37 °C was done for 18-20hrs. The diameters of the zones of inhibition were then measured and interpreted according to Clinical Laboratory Standard Institute (CLSI 2020) guidelines. *Escherichia coli* (ATCC-25922) strain was used as the control to test the antibiotic discs potency as well as the media quality.

Objective 2: To evaluate the risk factors associated with Non-Typhoidal Salmonella infection among children under 5 years of age in Mukuru slums

3.12 Administering questionnaires to obtain social-demographic data

Patients who matched the inclusion criteria were recruited in the study after their guardians gave written informed consent. A thorough history and physical examination were documented on a structured data form (Case Report Form) by a clinician from the health facilities in the study sites. A questionnaire was administered to the guardians which captured data on their background information, environmental risks, infrastructural risks, general wash, and social-economic risk factors. Epicollect 5 which is a mobile and web-based application was used to administer the questionnaires.

Objective 3: To identify the genetic determinants of resistance in Nontyphoidal Salmonella isolated from children under 5 years of age in Mukuru slums.

3.13 Extraction of total DNA from NTS resistant phenotypes.

Isolates that were resistant to multiple classes (>2 antibiotics) of drugs including fluoroquinolones and/or 3rd generation cephalosporin were regarded as multidrug-resistant. The resistant isolates DNA was then extracted for further molecular analysis using PCR. Bacteria were cultured on Muller Hinton agar at 37°C. DNA extraction from pure isolates was performed using a 12-minute boiling method. Briefly, an aliquot from the bacterial culture was collected using a 20µl sterile loop and transferred to an eppendorf tube containing 500µl of sterile PCR water. After 12 minutes of heating and subsequent cooling, the tubes were centrifuged at 14000 rpm for 5 minutes. The resulting supernatant, containing extracted DNA, was stored in a sterile tube at 4°C for PCR analysis.

3.14 PCR detection of Extended Spectrum Beta Lactams (ESBLs) resistant genes

Primers that were used for amplifying ESBL resistance genes are shown in **Table 1**. The primer sets included TEM, CTX-M and SHV for the detection of Beta lactams. In this multiplex PCR assay, a known positive control and a negative control containing PCR water in place of the DNA template were included. The PCR reaction was conducted using 2.5 µl of Taq 5x master mix (New England Bio Labs), along with 0.25 µl each of forward and reverse primers, 8.5 µl of PCR water and 1 µl of the DNA template. Subsequently, the reaction mixture (25 µl) was amplified under the appropriate conditions (**Table 1**).

Amplification conditions consisted of 30 cycles, with the initial denaturation lasting 2 minutes at 95 °C, followed by subsequent denaturation steps lasting 30 sec at 95°C. Primer annealing occurred for 30 sec, followed by a final extension of 5 minutes at 72 °C. The samples were then maintained at 4°C before undergoing electrophoresis.

Table 1: PCR Primers used for detecting Beta lactams and their annealing temperature conditions

Target gene	Primer name	Primer sequence	Annealing Temperature	Product size	References
bla TEM	TEM F	5-GCGGAACCCCTATTTG-3	50	865bp	(Hasman, et al, 2005)
	TEMR	5-TCTAAAGTATATATGAGTAAACTTGGTCTGAC-3			
bla SHV	SHV F	5'- TTCGCCTGTGTATTATCTCCCTG- 3	50	795 bp	(Hasman, et al, 2005)
	SHV R	5'- TTAGCGTTGCCAGTGYTCG- 3'			
bla CTXM	CTXM F	5-ATGTGCAGYACCAGTAARGTKATGGC-3	60	593 bp	(Hasman, et al, 2005)
	CTXM R	5-TGGGTRAARTARGTSACCAGAAYCAGCGG-3			

3.14 Gel electrophoresis

After obtaining the PCR products, they were electrophoresed for 30 minutes on a 5% agarose gel stained with SYBR-safe at a voltage of 100. Following electrophoresis, the amplified DNA bands were visualized using a gel imager (Azure 200 bio systems).

3.15 Ethical considerations

This study was approved by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (P244/03/2023) and Kenya Medical Research Institute Scientific and Ethics Review Unit (KEMRI/SERU/CMR/P00230-011-2022/4672). Confidentiality was observed during the study period and when it was completed. All collected samples were given special codes that could only be identified by the principal investigator.

3.16 Data management and analysis

Data collected both in the field and laboratory was entered into Epicollect5 a free-web based software using a smartphone. The isolates acquired from the study were given unique identifiers. The data obtained was protected with password access and only accessible to the Principal Investigator and Co-investigators. A descriptive statistical analysis of the data was done using SPSS version 28.0. Counts and percentages were used to present this data. Pearson chi-square was used to test significant associations between risk factors and non-Typhoidal *Salmonella* infections. The strength of association was measured using the odds ratio and its related confidence interval (95% CI). All significant risk factors (p-value ≤ 0.05) were adjusted for confounders and risk modifiers using multivariable binary logistic regression with backward conditional as the removal method. A significance level of ≤ 0.05 was utilized and all results were interpreted at a 95% confidence interval.

CHAPTER FOUR: RESULTS

4.1 Prevalence of non-Typhoidal *Salmonella*

A total of 3071 participants were recruited in the study. The serotypes isolated were *Salmonella* Enteritidis and *Salmonella* Typhimurium. The prevalence of *Salmonella* Enteritidis was 0.8 % (25/3071) while that of *Salmonella* Typhimurium was 0.6 % (18/3071).

4.2 Demographic characteristics of the study participants

The male gender had the highest number of participants (52.6%) compared to the females (47.4%). The majority of the children recruited were aged 12 to 24 months (33%) as shown in **Table 2**. Infection was highest in children aged 12 to 24 months (2.1%) and in male children (1.7%).

Table 2. Demographic characteristics of the study participants with and without NTS infection

		NTS		
Variables	**Total number of participants (N=3071)**	**Positive**	**Negative**	**p-value**
Gender				
Female	1456 (50.3%)	16 (1.1%)	1440 (98.9%)	0.149
Male	1615 (52.6%)	27 (1.7%)	1588 (98.3%)	-
Age in months				
<12 months	716 (23.3%)	8 (1.1%)	708 (98.9%)	0.765
12 to 24 months	1013 (33.0%)	21 (2.1%)	992 (97.9%)	0.212
25 to 36 months	550 (17.9%)	4 (0.8%)	546 (99.2%)	0.457
>36 months	792 (25.8%)	10 (1.3%)	782 (98.7%)	-

4.3 Distribution and positivity in relation to selected variables in households

Almost half of the participants (48.7%) said that they treat their drinking water before consuming it. And out of these, boiling the water was the most common method chosen (48.7%). In terms of water storage containers, jerry cans were the most common type (91%). Regarding toilet facilities, the majority of participants reported using shared flash toilets with other households (48.4%), while none resorted to using carrier bags as a toilet. When it came

to vegetable shopping, a significant proportion preferred purchasing from vendors (91.7%). As for domestic animals, cats (47.8%), chickens (29.9%), and dogs (24.3%) were the most common animals present in the households. Furthermore, the data demonstrated that a substantial portion of participants consistently practiced hand hygiene. This included handwashing after defecation (65.6%), before food preparation (61.5%), and prior to eating (76.7%), as detailed in **Table 3**. The number of children infected with *Salmonella* Enteritidis and *Salmonella* Typhimurium was significantly higher in participants who used a drum as a method of water storage (2.0%; $p = 0.040$) and in households that never washed their hands before eating (7.7%; $p = 0.039$). There was no significant association between the keeping of animals and infection with NTS (**Table 3**)

Table 3. Distribution and positivity in relation to selected variables in households

		NTS		
Variables	**Total number of participants(N=3071)**	**Positive**	**Negative**	**p-value**
Generally treat water before drinking				
Yes	1496 (48.7%)	15 (1.0%)	1481 (99.0%)	0.106
No	1575 (51.3%)	28 (1.8%)	1547 (98.2%)	-
Method of water treatment				
Boiling	1496 (48.7%)	22 (1.5%)	1474 (98.5%)	-
Water guard/ aqua tab	1379 (44.9)	11 (0.8%)	1368 (99.2%)	-
Filtration	304 (9.9%)	2 (0.8%)	302 (99.3%)	-
Types of water storage containers				
Tap	436 (14.2%)	3 (0.8%)	433 (99.2%)	0.241
Water pot	80 (2.6%)	1 (1.4%)	79 (98.6%)	1.000
Drum	1241 (40.4%)	25 (2.0%)	1216 (98.0%)	**0.040**
Bucket	1259 (41%)	19 (1.5%)	1240 (98.5%)	0.744
Jerry can	2795 (91.0%)	39 (1.4%)	2756 (98.6%)	0.782
Type of toilet used by the household				
Private flush toilet	494 (16.1%)	11 (2.2%)	483 (97.8%)	0.103
Private pit latrines (Shared)	25 (0.8%)	1 (4.3%)	24 (95.5%)	0.230
Public toilet (Fresh life)	365 (11.9%)	3 (0.9%)	362 (99.1%)	0.415
Flush toilets shared with other households	1486 (48.4%)	19 (1.3%)	1467 (98.7%)	0.773
Shared pit-latrines (Shared)	755 (24.6%)	8 (1.0%)	747 (99.0%)	0.334
Bush/river/open drain	9 (0.3%)	0 (0.0%)	9 (100.0%)	ND
Carrier bags	0 (0.0%)	0 (0.0%)	0 (0.0%)	ND
Grow in backyard	21 (68.4%)	0 (0.0%)	21 (100.0%)	ND
Buy from market	519 (16.9%)	8 (1.5%)	511 (98.5%)	0.856
Buy from shop	43 (1.4%)	0 (0.0%)	43 (100.0%)	ND
Buy from vendor	2816 (91.7%)	42 (1.5%)	2774 (98.5%)	ND
Wash and cook	1658 (54%)	22 (1.3%)	1636 (98.7%)	0.501
Eat raw salad after washing	574 (18.7%)	10 (1.7%)	564 (98.3%)	0.48
Buy and cook directly (from Mama Mboga)	1585 (51.6%)	24 (1.5%)	1561 (98.5%)	0.64
Households use waste containers	2927 (95.3%)	44 (1.5%)	2883 (98.5%)	ND

Location of the container for waste disposal				
Inside the house	418 (13.6%)	5 (1.1%)	413 (98.9%)	-
Outside the house	2653 (86.4%)	40 (1.5%)	2613 (98.5%)	-
Households wash hands after defecation				
Always	2015 (65.6%)	30 (1.5%)	1985 (98.5%)	0.447
Never/Don't know	21 (0.7%)	0 (0.0%)	21 (100.0%)	ND
Sometimes	1035 (33.7%)	12 (1.2%)	1023 (98.8%)	-
Households wash hands before food preparation				
Always	1889 (61.5%)	28 (1.5%)	1861 (98.5%)	0.765
Never/Don't know	98 (3.2%)	1 (1.1%)	97 (98.9%)	0.880
Sometimes	1087 (35.4%)	14 (1.3%)	1073 (98.7%)	-
Households wash hands before eating				
Always	2355 (76.7)	38 (1.6%)	2317 (98.5%)	0.158
Never/Don't know	15 (0.5%)	1 (7.7%)	14 (92.3%)	**0.039**
Sometimes	700 (22.8%)	6 (0.8%)	694 (99.2%)	-
Domestic animals				
Cattle				
Present	3599 (11.7%)	5 (1.5%)	354 (98.5%)	0.827
Not present	2712 (88.3%)	38 (1.4%)	2674 (98.6%)	-
Chicken				
Present	918 (29.9%)	10 (1.1%)	908 (98.9%)	0.350
Not present	2153 (70.1%)	32 (1.5%)	2121 (98.5%)	-
Goat				
Present	396 (12.9%)	4 (1.1%)	392 (98.9%)	0.625
Not present	2675 (87.1%)	40 (1.5%)	2635 (98.5%)	-
Dog				
Present	746 (24.3%)	5 (0.7%)	741 (99.3%)	0.093
Not present	2325 (75.7%)	37 (1.6%)	2288 (98.4%)	-
Cat				
Present	1468 (47.8%)	21 (1.4%)	1447 (98.6%)	0.908
Not present	1603 (52.2%)	22 (1.4%)	1581 (98.6%)	-
Pig				
Present	289 (9.4%)	3 (1.2%)	286 (98.8%)	0.719
Not present	2782 (90.6%)	39 (1.4%)	2743 (98.6%)	-
Sheep				
Present	193 (6.3%)	3 (1.7%)	190 (98.3%)	0.708
Not present	2878 (93.7%)	40 (1.4%)	2838 (98.6%)	-
Duck				
Present	206 (6.7%)	2 (1.1%)	204 (98.9%)	0.695
Not present	2865 (93.3%)	40 (1.4%)	2825 (98.6%)	-
Turkey				
Present	104 (3.4%)	0 (0.0%)	104 (100.0%)	ND
Not present	2967 (96.7%)	45 (1.5%)	2922 (98.5%)	-
Dove				
Present	55 (1.8%)	0 (0.0%)	55 (100.0%)	ND
Not present	3016 (98.2%)	42 (1.4%)	2974 (98.6%)	-
The presence of any animal in the compound	1843 (60.0%)	24 (1.3%)	1819 (98.7%)	0.429

ND=Not determined

4.4 Distribution and positivity in relation to the clinical characteristics

For clinical presentations, the majority of the participants had persistent fever (61.9%) while (18.6%) of them did not have a fever. Diarrhea was observed in 82.4% of the participants with only 3.1% of them having bloody diarrhea (**Table 4**). The occurrence of *Salmonella* Enteritidis and *Salmonella* Typhimurium was significantly higher in participants who had bloody diarrhea (4.6%; $p = 0.010$) and malignancy (33.3%; $p = 0.042$). There was no significant association between having NTS disease and experiencing presentations such as vomiting, abdominal cramps, headaches, diarrhea or dysuria.

Table 4: Distribution and positivity in relation to the clinical characteristics

		NTS		
Variables	**Total number of participants(N=3071)**	**Positive**	**Negative**	**p-value**
Fever				
Yes, continuous	1904 (62.0%)	34 (1.8%)	1870 (98.2%)	0.217
Yes, not continuous	596 (19.4%)	4 (0.7%)	592 (99.3%)	0.692
No	571 (18.6)	6 (1.0%)	565 (99.0%)	-
Cough				
Yes	1646 (53.6%)	20 (1.2%)	1626 (98.8%)	0.347
No	1425 (46.4%)	23 (1.6%)	1402 (98.4%)	-
Haemoptysis				
Yes	37 (1.2%)	0 (0.0%)	37 (100.0%)	ND
No	3034 (98.8%)	42 (1.4%)	2992 (98.6%)	-
Expectoration				
Yes	271 (8.8%)	1 (0.4%)	270 (99.6%)	0.166
No	2800 (91.2%)	42 (1.5%)	2758 (98.5%)	-
Vomiting				
Yes	1869 (60.9%)	22 (1.2%)	1847 (98.8%)	0.365
No	1202 (39.1%)	20 (1.7%)	1182 (98.3%)	-
Abdominal pain				
Yes	1580 (51.4%)	21 (1.3%)	1559 (98.7%)	0.506
No	1491 (48.6%)	24 (1.6%)	1467 (98.4%)	-
Distension				
Yes	93 (3.0%)	1 (1.2%)	92 (98.8%)	0.863
No	2978 (96.9%)	42 (1.4%)	2936 (98.6%)	-
Diarrhea				
Yes	2530 (82.4%)	38 (1.5%)	2492 (98.5%)	0.427
No	541 (17.6%)	5 (1.0%)	536 (99.0%)	-
Bloody diarrhea				
Yes	97 (3.1%)	4 (4.6%)	93 (95.3%)	**0.010**
No	2974 (96.9%)	39(1.3%)	2935 (98.7%)	-
Constipation				
Yes	43 (1.4%)	0 (0.0%)	43 (100.0%)	ND
No	3028 (98.6%)	42 (1.4%)	2986 (98.6%)	-
Dysuria				
Yes	34 (1.1%)	1 (3.2%)	33 (96.8%)	0.388

No	3037 (98.8%)	43 (1.4%)	2994 (98.6%)	-
Pass urine more frequently than usual				
Yes	37 (1.2%)	0 (0.0%)	37 (100.0%)	ND
No	3034 (98.9%)	42 (1.4%)	2992 (98.6%)	-
Headache				
Yes	412 (13.4%)	5 (1.1%)	407 (98.9%)	0.561
No	2659 (86.6%)	40 (1.5%)	2619 (98.5%)	-
Seizure				
Yes	14 (0.5%)	0 (0.0%)	14 (100.0%)	ND
No	3057 (99.5%)	43 (1.4%)	3014 (98.6%)	-
Sickle cell disease				
Yes	4 (0.1%)	0 (0.0%)	4 (100.0%)	ND
No	3067 (99.9%)	43 (1.4%)	3024 (98.6%)	-
Tuberculosis				
Yes	8 (0.3%)	0 (0.0%)	8 (100.0%)	ND
No	3063 (99.7%)	43 (1.4%)	3020 (98.6%)	-
Malignancy				
Yes	3 (0.1%)	1 (33.3%)	2 (66.7%)	**0.042**
No	3068 (99.9%)	43 (1.4%)	3025 (98.6%)	-
HIV/AIDS				
Yes	6 (0.2%)	0 (0.0%)	6 (100.0%)	ND
No	3065 (99.8%)	43 (1.4%)	3022 (98.6%)	-
Any form of infection				
Yes	2988 (97.3%)	42 (1.4%)	2946 (98.6%)	0.955
No	83 (2.7%)	1 (1.3%)	82 (98.7%)	-

ND=Not determined

4.5 Antimicrobial susceptibility patterns for *S*. Typhimurium

Resistance to various antibiotics was evident, with 22.2% demonstrating resistance to ampicillin, 16.7% to trimethoprim-sulfamethoxazole, 16.7% to azithromycin, and a substantial 44.4% to tetracycline. None of the isolates were multidrug resistant (combined resistance to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole). Additionally, reduced susceptibility to azithromycin was observed in 22.2% of cases as shown in **Figure 3**.

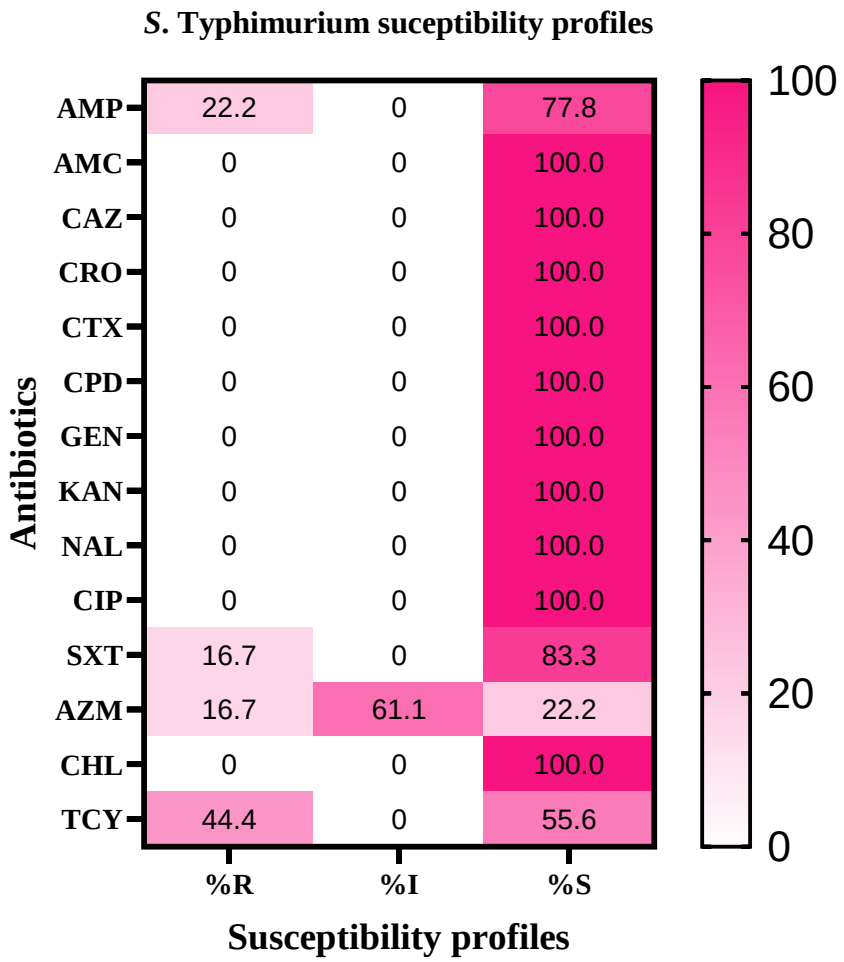


Figure 3: *S*. Typhimurium antimicrobial susceptibility profiles

N=18 isolates exposed to 14 antibiotics; Ampicillin (AMP), Amoxicillin Clavulanate (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefpodoxime (CPD), Gentamicin (GEN), Kanamycin (KAN), Nalidixic Acid (NAL), Ciprofloxacin (CIP), Sulfamethoxazole-trimethoprim (SXT), Azithromycin (AZM), Chloramphenicol (CHL), and Tetracycline (TCY), R-Resistant, I-Intermediate, S-Susceptible

4.6 Antimicrobial susceptibility patterns for *S*. Enteritidis

Most *Salmonella* Enteritidis isolates 92% (23/25) exhibited complete susceptibility to the antibiotics used, while a subset displayed varying levels of resistance and reduced susceptibility. Specifically, 8% were resistant to nalidixic acid, 4% to cefpodoxime, and 4% to azithromycin as shown in figure 4. Furthermore, 4% showed intermediate susceptibility to ceftriaxone and kanamycin, while 16% exhibited intermediate susceptibility to ciprofloxacin, and a substantial 88% displayed intermediate susceptibility to azithromycin (**Figure 4**).

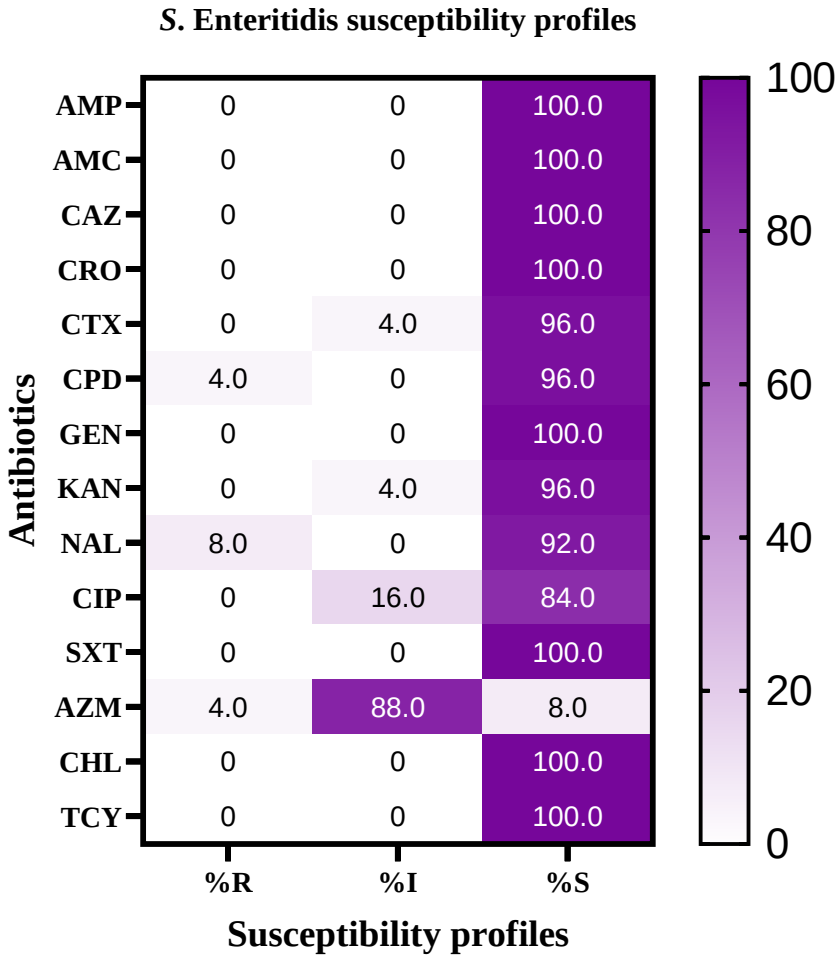


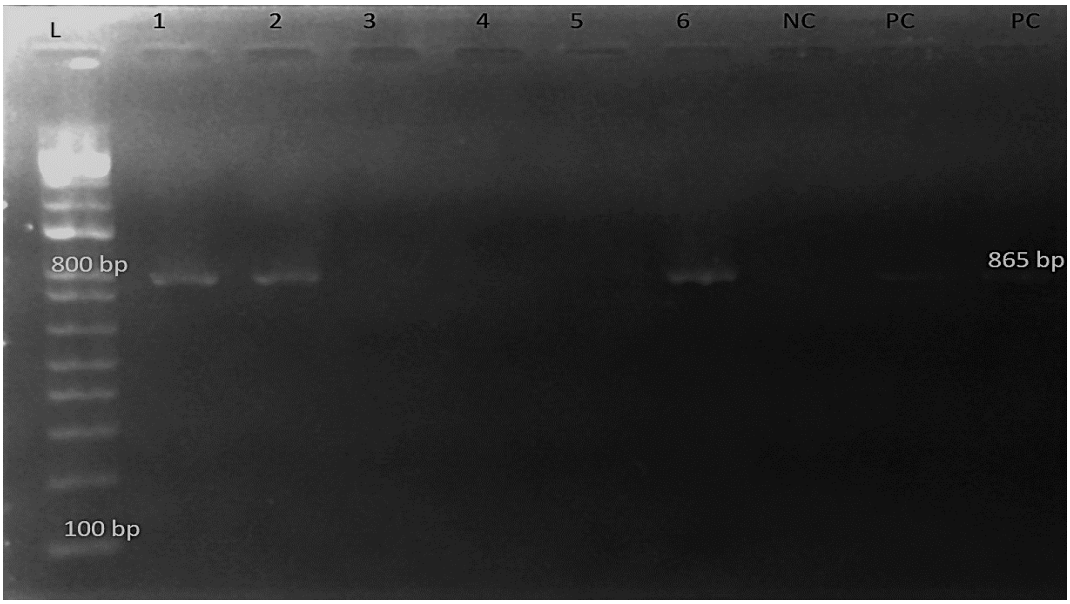
Figure 4: *S*. Enteritidis antimicrobial susceptibility profiles

N=25 isolates exposed to 14 antibiotics; Ampicillin (AMP), Amoxicillin Clavulanate (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefpodoxime (CPD), Gentamicin (GEN), Kanamycin (KAN), Nalidixic Acid (NAL), Ciprofloxacin (CIP), Sulfamethoxazole-trimethoprim (SXT), Azithromycin (AZM), Chloramphenicol (CHL), and Tetracycline (TCY), R-Resistant, I-Intermediate, S-Susceptible

4.7 PCR findings for ESBLs resistant genes

A total of 11/43 (25.6%) isolates were subjected to PCR to detect ESBLs resistance genes and 3/11 (27.3%) were found to have the resistance gene bla*TEM*. This gene was present in isolates number 1, 2 and 6 (**PLATE A**), all *S.* Typhimurium. None of the 11 isolates tested positive for the bla*SHV* and bla*CTX-M* genes (**PLATE B** and **PLATE C**).

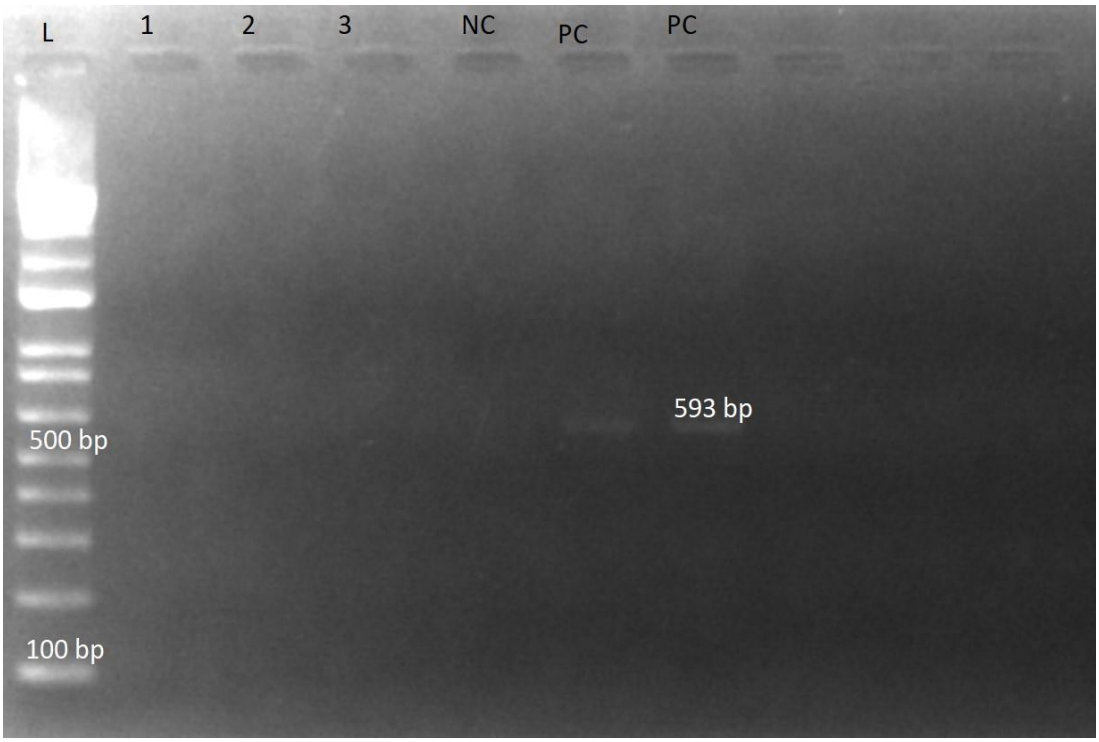
A



A (bla TEM) 865bp L: DNA Marker (Ladder); **1**: *S.* Typhimurium (031528*); **2**: *S.* Typhimurium (044043*); **3**: *S.* Enteritidis (038055); **4**: *S.* Typhimurium (022138); **5**: *S.* Typhimurium (048245); **6**: *S.* Typhimurium (022578*);

L-Molecular Weight Ladder(100bp); **NC**-Negative Control (PCR water); **PC**-Positive Control (consisting of known positive control strains)

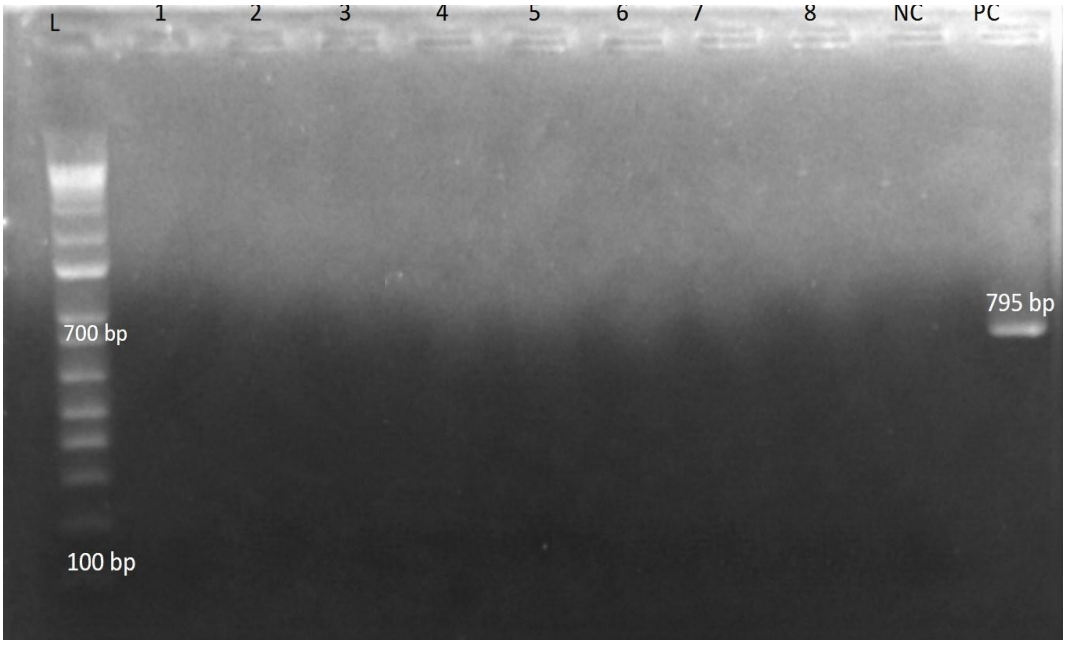
B



B (bla*CTX-M)* 593bp L: DNA Marker (Ladder); **1**: *S.* Typhimurium (031528); **2**: *S.* Typhimurium (044043); **3**: *S.* Enteritidis (038055);

L-Molecular Weight Ladder(**100bp**); **NC**-Negative Control (PCR water); **PC**-Positive Control (consisting of known positive control strains)

C



C (**bla** $_{SHV}$) **795bp L**: DNA Marker (Ladder); **1**: *S.* Typhimurium (031528); **2**: *S.* Typhimurium (044043); **3**: *S.* Enteritidis (038055); **4**: *S.* Typhimurium (022138); **5**: *S.* Typhimurium (048245); **6**: *S.* Typhimurium (022578); **7**: *S.* Typhimurium (030432); **8**: *S.* Typhimurium (024661);

L-Molecular Weight Ladder (**100bp**); **NC**-Negative Control (PCR water); **PC**-Positive Control (consisting of known positive control strains

Figure 5 (A, B, C): Agarose Gel Electrophoresis of PCR Detection of Beta lactam Resistance Genes for representative isolates.

4.8 Factors associated with non-Typhoidal *Salmonella*

After adjusting for other factors, using drums and other open containers for water storage potentially predicted infection with NTS (2.0%; OR = 1.95, p = 0.040). Participants who had malignancy were predicted to be at risk of having NTS (33.3%; OR = 39.10, p = 0.003) (**Table 5**). Not washing hands before eating (7.7%; OR = 10.43, p = 0.039) and bloody diarrhea (4.6%; OR = 3.64, p = 0.010) were found to have confounders after adjusting for other factors.

Table 5: Factors associated with non-Typhoidal *Salmonella*

	Univariable analysis			Multivariable analysis		
variables	**cOR**	**(95% CI)**	**p-value**	**aOR**	**(95% CI)**	**p-value**
Gender						
Male	0.618	(0.32-1.20)	0.149	ND		
Female	Ref.					
Age in months						
12 to 24 months	1.663	(0.75-3.70)	0.212	ND		
>36 months	Ref.					
Generally treat water before drinking						
Yes	0.58	(0.30-1.13)	0.106	ND		
No	Ref					
Types of water storage containers						
Drums						
Yes	1.93	(1.02-3.65)	**0.040**	1.95	(1.03-3.71)	**0.040**
No	Ref					
Type of toilet used by the household						
Private flush toilet						
Yes	1.81	(0.88-3.75)	0.103	ND		
No	Ref					
Households wash hands before eating						
Always	1.98	(0.77-5.08)	0.158	ND		
Never/Don't know	10.43	(1.13-96.23)	**0.039**	-		
Sometimes	Ref					
Domestic animals						
Dog						
Yes	0.45	(0.18-1.17)	0.093	ND		
No	Ref.					
Clinical presentations						
Expectoration						
Yes	0.27	(0.04-1.97)	0.166	ND		
No	Ref.					
Bloody diarrhea						
Yes	3.64	(1.27-10.49)	**0.010**	-		
No	Ref.					
Comorbidities						
Malignancy						
Yes	35.88	(3.19-404.21)	**0.042**	39.10	(3.36-454.33)	**0.003**
No	Ref.					

cOR =crude Odds Ratio, aOR= adjusted Odds Ratio, CI=Confidence Interval ND=Not determined

CHAPTER FIVE: DISCUSSION

The observed prevalence of 1.4% of NTS is similar to the prevalence rate of NTS (1.3%) reported in a comparable study conducted in 2020 in the same setting (Mbae et al., 2020b). This indicates that the prevalence of NTS has remained almost constant in this disease endemic setting.

The predominant serovar was *Salmonella* Enteritidis 58.1 % (25/43) compared to *Salmonella* Typhimurium 41.9% (18/43) which is similar to a study done by (Cetin et al., 2019) that also found the predominant serovar to be *S.* Enteritidis. Previously, a study done in Mukuru informal settlement found a higher proportion of *S.* Typhimurium (Kariuki et al., 2020c) an indication of a change of variation of the species which could be linked to changes in host adaptations which could influence the prevalence of the serovars (Cheng et al., 2019).

The prevalence of NTS was higher in the males (1.7%) compared to the female and was also highest in the age group 12-24 months (2.1%). This could be because males are more likely to consume contaminated food and water away from the house due to their tendency of playing in slum areas with poor sanitation This is consistent with previous studies which found the male gender to have the highest positivity of NTS disease compared to the female (Mbae et al., 2020c) . The age group of 12-24 months is typically characterized by increased mobility in children, who may also exhibit a tendency to pick up and eat items from the ground, possibly contributing to the higher positivity rate of NTS in this age range.

Several studies have shown that several *Salmonella* transmissions occur along the food chain such as consuming raw vegetables (Silva et al., 2014). This was demonstrated in this study from the prevalence of *Salmonella* Enteritidis and Typhimurium which was highest in participants who ate raw salad even after washing prior to preparation (1.7%). This could be attributed to using contaminated water when washing the vegetables as lack of access to clean water is common in informal settlements. It was however not significantly associated with having NTS disease.

Non-Typhoidal *Salmonella* is known to be a zoonotic disease and previous studies have found keeping pets and contact with animals especially reptiles and cats to be risk factors for the acquisition of the disease (Younus et al., 2010). This was not the case in this study as contact with animals was not significantly associated with having NTS (*Salmonella* Enteritidis or Typhimurium). This could therefore be an indication that the reservoirs of NTS within this study population may not be zoonotic.

The overall resistance rate to the tested antibiotics was 25.6% (11/43). Resistance to ampicillin, trimethoprim-sulfamethoxazole, azithromycin and tetracycline was observed in 81.8% of *Salmonella* Typhimurium isolates. Highest resistance was to tetracycline (44.4%). Resistance to nalidixic acid, cefpodoxime and azithromycin was seen in 18.2% of *Salmonella* Enteritidis.

This study did not find any multi drug resistant isolates (combined resistance to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole). This was not the case in the study done in 2019 in an endemic setting in Kenya which reported an MDR rate of 34.2% (Kariuki et al., 2019b). Resistance to cefpodoxime a 3rd generation cephalosporin was seen in *Salmonella enterica* serotype Enteritidis (9.1%) which has also been reported in various sub-Saharan African countries (Gilchrist & MacLennan, 2019).

This study highlighted 22.2 % resistance to azithromycin in *Salmonella* Typhimurium isolates and 4% in *Salmonella* Enteritidis. It also highlighted reduced susceptibility to azithromycin with only 22.2% of *S.* Typhimurium and 8% *S.* Enteritidis being susceptible. These findings emphasize the need for continued surveillance on AMR noting that a systematic review had recommended a switch to oral fluoroquinolones or azithromycin as the choice of treatment for NTS (Tack et al., 2020). A recent study also highlighted emerging resistance to azithromycin in *S.* Typhimurium which agrees with our study findings (Van Puyvelde et al., 2023). These finding indicates possible over use of azithromycin in treatment of NTS in the face of emerging MDR strains.

In 8% of *S.* Enteritidis isolates, resistance to nalidixic acid was detected, while all isolates exhibited complete susceptibility to ciprofloxacin predicting reduced ciprofloxacin

susceptibility. This was also reported in a systematic review that found NTS isolates that were resistant to nalidixic acid in Kenya (n=14) which were predicted to be DCS (reduced ciprofloxacin susceptibility) as they were nalidixic acid resistant but ciprofloxacin susceptible (Tack et al., 2020).

Phenotypically there was no resistance to 3rd generation cephalosporins which is in line with genotypic data where bla$_{SHV}$ and bla$_{CTX\text{-}M}$ genes that code for this resistance were absent. The gene CTX-M regulates resistance to 3rd generation cephalosporins while the gene SHV codes for resistance against ampicillin mostly in *Klebsiella* and *Escherichia coli* (Dirar et al., 2020). Resistance to ampicillin was associated with the presence of bla$_{TEM}$ gene. Three out of the eleven isolates that were tested were found to carry bla$_{TEM}$ genes. This finding agrees with a study done in Asia on hospitalized children that also found the gene bla$_{TEM}$ in isolates that were resistant to ampicillin (Duong et al., 2020).

After adjusting for other factors storage of water in drums and malignancy were found to be potential predictors of infection with non-Typhoidal *Salmonella*.

In a study carried out in Taiwan malignancy was a contributing factor to NTS occurrence in adults (Chen et al., 2012b). This could be as a result of having a weakened immune system which has been associated with having NTS. Correspondingly, our study also reveals a significant association between malignancy and the presence of *Salmonella* Enteritidis or Typhimurium, establishing a parallel connection between these factors in both children and adults.

Mukuru is an informal settlement and storage of water in drums and other open containers for household use and consumption is common. Members of the households are likely to contaminate the stored water when fetching using cups and jars possibly contributing to acquisition of NTS.

STUDY LIMITATIONS

The study employed a cross-sectional design, which hinders the ability to conclusively establish a cause-and-effect relationship between the identified risk factors and non-Typhoidal *Salmonella* infection. Additionally, the data gathered via the questionnaire relied on self-reports from caregivers, introducing the possibility of recall bias, as individuals may not accurately recall or report certain information. The sample size in this study was relatively small which potentially limited the statistical power of the analyses, impacting the precision of the estimates derived from this study.

CONCLUSION

In this disease-endemic region, the prevalence of non-Typhoidal *Salmonella* was found to be 1.4%, indicating stability in NTS prevalence. *Salmonella* Enteritidis was the most prevalent serovar.

Salmonella Typhimurium exhibited resistance to multiple antibiotics, particularly tetracycline, with an overall resistance rate of 25.6%. No multi-drug resistant isolates were detected in this study. The presence of the bla_{TEM} gene was linked to resistance to ampicillin. Additionally, both serovars displayed reduced susceptibility to azithromycin.

This study highlights the critical importance of enhancing access to water and sanitation infrastructure. The provision of proper facilities for hygiene practices is crucial for effective control and prevention of the spread of *Salmonella* infections.

RECOMMENDATIONS

Given the observed resistance to multiple antibiotics among non-Typhoidal *Salmonella* (NTS) infections, regular reviews and updates of treatment guidelines are imperative. The responsibility for maintaining effective treatment options lies with healthcare professionals and policymakers.

To address the challenges posed by antimicrobial resistance, there is need to enhance antimicrobial stewardship efforts and rapid diagnostics for clinical use, designed to promptly detect non-susceptibility.

The observed reduced susceptibility to azithromycin prompts concerns regarding its efficacy as a treatment choice for NTS infections. Healthcare policymakers should consider a comprehensive reevaluation of treatment guidelines ensuring that treatment strategies align with the evolving susceptibility patterns of non-Typhoidal *Salmonella* disease.

There is need to improve Water Sanitation and Hygiene (WASH) infrastructures to reduce risk factors associated with transmission of NTS in the community which requires collaboration between policymakers, and community stakeholders to implement effective measures that curb the spread of non-Typhoidal *Salmonella*.

Regulatory bodies, and public health policymakers need to embark in the production and introduction of vaccines for the prevention and control of non-Typhoidal *Salmonella* in the short-to medium-term.

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APPENDICES

Appendix 1: Informed consent form for guardians/parents

English version

STUDY TITLE: **Risk factors associated with non-Typhoidal *salmonella* infections and determination of their antimicrobial resistant phenotypes and genotypes in children aged 0- 5 years in Mukuru slums**

Principal investigator: Georgina Adongo Odityo

Informed consent.

(i) What is the purpose of this research?

We aim to obtain information about a disease that is common in children below the age of 5 years which may cause severe bloodstream infection or diarrhea illness that may require admission into hospital. This illness is brought on by a bacteria called Salmonella, which can be spread by handling food with unwashed hands, drinking contaminated water, and coming into contact with animal feces. If we isolate salmonella from your child, we will do further laboratory tests to know which is the best drug to use for treating your child. It is also important to know the HIV status of your child. This is because the diseases we are investigating may be more common in people who are HIV Positive or could be more dangerous in those who are HIV positive than those who are not. Moreover, people who are HIV positive will require medication for HIV and bacterial disease. To understand the extent and type of these germs infecting children in Kenya, either HIV positive or negative, we need to carry out an HIV test for the children and also collect stool samples from children with diarrhea for thorough laboratory tests. The knowledge accrued from this study will go a long way in identifying the types of such germs infecting these children to identify possible sources of infections and provide data on appropriate modes of prevention and control of such diseases. Bacteria isolated from your child will be tested in a laboratory in KEMRI to understand how the disease spreads in the community – no samples from

your child or any data containing your child's 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 which the disease may be spreading within your community.

(ii) Your part in the research

Your child will be investigated for bacteria that cause blood infection and/or diarrhea in children. We ask that you consent to you and your child being investigated for these diseases. The process will involve taking 3-5 ml of blood ONCE only from your child, and/or a stool sample or rectal swab for laboratory testing. The child might experience some slight discomfort during the sample collection process. You will get HIV testing. Along with HIV testing, you will also get information about HIV and how to prevent it from someone with special training as a counselor. If you find out you have HIV, we will refer you to a clinic where doctors would help you stay healthy. 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 from spreading in the community. The experiments will be done under the care of the Principal Investigator.

(iii) What are additional procedures that the involved participants need to know?

Blood Samples will be taken once only and results will be given to the doctor treating your child to advise you on how best to treat your child.

(ivHow will participants in the study be selected, and how many people will they be involved?

We anticipate obtaining samples from about 2300 children. This is a group of patients who will be identified by criteria for severe or enteric salmonella disease and contact with the patient.

(v) Benefits

If we find any illness in you through our laboratory tests, we will treat it with the proper

medications in accordance with hospital policies. We will also provide you with post-treatment counselling and health education on how to avoid infections that may occur due to sloppy food and water handling and poor sanitation at home. Following the investigation, we will be able to create a database on the most effective medications for treating blood poisoning infections, diarrhoea that affects populations, and how the bacteria may be entering the community. The Ministry of Health and caregivers will get access to this data in order to better population-wide treatment of these illnesses.

(vi) Risks

Your participation in the study entails no additional risk or expense. By utilizing the most recent stool collection methods, such as moistening the rectal swab, we want to reduce any discomfort you may have while having these samples taken. The attending clinicians will offer medical care at project expense if there is any injury sustained while collecting specimen for this study. Your care in this facility won't be in any way compromised if you refuse to participate.

(vii) What will happen after the study?

If your child has any illness that we discover through our laboratory tests, he/she will be treated with 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. The study will only be responsible for the costs of medication and not treatment in case of referrals. After the study, a database will be created on the most effective medications for treating children's diarrhea and blood poisoning infections as well as how the bacteria may be entering the community. For the purpose of improving the overall management of these infections in children, the Ministry of Health and caregivers will have access to these data.

(ix) What if I decide not to assist in this research?

You are free to leave the research at any moment if you accept to participate in the research but then not want to do it. If you later decide not to participate or do not agree to participate,

you will not be subjected to any form of discrimination in the future.

(x) Data confidentiality and protection.

In order to prevent individual children from being identified, the data gathered about the kids who participate in this study will be coded. The research team will be the only ones with access to the computers holding the coded information. Would you like me to answer any questions for you right now? You can get in touch with any of the following people at KEMRI, Centre for Microbiology Research, PO Box 43640-00100, Nairobi if you want to get more information about the study or if you have any problems that need to be resolved in the future.

Georgina Odityo– Tel 0726516813, or Secretary, KEMRI/National Ethical Review Committee, PO Box 54840-00200, Nairobi, TEL: +245-20-2722541

I certify that I understood the data provided for the aforementioned study and that I had the opportunity to ask any questions I may have had. I understand that my child's involvement in the research is completely voluntary, and I am free to withdraw him or her from it at any time without having to give a reason or be concerned about how doing so could affect my medical care or legal rights.

I agree to allow my child, named _____ (Study Number) _____, to take part in this study.

YES

NO

Signature of the guardian _____ Date

_____

Or Thumbprint:

Name of the guardian _____ Date

_____

If the participant does not know how to read:

I have read/ been explained to the consent form and I've had the chance to pose inquiries.

I certify that I gave my consent voluntarily and without being coerced.

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: _____

Swahili Version of Patient Information and Consent seeking:

Habari kuhusu utafiti juu ya magonjwa ya damu kwa watoto wachanga

(i) Lengo la utafiti huu ni lipi?

Kuna ugonjwa hatari unaoenezwa na viini vya bacteria aina ya salmonella ambao husabibisha sumu kwa damu ya mtoto. Mara nyingi viini hivi huuguza watoto chini ya umri wa miaka mitano pamoja na wale walio chini ya umri wa miaka kumi na sita. Katika jamii ugonjwa huu mara nyingi huenezwa kwa kula chakula ama kutumia vinywaji venye viini kutokana na mazingara ambayo yamechafuliwa na mavi ama mkojo wa wanyama, ama kwa kutumia mikono michafu wakati wa mankuli. Kutokana na utafiti utakaofanyiwa damu na choo cha mtoto wako katika maabara yetu, iwapo tutapata yakwamba anaugua ugonjwa huu wa Salmonella, basi daktari ataendelea kumtibu mpaka apone. Mtoto anaweza kupata usumbufu kidogo wakati wa mchakato wa kukusanya sampuli.

(ii) Utahitajika kufanya nini?

Tungehitaji wewe ukubali taakwimu za mtoto wako kama vile umri, uzito, hali ya afya na sehemu anakoishi zitumiwe katika utafiti KEMRI. Pia tutatuma viini vya bakteria ng'ambo kwa utafiti zaidi.

(iii) Watoto wangapi kwa jumla watahusika katika utafiti?

Karibu watoto elfu mbili na mia sita kutoka sehemu mbali mbali nchini na Africa Mashariki watahusishwa katika utafiti huu.

Sampuli hizi takriban elfu tano na mia sita zitachukuliwa kila mwaka kwa muda wa miaka minne.

(iv) Faida

Ikiwa una ugonjwa wowote ambao tunagundua kwa njia ya vipimo vya maabara yetu, utatibiwa na madawa sahihi kulingana na miongozo ya hospitali na pia kutoa ushauri,na

elimu ya kiafya juu ya jinsi ya kuzuia maambukizi ambayo yanaweza kutokea kutokana na taka, usafi wa mazingira na utunzaji wa vyakula na maji nyumbani. Baada ya utafiti tunaweza kuanzisha taarifa juu ya madawa bora kutumia kwa ajili ya maambukizi ya bakteria ya damu na kuhara inayoathiri idadi ya watu na jinsi bakteria inaweza kuenea katika jamii. Takwimu hizi zitapatikana kwa wataalamu wa wizara ya Afya kwa kuboresha kwa ujumla matibabu ya maambukizi humu nchini

(v) Hatari

Utafiti huu hauna hatari zaidi au gharama kwako kushiriki kwa utafiti huu. Kuchukua sampuli hizi kunaweza kukufanya usumbuke kidogo na tunalenga kupunguza hii kwa kutumia mbinu nyingi za sasa za kuchukua kinyesi, ikiwa ni pamoja na kutumia kijiti maalum. Ikiwa kuna madhara yoyote katika mchakato wa kupata sampuli kwa madhumuni ya utafiti huu, huduma za matibabu zitatolewa na daktari anayekushughulikia kwa gharama ya mradi. Skanning Kukataa kushiriki bila njia yoyote hakuhatarisha matibabu yako katika kituo hiki kwa njia yoyote.

(vi) Ni nini kitakachofanyika baada ya utafiti huu?

Watafiti wa KEMRI wataandika vitabu na maelezo aina mbali mbali ambayo yatapelekewa wauguzi katika sehemu zote ambako ugonjwa huu umeenea ili kuwashauri juu ya matibabu murwa na njia za kuzuia ugonjwa huu hatari. Matibabu pia yatatolewa kwa watoto watakaopatikana wakiugua ugonjwa huu.

(vii) Itakuaje iwapo sitaki kuhusishwa?

Kama hutaki kuhusishwa kwa utafiti huu, mtoto wako ataendelea kutibiwa mpaka apone, na hakuna madhara yoyote ama masharti mengine utakayowekewa.

(viii) Ni nani atakayesoma habari juu ya mtoto wangu?

Habari na taakwimu za mtoto wako zitawekwa na kulindwa vyema katika KEMRI. Jina la mtoto wako halitahusishwa kamwe na maandishi ama ripoti yoyote ambayo itapewa idara

za afya.

Ukiwa na swali lingine lolote juu ya utafiti huu tafadhali wasiliana na madaktari wafuatao katika chuo cha utafiti cha KEMRI:

Georgina Odityo – Tel 0726 516813

Ama, KEMRI/National ERC, PO Box 54840-00200 Nairobi

Simu: 020-2722541

Unaweza pia kumuuliza daktari anayekutibu maswali yoyote kuhusu utafiti huu. Pia unaweza kuwasiliana na Mwandishi mkuu (secretary) katika kamati inayoangalia maslahi ya wanaohusishwa kwa utafiti hapa KEMRI, Nambari ya simu ni 020-2722501

Kukubali kuhusika katika utafiti:

Nimeelewa yale niliyoelezewa juu ya utafiti ambao unaendelea hivi sasa, na nimepata fursa ya kuuliza maswali. Nimeelewa kwamba kuhusika kwangu ni kwa hiari yangu mwenyewe na nina uhuru wa kukataa kuhusika bila kutoa sababu zozote wala haki za kimatibabu za mtoto wangu kuathirika kwa vyovyote vile.

Nimekubali mtoto wangu _____ (nambari ya utafiti) _____ ahusike katika utafiti huu:

Ndio La

Nimekubali taakwimu na habari juu ya ugonjwa wa mtoto wangu, na viini vitumiwe katika utafiti hapa nchini na Ng'ambo:

Ndio La

Sahihi ya mzazi _____ Tarehe _____

Ama kidole

Jina la mzazi _____

(Tafadhali andika jina)

Ikiwa mshiriki hajui kusoma:

Nimeshuhudia kusomwa kwa uhakika fomu ya kibali kwa mshiriki huyu amekuwa na fursa ya kuuliza maswali. Nadhibitisha kuwa mtu huyu ametoa kibali bila kushurutishwa.

Jina la shahidi: _____

Sahihi ya shahidi: _____ Tarehe _____

Jina la mfanyikazi wa utafiti anayefanya usajili huu: _____

Sahihi ya mfanyikazi wa utafiti anayefanya usajili huu: _____Tarehe: _____

Appendix 2: Interviewer administered Questionnaire

Study title: Risk factors associated with non-Typhoidal *salmonella* and antimicrobial resistant phenotypes and genotypes in children aged 0-5 years in Mukuru slums

1.Patient's Barcode
2.Study ID
3.Name of Health facility
MMM
MCC
MLK
MR/NMS (Mukuru Maendeleo)
4.Mode of Presentation
0ut-patient
Emergency room
In-patient
5.Date of presentation
6.Name of study participant (at least two names)
7.Date of Birth
8.Age
9.Gender
Male
Female
10.Telephone Number
11.Name of household head /2nd member of the family/guardian
12.Education of the household head
Illiterate
Elementary
Primary school
Junior high school/technical secondary school

College and above

13.The telephone number for contacts within the household

14.Name of Village

Mukuru Kwa Njenga

Mukuru Kwa Reuben

Pipeline

Other (Outside mapped areas)

Indicate the Name of the other village

15.Zone

16.Nearest Landmark

17.No. of family members in the household

18.No. of children less than 5 Years old

19.No. of children above 5 years old

20. What is the main component of your house?

Masonry

Mud walled

Timber

Corrugated Iron

21. What is the type of house ownership?

Owned

Rented

22 .How many rooms are used for your family?

23. What is the main fuel used for cooking in the house?

Electricity

Gas

Firewood

Kerosene

Charcoal

24. Do you have electricity in your house?

Yes

No

25. Do you have a sofa in your house?

Yes
No
26 .Do you have a Television in your house?
Yes
No
27. Do you have a sewing machine in your house?
Yes
No
28. Do you have a refrigerator?
Yes
No
29. For how long have you lived in this area?
30. What is the average cash income of the household per month?
Nothing (No cash)
Less than Ksh. 3000
Ksh 3,000 to Ksh 9,900
Ksh 10,000 to Ksh. 24,900
Ksh 25,000 to Ksh 49,900
Ksh 50,000 to Ksh 74, 900
Ksh 75,000 to Ksh 100,000
31. What is the main source of drinking water in the house?
Own tap
Communal/ Municipal tap
Own well/pump
Communal well/pump
River/spring/rainwater
Water Vendor
Borehole
Bottled water
32 .Do you generally treat water before drinking?
Yes
No
33. Do you treat water by boiling it?
Yes
No
Sometimes

34. Do you treat water by use of Water guard/Aqua tabs?
Yes
No
Other
35. Do you treat water by use of Filtration?
Yes
No
36. Do you use water directly from the tap?
Yes
No
37. Do you use a Jerrycan for water storage in your house?
Yes
No
38. Do you use a Bucket for water storage in your house?
Yes
No
39 .Do you use a Drum for water storage in your house?
Yes
No
40. Do you use a water pot for water storage in your house?
Yes
No
41. Are there any contamination sources (toilet, barn, cesspit, and sump) around the water source within 20 meters?
Yes
No
42. Are there any open drains, flood water within 20 meters from your household?
Yes
No
43. Do you use a private flush toilet?
Yes
No
44. Do you use a private pit latrine?
Yes
No
45. Do you use a flush toilet shared with other households?
Yes
No
46. Do you use (shared) pit latrines?
Yes
No

47. Do you use a public toilet (Fresh life)?

Yes

No

48. Do you go to the bush/river/open drain as a toilet?

Yes

No

49. Do you use carrier bags as a toilet?

Yes

No

50. Do you wash your hands after visiting the toilet?

Always

Sometimes

Never

Don’t Know

51. Do you clean your hands before preparing food?

Always

Sometimes

Never

Don’t Know

52. Before eating, do you wash your hands?

Always

Sometimes

Never

Don’t Know

53. Do you obtain your vegetables from a Vendor (Mama Mboga?)

Yes

No

54. Do you obtain your vegetables from the market?

Yes

No

55. Do you grow your vegetables in your backyard?

Yes

No

56. Do you obtain your vegetables from the shop?

Yes

No

57. Do you obtain your vegetables from your neighbor?

Yes

No
58. Do you cook your vegetables directly without washing them?
Yes
No
59. Do you wash your vegetables before cooking?
Yes
No
60. Do you eat your vegetables raw as a salad (Kachumbari) after washing?
Yes
No
61. How often does your family eat street foods?
Never
1-2 times/week
3-5 times/week
6 or more/per week
Rarely
62. Does the household use waste containers?
Yes
No
63. Where is the container for your waste disposal?
Inside the house
Outside the house
64. Are there any cattle in the compound/homestead?
Yes
No
65. Is there any Chicken in the compound/homestead?
Yes
No
66. Are there any goats in the compound/homestead?
Yes
No
67. Are there any dogs in the compound/homestead?
Yes
No
68. Are there any cats in the compound/homestead?
Yes
No
69. Are there any pigs in the compound/homestead?

Yes
No
70. Are there any sheep in the compound/homestead?
Yes
No
71. Are there any Ducks in your homestead/compound?
Yes
No
72. Are there any Turkeys in your household/compound?
Yes
No
73. Are there any Doves in your homestead/compound?
Yes
No
74. How long does it take from your house to the nearest health facility on foot?
10 min
20 min
30 min
40 min
50 min
60 min
More than 1 hour
75. Has anybody in the house taken the typhoid vaccine?
Yes
No
Don't Know
76. How many members of the household took the vaccine?
77. Was a Stool sample taken from the participant?
Yes
No
78. Was a Blood sample taken from the participant?
Yes
No
79.Date of interview
80.Time of interview
81.Name of Interviewer

Appendix 3: Case Report Form

Study title: Risk factors associated with non-Typhoidal *salmonella* and antimicrobial resistant phenotypes and genotypes in children aged 0-5 years in Mukuru slums

1. Patient Bar code
2. Study ID
3. Date of Visit
4. Name of patient (At least two names)
5. Date of birth
6. Age
7. Gender
Male
Female
8. Telephone number
9. Telephone number of a contact
10. Did you have a fever in the past 3 days?
Yes
No
Not Sure
11. Was it continuous/Intermittent fever?
Yes
No
Not sure
12. How many days?
13. Has the participant had in the last four weeks or currently has a cough?
Yes
No
Don't Know
14. Has the participant had in the last four weeks or currently have Hemoptysis?

Yes

No

Don't Know

15. Has the participant had in the last four weeks or currently have Expectoration?

Yes

No

Don't Know

16. Has the participant had in the last four weeks or currently vomiting?

Yes

No

Don't Know

17. Has the participant had in the last four weeks or currently abdominal pain?

Yes

No

Don't Know

18. Has the participant had in the last four weeks or currently have Distension?

Yes

No

Don't Know

19. Has the participant had in the last four weeks or currently has Diarrhea?

Yes

No

Don't Know

20. Has the participant had in the last four weeks or currently has Bloody Diarrhea?

Yes

No

Don't Know

21. Has the participant had in the last four weeks or currently have constipation?

Yes

No

Don't Know

22. Has the participant had in the last four weeks or currently have Dysuria?

Yes

No

Don't Know

23. Has the participant had in the last four weeks or currently passed urine more frequently than usual?

Yes

No

Don’t Know

24. Has the participant had in the last four weeks or currently has Headache?

Yes

No

Don't Know

25. Has the participant had in the last four weeks or currently has a seizure?

Yes

No

Don't Know

26. Has the subject had or currently had Sickle cell disease?

Yes

No

Present

27. Has the subject had or currently had Tuberculosis?

Yes

No

Present

28. Has the subject had or currently had malignancy?

Yes

No

Present

29. Has the subject had or currently has HIV/AIDS?

Yes

No

Present

30. Did you take any medication in the last 8 weeks?

Yes

No

Not sure

31. Chloramphenicol (Chloromycetin/Econochlor)

Yes

No

32. Amoxicillin (Amoxil, Moxotag, Trimox)

Yes

No

33. Co-amoxiclav (Augmentin

Yes

No

34. Ciprofloxacin (Cipro/Cipro XR/Ciprobay
Yes

No

35. Ceftriaxone (Rosephin)
Yes

No

36. Antimalarial Drugs
Yes

No

37. If others, which ones?

38. Heart Rate (beats per minute)

39. Temperature (Degrees Celsius)

40. Respiratory rate (breaths per minute)

41. Height (cm)

42. Weight (Kg)

43. Mid arm circumference (cm) (Patients below 5 Years)

44. Presence of Cyanosis
Yes

No

45. Presence of edema
Yes

No

46. Dehydration
None

Mild

Moderate

Severe

47. Jaundice?
Yes

No

48. Pallor (Conjunctiva)
Yes

No

49. Coated tongue
Yes

No

50. Throat
Normal

Pharyngotonsillitis

51. Others

Indicate others

52. Neck stiffness
Yes

No

53. Focal Neurological signs
Yes

No

54. Apathy
Yes

No

55. Disorientation
Yes

No

56. Delirium
Yes

No

57. Stupor
Yes

No

58. Distension
Yes

No

59. Tenderness
Yes

No

60. Palpable Liver
Yes

No

61. Palpable Spleen
Yes

No

62. Decreased abdominal sound

Yes

No

63. Rebound Tenderness

Yes

No

64. Bronchial breath

Yes

No

65. Rhonchi

Yes

No

66. Crepitation

Yes

No

67. Chest indrawing

Yes

No

68. Was therapy initiated?

Yes

No

Not applicable

69. Patient disposition

Sent home

Admitted

Died

Absconded/Lost

Transferred to Mama Lucy Kibaki Hospital

Transferred elsewhere

If admitted, where?

If elsewhere, specify

Patient examined by

70. Was an HIV test done?

Yes

No

Decline

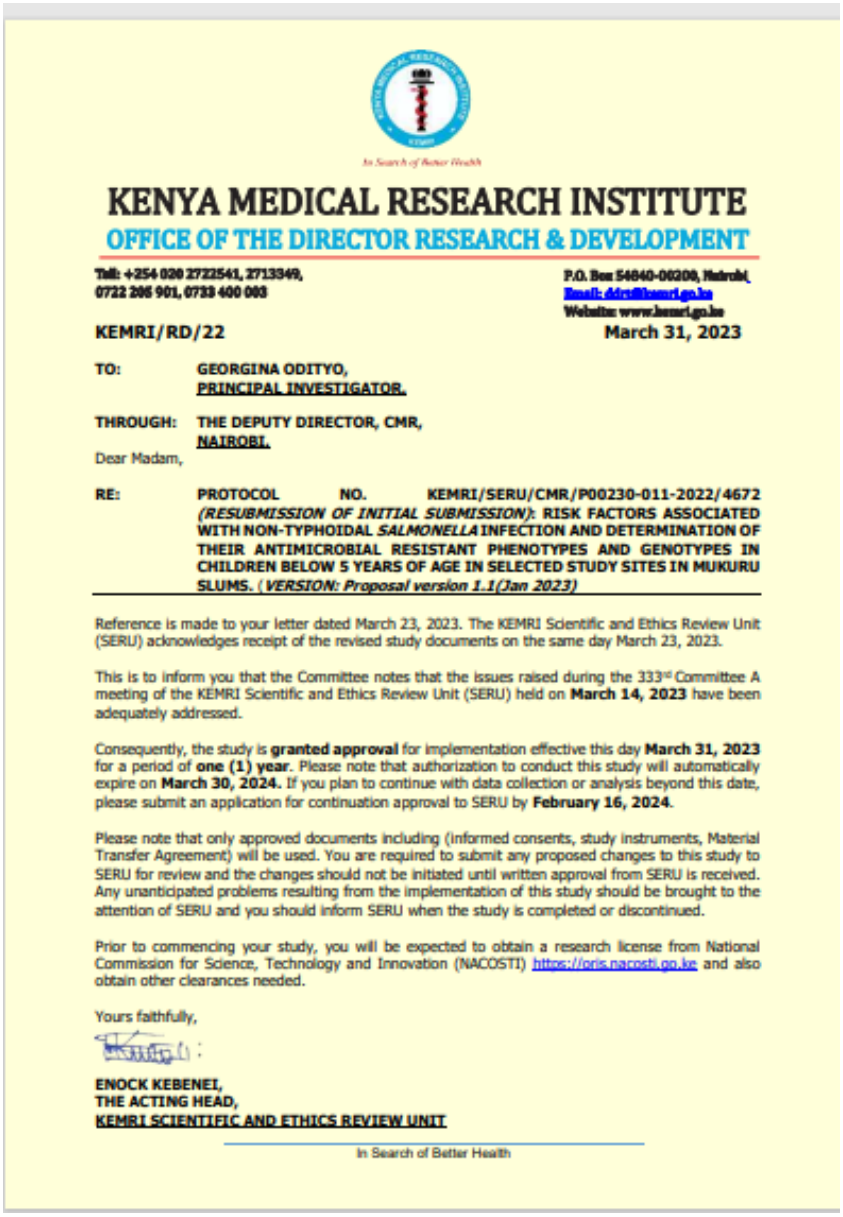
Known Positive

71. What was the result?

Reactive
Non-reactive
72. Sample type taken
Blood
Stool
73. Take a photo of the CRF
74. Time of data entry (24HR Clock)
75. Date of Data entry
76. Data entered by

Appendix 4: Ethical approvals (SERU and KNH-UON ERC)

SERU



In Search of Better Health

KENYA MEDICAL RESEARCH INSTITUTE

OFFICE OF THE DIRECTOR RESEARCH & DEVELOPMENT

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P.O. Box 54840-00200, Nairobi,
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KEMRI/RD/22 **March 31, 2023**

TO: **GEORGINA ODITYO,**
PRINCIPAL INVESTIGATOR.

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

Dear Madam,

RE: **PROTOCOL NO. KEMRI/SERU/CMR/P00230-011-2022/4672** ***(RESUBMISSION OF INITIAL SUBMISSION)*****: RISK FACTORS ASSOCIATED WITH NON-TYPHOIDAL *SALMONELLA* INFECTION AND DETERMINATION OF THEIR ANTIMICROBIAL RESISTANT PHENOTYPES AND GENOTYPES IN CHILDREN BELOW 5 YEARS OF AGE IN SELECTED STUDY SITES IN MUKURU SLUMS. (*VERSION: Proposal version 1.1(Jan 2023)***

Reference is made to your letter dated March 23, 2023. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on the same day March 23, 2023.

This is to inform you that the Committee notes that the issues raised during the 333rd Committee A meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **March 14, 2023** have been adequately addressed.

Consequently, the study is **granted approval** for implementation effective this day **March 31, 2023** for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **March 30, 2024.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **February 16, 2024**.

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 you should 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,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

KNH-UON ERC

UNIVERSITY OF NAIROBI
FACULTY OF HEALTH SCIENCES
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Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/273

30th June, 2023

Georgina Adongo Odityo
Reg No. H56/40863/2021
Dept. of Medical Microbiology
Faculty of Health Sciences
University of Nairobi

APPROVED
30 JUN 2023
KNH/UoN-ERC

Dear Georgina,

ETHICAL APPROVAL-RESEARCH PROPOSAL: RISK FACTORS ASSOCIATED WITH NON-TYPHOIDAL SALMONELLA INFECTION AND DETERMINATION OF THEIR ANTIMICROBIAL RESISTANT PHENOTYPES AND GENOTYPES IN CHILDREN AGED 5 YEARS AND BELOW IN MUKURU INFORMAL SETTLEMENT (P244/03/2023)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P244/03/2023.** The approval period is 30th June 2023 –29th June 2024.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Protect to discover

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

Yours sincerely,

DR. BEATRICE K.M. AMUGUNE
SECRETARY, KNH- UoN ERC

c.c. The Dean, Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information Dept., KNH
The Chair, Dept. of Medical Microbiology, UoN
Supervisors: Dr. Winnie Mutai, Dept. of Medical Microbiology, UoN
Prof. Samuel Kariuki, Dept. of Medical Microbiology, UoN.