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Seroepidemiology of Non-Typhoidal Salmonella in Kenya; Spatial Patterns, Determinants of Exposure and Immune Correlates of Protection in Apparently Healthy Children Under Five Years in Mukuru Informal Settlement

Mercy Ngetich[1*], Schola Kamwethya[1], Helen Dale[2, 3], Evans Kiptanui1, Osindi Bonface[7], Omar Rossi[8], Rocio Canals[8], Niza Silingwe[3], Chifundo Mkwangwanya[3], Tonney Nyirenda[4], Evans Kibet[1], Diana Imoli1, Christine Kioko[1], Rael Too[1], Cecilia Mbae[1], Robert Onsare[1], Melita Gordon[2, 3], Samuel Kariuki1 [5, 6] and Vacc-iNTS Consortium Collaborators.

[1]Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya
[2]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, United Kingdom
[3]Clinical Research, Malawi-Liverpool-Wellcome Clinical Research-Program, Blantyre, Malawi
[4]Pathology Department, Kamuzu University of Health Sciences, Blantyre, Malawi
[5]Wellcome Sanger Institute, Hinxton, United Kingdom
[6]Drugs for Neglected Diseases initiative, Nairobi, Kenya
[7]Washington State University Global Health Program- Kenya, Nairobi, Kenya
[8]GSK Vaccines Institute for Global Health (GVGH), Siena, Italy

[*]**Corresponding Author:** Mercy Ngetich, 1Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya, Email: mercynge2030@gmail.com

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Abstract

Background: Invasive non-typhoidal Salmonella disease caused by Salmonella enterica serovars Typhimurium (STm) and Enteritidis (SEn), remains a major cause of morbidity and mortality in sub-Saharan Africa affecting children under five years of age. We assessed seroprevalence, spatial patterns of exposure, associated risk factors, and fuctional activity of naturally acquired antibodies among children in Mukuru informal settlement, Nairobi, Kenya.

Methods: Cross-sectional study conducted among 1000 apparently healthy children aged 0-≤5 years. Immunoglobulin G (IgG) assessed using Enzyme-Linked Immunosorbent Assay, functional activity evaluated using serum bactericidal assay. Spatial clustering of exposure assessed using Moran's I statistics and co-distribution with water, sanitation, hygiene and socioeconomic indicators examined using bivariate choropleth maps. Logistic regression identified determinants of exposure; Spearman correlation assessed IgG levels-bactericidal activity relationship.

Results: High seroprevalence; 96.3% for STm, 93.6% for SEn and 90.4% for both serovars combined. Significant spatial clustering of exposure (both serovars, Morans I = 0.533, p = 0.0003; SEn, I = 0.473, p = 0.0009). Age associated with higher odds of exposure to both serovars (aOR 1.12, p < 0.001); STm (aOR 1.12, p = 0.003). Significantly lower odds of exposure associated with higher household wealth (both serovars, aOR 1.12, p = 0.040; SEn, aOR 0.07, p = 0.025), male (SEn, aOR 0.48, p = 0.045), HIV exposure (both serovars, aOR 0.17, p = 0.005; STm, aOR 0.13, p = 0.004), recent positive malaria test (both serovars, aOR 0.40, p = 0.043) and use of pit latrines with wood/soil flooring (STm, aOR 0.35, p = 0.048). Bactericidal activity; 91.0%-SEn and 99.7%-STm though weak correlation with IgG levels (ρ=0.106 for SEn, ρ=0.345 for STm).

Conclusion: These findings support prioritizing iNTS vaccines in high-risk populations and further assessment of other serological markers and linkage to clinical outcomes. Spatial clustering highlights localized transmission warranting targeted interventions to improve sanitation, water quality and hygiene.

Keywords: Non-typhoidal Salmonella; seroprevalence; spatial clustering; functional immunity; informal settlements.

List of Abbreviations

AOR: Adjusted odds ratio; BRC: Baby Rabbit Complement; BSA: Bovine serum albumin; CBD: Central Business District; CFU: Colony-forming unit; CHVs: Community Health Volunteers; CI: Confidence interval; CMR: Centre for Microbiology Research; ELISA: Enzyme-Linked Immunosorbent Assay; EU: ELISA unit; Fab: Fragment antigen-binding ; GMMA: Generalized Modules for Membrane Antigen; HI: Heat Inactivated; HIV: Human Immunodeficiency Virus; IC50: Inhibitory Concentration50; IgA: Immunoglobulin A; IgG: Immunoglobulin G; iNTS: Invasive Non-typhoidal Salmonella; KEMRI: Kenya Medical Research Institute; L-SBA: Luminescence-based Serum Bactericidal Assay; LB: Luria–Bertani (medium); LISA: Local Indicators of Spatial Association; LLoQ: Lower Limit of Quantification; LMICs: Low- and middle-income countries; LPS: Lipopolysaccharide; LSCA: Limits of Standard Curve Accuracy; MCC: Municipal, County Council Clinic; MDR: Multidrug Resistance; MLW: Malawi-Liverpool-Wellcome; MMM: Medical Missionaries of Mary ; MR: Mukuru kwa Reuben ; MTA: Material Transfer Agreement; MUAC: Mid-Upper Arm Circumference; NACOSTI: National Commission for Science, Technology and Innovation; NTS: Non-typhoidal Salmonella; OAg: O-antigen; OD: Optical Density; OR: Odds Ratio; PBS: Phosphate-Buffered Saline; QC: Quality Control; SBA: Serum bactericidal Assay ; SEn: Salmonella Enteritidis; SERU: Scientific and Ethics Review Unit; ST313: Sequence Type 313; STm: Salmonella Typhimurium; STRATAA: Strategic Typhoid Alliance across Africa and Asia; TLR: Toll-Like Receptor; WASH: Water, sanitation, and hygiene; WHO: World Health Organization.

Introduction

Non-typhoidal Salmonella (NTS) infections significantly contribute to the global disease burden, particularly in low- and middle-income countries (LMICs). These infections, caused mainly by Salmonella enterica serovars Typhimurium (STm) and Enteritidis (SEn), present as enterocolitis or more severe invasive disease [1,2]. In parts of sub-Saharan Africa, NTS serovars are responsible for up to 39 % of community-onset bloodstream infections with case-fatality ratios of 19 % [3]. Of particular concern is invasive non-typhoidal Salmonella (iNTS) disease, which is endemic in sub-Saharan Africa and associated with high morbidity and mortality, particularly among infants and young children, the elderly, and immune-compromised, malnourished, or anaemic individuals [1,4]. Globally, it is estimated that iNTS caused about 76,700 deaths in 2023 with the age-standardized death rate of 1.1 per 100,000 population [5]. Sub-Saharan Africa accounts for a higher burden than other regions, with an estimated incidence of 34.5 cases per 100,000 persons annually and a case fatality rate of 13.5% among children under five years [4,6].

The growing threat of antimicrobial resistance has complicated treatment options for NTS infections. Multidrug resistance (MDR) is increasingly reported, with resistance to commonly used antibiotics, including third-generation cephalosporins and fluoroquinolones [7]. Extensively drug-resistant strains such as Salmonella enterica serovar Typhimurium sequence type 313 (ST313) have caused epidemics of NTS bacteremia [8,9]. The emergence of iNTS lineages with resistance to multiple antimicrobial classes, combined with non-specific clinical presentations, the absence of accurate diagnostics, and the lack of vaccine for NTS in humans, contributes to substantial morbidity and mortality in Africa. The development of effective vaccines and alternative treatment options for NTS is urgently needed. Several vaccine candidates for iNTS including glycoconjugate vaccines, live attenuated vaccines, trivalent iNTS-typhoid conjugate vaccines and the Generalized Modules for Membrane Antigen (GMMA) vaccine are currently in the preclinical or early clinical phases of development [10–12]. However, there is no vaccine licensed to prevent NTS infection.

The immune response to NTS involves both innate and adaptive components, with Immunoglobulin G (IgG) and Immunoglobulin A (IgA) playing a central role in mediating protection [13]. These antibodies recognize specific surface antigens on NTS bacteria, such as the O-antigen of lipopolysaccharides (LPS) and flagellar proteins, and mediate immune protection through various mechanisms. IgG antibodies bind to NTS antigens via their fragment antigen-binding (Fab) regions facilitating opsonization, activating the classical complement pathway and enabling antibody-dependent cellular cytotoxicity [13,14]. IgA antibodies play a pivotal role at the mucosal surfaces, where they neutralize the pathogen, preventing its attachment and invasion into the gut epithelium [15,16].

Despite the importance of these immune mechanisms, there is insufficient information on serological prevalence and correlates of protection against NTS especially in low- and middle-income countries (LMICs) like Kenya. Also, informal settlements such as Mukuru represent unique epidemiologic environments characterized by inadequate access to safe water, sanitation, and hygiene (WASH) infrastructure, high population density, and substantial socioeconomic inequalities [17]. Data on t factors associated with exposure to NTS remain limited. Recent data in these settings indicate that asymptomatic carriage and environmental transmission may contribute to community propagation of invasive iNTS [18,19].Asymptomatic fecal shedding of non-typhoidal Salmonella among apparently healthy individuals highlights the potential role of human reservoirs in sustaining transmission cycles [18]. Additionally, environmental sampling demonstrated higher NTS contamination in household water and effluent associated with infection risk, highlighting the roles of environmental reservoirs and infrastructure in shaping exposure [19]. While hospital-based surveillance and culture-confirmed case reports provide essential data on clinical disease, they inherently underestimate true exposure to the pathogen and transmission dynamics, particularly in settings with limited access to blood culture diagnostics. Understanding the exposure status and serum bactericidal activity of IgG antibodies against NTS is crucial in paving the way for early-phase field trials including the potential introduction of vaccines targeting specific serovars

prevalent in the region.

This study aimed to investigate the seroprevalence, spatial distribution of serological exposure to NTS measured by antibody responses to the O Antigen (OAg) of S. Enteritidis (SEn) and S. Typhimurium (STm) and to assess exposure-associated factors in apparently healthy children under five in Mukuru informal settlement, Kenya. In addition, the study, aimed to correlate serum antibody levels to OAg with bactericidal function against these serovars among the recruited participants.

Materials and methods

Study area

This study was conducted in Mukuru informal settlement, located 20 km east of Nairobi's Central Business District (CBD) and home to about 250,000 persons [15]. About 92% of residents are tenants living in congested 10 x 10 feet metal sheet structures with dirt floors [21]. Most work as casual laborers in nearby industrial areas, while others are hawkers or small-scale business owners. The settlement faces high rates of infectious diseases and mortalities in children under 5 years driven by overcrowding, lack of clean water, consumption of street food, poor sanitation, inadequate sewage systems, open defaecation and use of shared pit latrines [9,17,22]. Mukuru informal settlement is divided into eight villages; Mukuru Sinai, Mukuru Lunga-Lunga, Kosovo, Mukuru kwa Reuben, Mukuru Kayaba, Mukuru North and Mukuru kwa Njenga (Figure 1). This study was conducted within Mukuru kwa Njenga and Mukuru kwa Reuben, two of the larger villages within the settlement.

Source: Map prepared by the author.

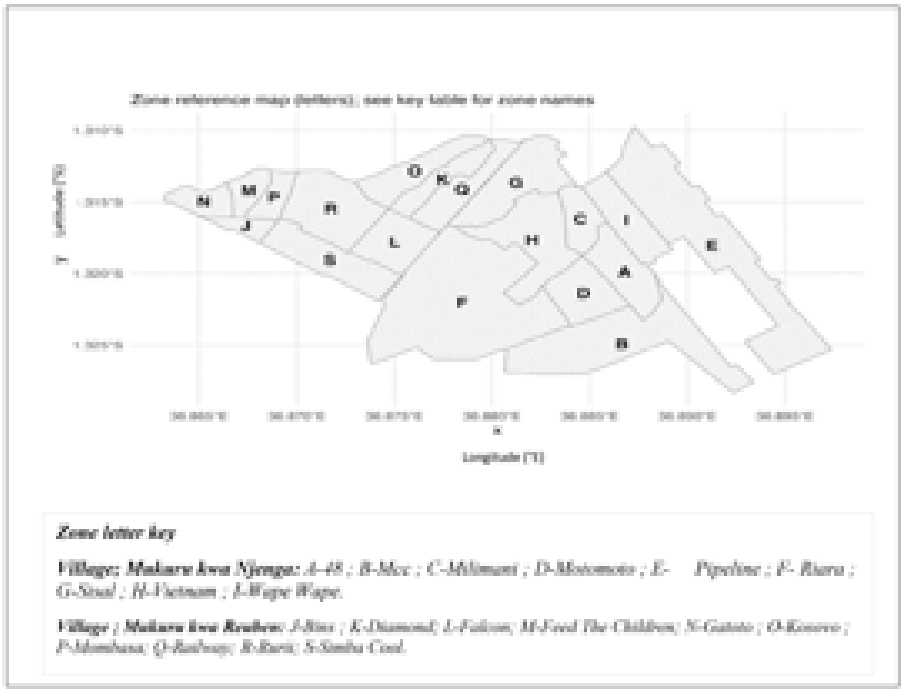

Figure 1: Zone reference map showing letter labels (A–S) for all 19 zones across Mukuru Kwa Njenga and Mukuru Kwa Reuben.

Study design and study participants

The study adopted a cross-sectional study design targeting apparently healthy children aged between 0-<5 years. Apparently healthy was defined as having an axillary temperature between 36.5°C and 37.5°C and no history of fever in the three days preceding recruitment, no visible signs of acute illness. Participants were selected from mapped and censused households within

the catchment area, with equal representation across each 1-year age stratum (0–<1 year, 1–<2 years, 2–<3 years, 3–<4 years, and 4–<5 years). Infants and children whose parents or guardians gave informed consent were eligible for inclusion.

Recruitment

The study employed purposive sampling to select participants. With the assistance of local administration and Community Health Volunteers (CHVs), a list of households was compiled from the mapped and censused populations within the study sites. Households with children aged five years and below were identified and approached for recruitment. Before enrollment, the study team shared detailed information about the study and obtained informed consent from the parents or guardians of eligible children. CHVs played a vital role in disseminating study information to the public and accompanied the research team during field visits. They also helped mobilize study participants, who were subsequently referred to the nearest healthcare centres including the Medical Missionaries of Mary Clinic (MMM), Municipal, County Council Clinic (MCC), and Mukuru kwa Reuben Clinic (MR) for data and sample collection.

Data and sample collection

Following informed consent, axillary temperatures and mid-upper arm circumference (MUAC) were measured thereafter, a questionnaire was administered to the parents and guardians of the eligible study participants at the health facilities. The questionnaire (Supplementary file 1) collected data on participant demographics (age, gender and location), socioeconomic (people in the household, housing, household items, wealth rank and animal ownership), past medical and vaccination history as well as water sanitation and hygiene (WASH) including source of cooking and drinking water and toilet type. Wealth was assessed using a six-step self-reported wealth ladder in which participants judgement of their wealth was based on where they perceived they stood on a six-step ladder, with the bottom step representing the poorest and the top step representing the richest. Water treatment was recorded as a binary variable (treated/untreated). Treatments considered included boiling, use of water purification tablets, filtration, and solar disinfection. Untreated water included water consumed directly from the source without any treatment. From each eligible participant, a venous blood sample of1.5-3mls was collected into a serum separator tube, labelled with the patient's identification number and collection date. The blood samples were transported daily in a cool box (4-8 ℃) to Kenya Medical Research Institute (KEMRI), Centre for Microbiology Research (CMR) where it was centrifuged at 3000rpm for 3 minutes to obtain serum. The serum aliquots were then stored at -80℃ awaiting laboratory analysis.

Laboratory testing

Enzyme-Linked Immunosorbent Assay (ELISA)

The aliquots of 1000 serum samples were shipped to Malawi Liverpool Wellcome Trust Clinical Research Programme (MLW), Malawi for Laboratory analysis. ELISA procedure was performed as described by Aruta et al. (2023) for the detection of IgG against S. Typhimurium and S. Enteritidis in the serum [23]. Briefly, a sufficient amount of each reagent was prepared for all the ELISA steps including coating, blocking, primary antibody, secondary antibody, and substrate. These reagents entailed coating buffer (0.05 M carbonate buffer, pH 9.6 (Sigma-Aldrich), Phosphate Buffer Saline at pH 7 (PBS) used for the preparation of different buffers: PBS + milk 5% (by adding 5% fat-free milk, Sigma, to PBS), washing buffer–PBS-T (by adding 0.05% Tween-20) and secondary antibody buffer by adding 0.1% BSA (Sigma) to PBS-T. Anti-human IgG-alkaline Phosphatase (Sigma) was used as the secondary antibody. The coating antigens used were O-antigens (OAg) extracted by direct acid hydrolysis from GMMA purified from S. Typhimurium and S. Enteritidis ΔtolRΔpagPΔmsbB strains [24]. Anti-STm OAg and anti-SEn OAg specific IgG were measured in sera samples using S. Typhimurium and S. Enteritidis OAg as coating antigens at a final concentration of 5 µg/mL or 15 µg/mL in carbonate buffer, respectively. Coating of ELISA plates was done by diluting the antigen in 1×coating buffer, vortex-mixed the coating solution and poured it into a reagent reservoir. Using a multi-channel

pipette, added 100 µL of the coating solution to each well of Nunc Maxisorp 96-well round bottom (Nunc) ELISA plates. The plates were stack in groups of 3 plates and the first one (of each stack) covered with a plate sealer and left overnight (16 h) at 2°C to 8°C in the fridge. Blocking of the ELISA plates was done by aspirating coating buffer from each well of the first plate using an ELISA washer and then adding 200 µl of blocking solution (PBS +5% Milk) into each well. After 2 minutes, add the blocking solution in the next coated plate until all have been processed. The plates were incubated at 25°C for 1 hour. Meanwhile, the sera dilution (1:100 dilution) in sera dilution plates and standard anti-serum preparation (dilution of the standard sera in diluent which is PBS + milk 5%) was done. The standard was prepared by pooling sera from highly seropositive Malawian adults enrolled in the STRATAA (Strategic Typhoid Alliance across Africa and Asia) epidemiological study [25]. Each serum sample was run in triplicate at different dilutions (1:100, 1:4000 and 1:160000 if needed for high-titer samples) in PBS+ milk 5%. Added 396 µL of diluent in 1:100 dilution plate and 390 µL of diluent in the 1:4000 and 1:160000 if needed. Prepared the initial 1:100 dilution of all sera by adding 4 µl of serum to 396 µl diluent on each well of dilution plate. With clean tips using multi-channel pipette mixed the content of each well. Using the multi-channel pipette, transfered 10ul of diluted test sera from the 1:100 dilution plate to the corresponding wells (with 390ul diluent) in the 1:4,000 dilution plate and mixed the content of each well. The incubated ELISA plates were washed 3 times using PBS-T on an ELISA washer. At the primary antibody coating step, transfered 100 µl/ well of diluted serum from the dilution plate to the same position in the ELISA plate for the first 6 rows, then transfered 100 µl each of the 10 standard sera to rows G and H. Blank wells with only diluent buffer are dispensed in wells G1/G12 and wells H1/H12 of the ELISA plate. Repeated the steps above for each plate leaving 2 minutes between plates. The plates were incubated for 2 h at 25 °C. Before reaching 2 hours of incubation, prepared secondary antibody by adding 1 part of secondary antibody to 10000 parts of antibody dilution buffer (PBS-T +0.1%BSA).The incubated ELISA plates were washed 3 times using PBS-T on an ELISA washer, added 100 µL secondary antibodies to each well of the ELISA plate and incubated for 1 h at 25 °C. To maintain an environment with constant temperature and humidity, minimized the times of opening of the incubator's door. Repeated the steps above for each plate leaving 2 minutes between plates. The incubated ELISA plates were washed 3 times using PBS-T on an ELISA washer, added 100 µL/ well of the p-Nitrophenyl phosphate substrate (Sigma--fast, Sigma-Aldrich, Massachusetts, United States) and incubated for 1 h at 25 °C. Using Biotek automatic plate reader connected to GEN5 software on PC, absorbances were read with a spectrophotometer at 405 and 490 nm and use the difference 405-490 nm for data analysis allowing 2 minutes between plates and ensuring the bottom of the plate is clean.

ELISA units for each plate were calculated by fitting a 5PL curve to the OD of the standard curve composed of 10 standard points and 2 blank wells (run in duplicate on each plate). ELISA unit is defined as the reciprocal of the dilution of the standard serum that gives an absorbance value (optical density (OD) measured at 405 nm subtracted to OD measured at 490 nm) equal to 1. Several QC criteria were applied on each run, in particular, the minimum R-square of 0.96 for the 5 PL curve fit of the standard dilution series, maximum background OD of 0.15, Minimum value for the maximum of the standard curve of 2.6, range of acceptance (0.5-2) in terms of OD for 1 EU/mL. If at least one of the criteria was not met, the entire layout was repeated under the same experimental conditions. Each sample was valid if the EU/mL determined as average from the values obtained in the three independent plates had a CV% < 30%. If not met, the sample was re-run under the same assay conditions. The limits of standard curve accuracy (LSCAs) were set at the nominal value of the standard at the last and the first serial dilutions respectively. The lower limit of quantification (LLoQ) was set as equivalent to lower LSCA multiplied by 100 (the lowest sera dilution tested in the assay). Seropositivity was expressed as a binary variable (1 = positive; 0 = negative) and defined as an antibody concentration greater than the LLoQ for each specific serotype. The LLoQ was 4 EU/ml for S. Typhimurium and S. Enteritidis serovars.

Serum Bactericidal Assay

The luminescence-based serum bactericidal assay (L-SBA) was performed as described by Aruta et al. (2022) to determine the ability of antibodies to mediate complement-mediated killing of bacteria (26). Briefly, bacterial strains namely Salmonella enter-

ica serovar Typhimurium D23580 (a Malawian clinical blood-culture isolate) and Salmonella enterica serovar Enteritidis CMC-C4314 (equivalent to ATCC 4931) were used (26). The bacteria were grown overnight at 37°C in Luria–Bertani (LB) medium stirring at 180 rpm. The overnight bacterial suspensions were then diluted in fresh LB and incubated at 37 °C for 2 hours with 180 rpm agitation in an orbital shaker to log phase (OD: 0.2-0.25) and then diluted to approximately 1 x 106 CFU/mL in phosphate-buffered saline (PBS). The test sera were prepared by aliquoting 30μL of each sample, heat at 56 °C for 30 minutes to inactivate the complement in the sera then store at 2-8 °C. L-SBA was performed in 96-well round-bottom sterile plates (Corning). In each well of the SBA plate, 120 μL sterile PBS was pipetted, 5 μl of the individual Heat Inactivated (HI) sera into the wells of the initial dilution plate then added 125 μl of standard sera into the wells of the standard. The serial dilutions (3-folds) of HI sera were prepared in PBS directly in the SBA plate by adding 25 μl of PBS to each well and adding 25 μl of the diluted sera from the initial dilution plate to wells in A (corresponding wells of the SBA plate). The 12.5 μl volumes from wells A to B, were mixed by gently aspirating and pipetting 3 times. The remaining dilutions were prepared by serially transferring and mixing 12.5 μL to the following corresponding wells (final volume 25μL). The premix (10 μL/well of buffer (PBS), 50 μL/well of Baby Rabbit Complement -BRC (Cederlane, CL3441-S100) and 15 μL/well bacteria at OD 0.20-0.25 pre-diluted 1:60). The reaction mix of 75μL/ well were added to each well of the SBA plate containing HI serum dilutions (final reaction volume 100 μL), mixed, and incubated for 3 h at 37 °C. At the end of the incubation (T180), the SBA plate was centrifuged for 10 min at 4000× g at room temperature. The supernatant containing ATP derived from dead bacteria and SBA reagents was discarded, and the remaining live bacterial pellets were resuspended in PBS (100 μL/well), transferred in a white round-bottom 96-well plate for luminescence reading (Greiner), and mixed at 1:1 (v:v) with BacTiter-Glo Reagent (Promega, Southampton, UK). The reaction was incubated for 5 min at room temperature on an orbital shaker, and the luminescent signal was detected by a luminometer (Synergy HT, Biotek, Swindon, UK). Bacterial luminescence detected is inversely proportional to functional antibody levels in serum. The inhibitory concentration 50 (IC50) was the serum dilution that inhibited 50% of bacterial growth (luminescence reduction). IC50 values were determined using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA). Samples with IC50 ≤4 (LLoQ at 1:100 dilution) were classified as not bactericidal as the killing was not detected at the LLoQ, while those with IC50 ≥4 were bactericidal indicating the serum retained killing activity beyond the starting dilution of 1:100. It should be noted that this threshold is an assay-defined relative measure and has not been correlated to clinical protection outcomes in children.

Data analysis

Statistical analysis was done using R (v4.5.3). Descriptive analysis was presented in frequencies, proportions and graphs. To maintain the sample size (n=996), missing values for IC50, and STM IgG were imputed using the median values for each category. Seroprevalence of NTS exposure (STm, SEn and both serovars combined) was calculated as the proportion of seropositive participants for specific serovars. Spatial coordinates were used to assign participants to predefined administrative zones within the two study villages via spatial join. Spatial clustering of NTS serovars seropositivity at the zone level was assessed using Global Moran's I statistic, with significance evaluated by permutation testing (999 iterations). Local indicators of spatial association (LISA / Local Moran's I) were subsequently computed to identify specific zones contributing to clustering. Zone-level choropleth maps were generated to visualize the geographic distribution of seroprevalence. Zones are identified by letter codes (A–S); see Figure 1 for the full legend. To explore the spatial co-distribution of NTS seropositivity with WASH and socioeconomic indicators, bivariate choropleth maps were constructed using the biscale R package. The mixed-effects logistic regression was used to test associations between NTS exposure and socioeconomic, WASH and medical history variables. Each variable assessed in a multivariable model adjusted for age (continuous, months), sex, caregiver education, household size, food insecurity, household wealth rank, drinking water source, household water treatment practice, toilet type, toilet sharing, recent fever, malaria test result, and HIV exposure status. Results are expressed as adjusted odds ratios (aOR) with 95% confidence intervals (CI) and p-values. Associations were considered to be statistically significant if they achieved a $p < 0.05$. A Spearman correlation was used to assess the relationship between IgG antibody levels and bactericidal activity.

Ethical consideration

Ethical approval for the study was obtained from the Scientific and Ethics Review Unit (SERU) of KEMRI (SERU No. KEMRI/SERU/CMR/P00149/4182 and National Commission for Science, Technology and Innovation (NACOSTI) - NACOSTI/P/21/10162. Permission to ship serum samples to Malawi Liverpool Wellcome Trust Clinical Research Programme (MLW), Malawi for Laboratory was sought from SERU, KEMRI, and shipping was performed under the material transfer agreement (MTA) guidelines. Written informed consent was sought from all parents or guardians of participating children before being recruited into the study. For anonymity, a unique study identification number and barcode label in place of the participant's names was used. All patient data was stored on password-protected computers with authorized access only to the study team.

Results

Socio-demographic characteristics of the study participants

A total of 1000 apparently healthy children were enrolled from the two villages (Mukuru Kwa Njenga and Mukuru Kwa Ruben) in Mukuru informal settlements. The results were available for 996 study participants as shown in (Table 1). The ages ranged from 0 to 60 months with a median age of 30 months with 506 (50.8%) female and 490 (49.2%) males. Most caregivers had attained at least secondary education, 551 (55.3%), followed by primary education, 335(33.6% /), while only 23(2.3% had no formal education. The highest proportion of households were concentrated in lower socioeconomic strata, particularly income Step 2 (poor), 436(43.8 %), followed by the poorest category, 251 (25.2%. Fewer households were in Step 3, 217(21.8 %), and Step 6 (richest), 4 (0.4 %). Animal ownership was low, reported in only 51(5.1 %) of households. The main sources of drinking water were public municipal taps, 503(50.5%), and private vendors, 436(43.8%). More than half of the households, 545(54.7%), reported treating their drinking water. Sanitation facilities were predominantly pit latrines with slabs, 536(53.8%), followed by flush or pour-flush toilets, 300(30.1%), and pit latrines with wooden floors, 155(15.6%). Toilet sharing was highly prevalent, with the vast majority of households, 968(97.2%), sharing toilet facilities with more than three households.

Table 1. Socio-demographic characteristics of the study participants

Characteristic	Frequency (n=996)	(%)
Residence		
Mukuru Kwa Njenga	466	46.8
Mukuru Kwa Reuben	530	53.2
Gender		
Female	506	50.8
Male	490	49.2
Education		
Never	23	2.3
Primary	335	33.6
Secondary	551	55.3
University/College/Tertiary	87	8.7
Wealth rank		
Step 1: Poorest	251	25.2

Step 2	436	43.8
Step 3	217	21.8
Step 4:	74	7.4
Step 5	14	1.4
Step 6 Richest	4	0.4
Animal Ownership		
No	945	94.9
Yes	51	5.1
Source of drinking water		
Public municipal tap	503	50.5
Private tap (individual selling water from their house)	436	43.8
Public municipal borehole (pump handle)	35	3.5
Bottled	13	1.3
Other sources	9	0.9
Water treatment		
Yes	545	54.7
No	451	45.3
Toilet used		
Flush/pour flush	300	30.1
Pit latrine with slab	536	53.8
Pit latrine with wood	155	15.6
Household toilet facility sharing		
Household use only	11	1.1
Shared with 3 households	11	1.1
Shared with more than 3 households	968	97.2

Seroprevalence of IgG antibodies against Non Typhoidal Salmonella (NTS) serovars

The overall seroprevalence of IgG antibodies to both NTS serovars was 900 (90.4%). Specifically, IgG seroprevalence to S. Typhimurium and S. Enteritidis was 959(96.3%) and 932(93.6%), respectively as shown in (Table 2). The difference in the exposure levels to STm and SEn was statistically insignificant (P=0.073). The majority of participants tested positive for IgG antibodies against both serovars indicating previous exposure to NTS.

At the zone level, seroprevalence of IgG antibodies against NTS serovars was generally high across all zones although some variability was observed between zones and serovars as shown in (Table 2). Seroprevalence of STm IgG ranged from 85.4% to 100%, while SEn IgG seroprevalence ranged from 79.6% to 100% across zones. Exposure to both serovars was also high with seroprevalence ranging from 77.1% to 100% across zones. These findings indicate widespread and sustained exposure to NTS serovars among apparently healthy children in this setting.

Table 2. Seroprevalence of IgG antibodies against NTS serovars among children under five years, Mukuru Kwa Njenga and

Mukuru Kwa Reuben villages, Nairobi, Kenya

Village Name; Mukuru kwa Njenga				
Zone	**N**	**STm prevalence (%)**	**SEn prevalence (%)**	**Both STm SEn prevalence (%)**
48 Zone	65	98.4	100.0	98.4
MCC Zone	14	92.9	100.0	92.9
Milimani	25	96.7	96.7	96.7
Motomoto	66	92.3	100.0	92.3
Pipeline	50	94.4	100.0	94.4
Riara	60	98.4	92.2	90.6
Sisal	40	97.4	100.0	97.4
Vietnam	114	96.1	100.0	96.1
Wape Wape	94	98.9	93.6	92.6
Subtotal	528	96.9	97.6	94.5
Village Name; Mukuru kwa Reuben				
Zone	**N**	**STm prevalence (%)**	**SEn prevalence (%)**	**Both STm SEn prevalence (%)**
Bins	19	100.0	88.9	88.9
Diamond	48	85.4	91.7	77.1
Falcon	30	98.1	79.6	79.6
Feed The Children	57	96.0	88.0	84.0
Gatoto	54	100.0	97.8	97.8
Kosovo	37	97.9	91.7	89.6
Mombasa	29	95.5	86.4	81.8
Railway	90	95.9	89.8	85.7
Rurii	76	96.0	86.7	85.3
Simba Cool	29	92.9	89.3	85.7
Subtotal	**469**	96.9	97.6	94.5
Overall		**96.3**	**93.6**	**90.4**

Spatial distribution and clustering of NTS exposure

Spatial analysis revealed substantial heterogeneity in serological exposure across Mukuru informal settlement. Mapping of household-level coordinates further demonstrated localized concentrations of seropositive individuals as shown in Figure 2. Global spatial autocorrelation analysis demonstrated statistically significant clustering of serological exposure. Global Moran's I analysis demonstrated significant positive spatial autocorrelation for both STm and SEn serovars seropositivity ($I = 0.533$, $p = 0.0003$). Also, strong positive spatial autocorrelation was observed in SEn seropositivity ($I = 0.473$, $p = 0.0009$) as shown in (Table 3). In contrast, STm seropositivity showed no significant spatial clustering ($I = -0.073$, $p = 0.539$).

Table 3. Global Moran's I statistics for NTS seropositivity

Outcome	Moran's I	Expected	Variance	P-value

Both (STm & SEn) seropositivity (%)	0.533	−0.059	0.029	0.0003
STm seropositivity (%)	−0.073	−0.059	0.022	0.539
SEn seropositivity (%)	0.473	−0.059	0.029	0.0009

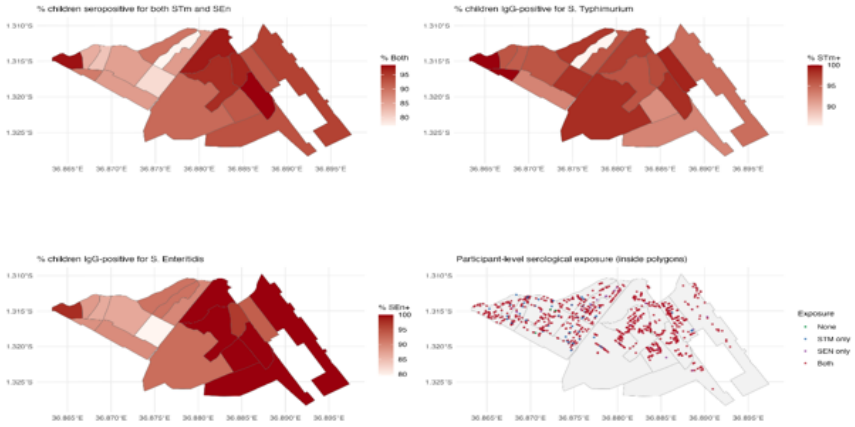

Figure 2. Spatial distribution of seroprevalence to S. Enteritidis (SEn) and S.Typhimurium (STm) serovars among the participants in Mukuru informal settlements

Spatial choropleth maps of NTS seropositivity: Both serovars (top-left), STm (top-right), SEn (bottom-left) seroprevalence results. The map on bottom-right presents the participant exposure with each point representing an individual involved, categorized by exposure status; Red (Individuals exposed to both serovars - S. Enteritidis and S. Typhimurium), Blue (Individuals exposed only to S. Typhimurium {STM}), Purple(Individuals exposed only to S. Enteritidis {SEN}) and Green (Individuals who were not exposed to either serovar).

Local Moran's I Spatial Analysis (LISA) identified several zones in Mukuru Kwa Reuben with statistically significant clusters (hotspots) for both NTS serovars seropositivity, including Rurii (Ii = 0.818, p = 0.003), Kosovo (Ii = 0.048, p = 0.007), and Falcon (Ii = 1.344, p = 0.014). In Mukuru kwa Njenga, Milimani (Ii = 1.099, p = 0.018) and Vietnam Zone (Ii = 0.836, p = 0.022) showed significant clusters (Figure 3). These reflects localized differences in exposure or population immunity.

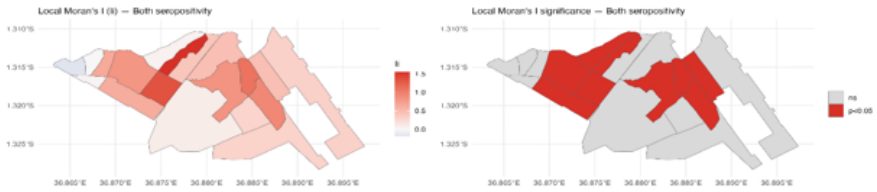

Figure 3. Local Moran's I (Ii) and significance for Both STm and SEn seropositivity, highlighting spatial clusters

Determinants of NTS exposure

Results from mixed-effects logistic regression models for STm, SEn and both serovars combined (STm and SEn) seropositivity are summarised in (Table 4). Age was significantly associated with higher odds of exposure: for both STm and SEn serovars (aOR 1.12, 95% CI 1.07–1.18, p < 0.001), STm (aOR 1.12, 95% CI 1.04–1.21, p = 0.003), and SEn (aOR 1.09, 95% CI 1.03–1.16, p = 0.006). Male children was associated with significantly lower odds of exposure to SEn (aOR 0.48, 95% CI 0.23–0.98, p = 0.045), although no significant association with STm or both serovars seropositivity was observed. Higher household wealth de-

monstrated a protective effect, with children in wealth rank step 5 having significantly lower odds of exposure to both STm and SEn serovars (aOR = 0.11, 95% CI: 0.01–0.90; p = 0.040) and SEn (aOR 0.07, 95% CI 0.01–0.72, p = 0.025), suggesting a modest protective effect of socioeconomic advantage. HIV exposure in children was associated with significantly lower odds of exposure to both STm and SEn serovars (aOR 0.17, 95% CI 0.05–0.59, p = 0.005) and STm (aOR 0.13, 95% CI 0.03–0.52, p = 0.004). A positive malaria test results were associated with significantly lower odds of exposure to both serovars (STm and SEn) (aOR 0.40, 95% CI 0.17–0.97, p = 0.043). These inverse associations is biologically interesting and may reflect confounding by health-seeking behaviour independently reducing NTS exposure risk. Use of pit latrines with wood/soil flooring was associated with significantly lower odds of exposure to STm (aOR 0.35, 95 % CI 0.12–0.99, p = 0.048). No significant associations were observed for source of drinking water for the households, drinking water treatment, Pit latrine with slab, toilet sharing or recent febrile illness. Some variables were reported by very few participants and therefore could not be included in the final model e.g. keeping livestock, medical history data including conditions such as sickle cell anemia and prior receipt of routine vaccines e.g. Typhoid vaccine were each reported by very small number of participants, limiting their suitability for analysis.

Table 4. Risk factors of exposure to NTS.

	Associations with BOTH (STM SEN) exposure		**Associations with STM exposure**		**Associations with SEN exposure**	
Variable	**Adjusted OR (95% CI)**	**p-value**	**Adjusted OR (95% CI)**	**p-value**	**Adjusted OR (95% CI)**	**p-value**
Age (months)	1.12 (1.07–1.18)	0	1.12 (1.04–1.21)	0.003	1.09 (1.03–1.16)	0.006
Sex: Male	0.73 (0.41–1.29)	0.275	1.79 (0.75–4.28)	0.192	0.48 (0.23–0.98)	0.045
Caregiver education						
Never been to school	14271060.76 (0.00–Inf)	0.998	12289514.83 (0.00–Inf)	0.999	5225778.40 (0.00–Inf)	0.998
Primary	1.01 (0.34–3.01)	0.987	0.83 (0.14–4.88)	0.832	0.85 (0.21–3.40)	0.817
Secondary	0.74 (0.27–2.02)	0.554	0.75 (0.14–4.11)	0.739	0.48 (0.13–1.71)	0.258
Household size	0.86 (0.70–1.06)	0.163	0.85 (0.63–1.15)	0.3	0.91 (0.71–1.19)	0.501
Food insecure: Yes	0.87 (0.46–1.65)	0.676	0.71 (0.26–1.90)	0.495	0.95 (0.43–2.12)	0.899
Wealth rank						
Step 2;Poor	0.68 (0.31–1.49)	0.33	0.40 (0.12–1.35)	0.141	0.67 (0.24–1.85)	0.438
Step 3	0.42 (0.17–1.02)	0.054	0.66 (0.15–2.85)	0.576	0.34 (0.12–1.02)	0.055
Step 4	0.34 (0.08–1.44)	0.142	0.44 (0.05–4.19)	0.474	0.30 (0.05–1.72)	0.178
Step 5	0.11 (0.01–0.90)	0.04	13369115.89 (0.00–Inf)	0.999	0.07 (0.01–0.72)	0.025
Step 6;Rich	9713403.91 (0.00–Inf)	0.999	5151490.25 (0.00–Inf)	0.999	3644378.22 (0.00–Inf)	0.999

Drinking water source							
Private tap (individual selling water from their house)	0.00 (0.00–Inf)	0.998		0.00 (0.00–Inf)	0.999	0.00 (0.00–Inf)	0.999
Public municipal borehole (pump handle)	0.00 (0.00–Inf)	0.998		0.00 (0.00–Inf)	0.999	0.00 (0.00–Inf)	0.999
Public municipal tap	0.00 (0.00–Inf)	0.998		0.00 (0.00–Inf)	0.999	0.00 (0.00–Inf)	0.999
Water treatment: Yes	1.40 (0.79–2.48)	0.243		1.15 (0.49–2.70)	0.757	1.75 (0.85–3.59)	0.127
Toilet type							
Pit latrine with slab	0.79 (0.40–1.58)	0.505		1.13 (0.41–3.13)	0.813	1.02 (0.42–2.45)	0.973
Pit latrine with wood/soil floor	0.45 (0.19–1.07)	0.069		0.60 (0.17–2.09)	0.424	0.35 (0.12–0.99)	0.048
Shared toilet							
Shared with 1-3 households	3.99 (0.09–168.84)	0.469		783858111.62 (0.00–Inf)	0.999	0.00 (0.00–Inf)	0.998
Shared with more than 3 households	4.78 (0.26–87.71)	0.292		20.62 (0.90–474.64)	0.059	0.00 (0.00–Inf)	0.998
Recent fever: Yes,	1.07 (0.56–2.03)	0.838		1.45 (0.55–3.86)	0.452	1.12 (0.51–2.47)	0.784
Malaria test positive: Yes	0.40 (0.17–0.97)	0.043		0.72 (0.16–3.18)	0.667	0.37 (0.13–1.07)	0.066
HIV exposed: Yes	0.17 (0.05–0.59)	0.005		0.21 (0.05–0.97)	0.045	0.13 (0.03–0.52)	0.004

The bivariate maps (Figure4) contextualize these zone-level differences against household wealth and sanitation coverage (toilet type) which are significant determinants identified in the regression model. Map A (colour classes represent quantile tertiles for each variable jointly (3×3 biscale scheme); dark teal zones indicate high seropositivity combined with low household wealth) shows that zones with the highest NTS seropositivity and lowest household wealth were concentrated in Mukuru Kwa Njenga, particularly Sisal, Milimani, and 48. Zones with comparatively lower seropositivity were predominantly located in Mukuru Kwa Reuben and tended to have higher relative wealth, including Simba Cool, Mombasa, and Rurii, though seroprevalence remained high even in these zones as shown (Table 2). These findings are consistent with the regression findings that higher wealth rank was associated with lower odds of seropositivity.

Map B (colour classes represent quantile tertiles for each variable jointly (3×3 biscale scheme); dark blue-purple zones indicate high seropositivity combined with low sanitation coverage) reveals a consistent pattern between seropositivity and toilet type. Zones such as Vietnam and Milimani showed high seropositivity alongside relatively lower sanitation coverage. However, several zones with high improved sanitation coverage also retained very high seropositivity e.g. Pipeline and Gatoto indicating that

access to improved toilet facilities does not in itself reduce NTS exposure, likely because shared toilet use, high population density and other environmental contamination pathways persist even where toilet infrastructure is present.

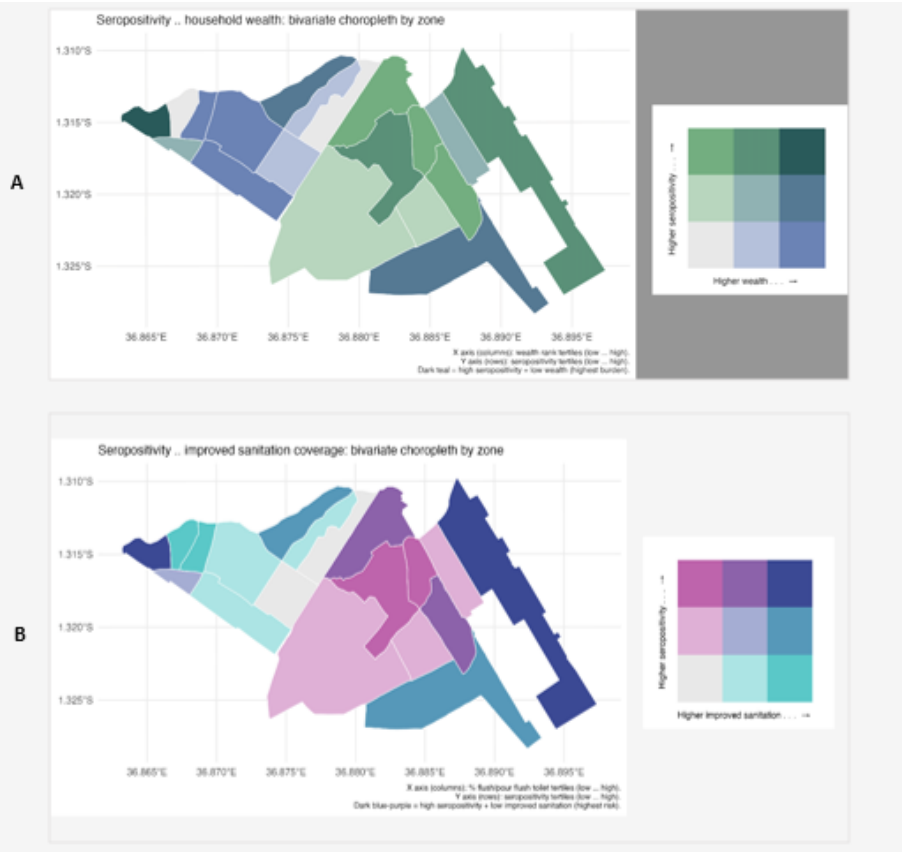

Figure 4. Bivariate maps showing zone-level association between NTS seropositivity and household wealth rank (A) and improved sanitation coverage by toilet type (B) among the participants in Mukuru informal settlements

Serum IgG antibody levels and bactericidal activity as a correlate of protection against NTS serovars

Bactericidal activity was determined by SBA and expressed as IC50 titers. The sera of participants across all ages exhibited relatively high serum bactericidal activity against S. Enteritidis and S. Typhimurium (Table 5). Serum bactericidal activity was observed in 906 (91.0%) of participants against S. Enteritidis and 993 (99.7%) against S. Typhimurium. The differences in the bactericidal activity between different age groups were statistically insignificant (P= 0.582). Results suggest a weak correlation between IgG antibody concentrations and bactericidal killing with a correlation value of ρ=0.106 for S. enteritidis and ρ=0.345 for S. Typhimurium (Figure 5). Interestingly, despite the widespread functional activity of antibodies against S. Enteritidis and S. Typhimurium,and measurable antibodies detected for the serovars, the biological significance of the observed bactericidal activity in terms of protection against clinical iNTS disease remains unclear,

Table 5. Comparison between the serum bactericidal activity against S. Enteritidis and S. Typhimurium in sera of participants in Mukuru Informal Settlement.

	S. Enteritidis					*S.* Typhimurium			
Age(Months)	**N**	**Bactericidal n (%)**	**P-Value**	**aOR**	**95% CI**	**Bactericidal n (%)**	**P-Value**	**aOR**	**95% CI**
0-12	198	175(88.4)	Ref	-	-	196(99)	Ref	-	-
13-24	199	182(91.5)	0.223	1.485	0.786 – 2.804	199(100.0)	-	-	-

25-36	200	191(95.5)	0.022	2.534	1.143 – 5.622	199(99.5)	0.497	2.353	0.200-27.724
37-48	200	174(87.0)	0.495	0.814	0.451 – 1.469	200(100.0)	-	-	-
49-60	199	184(92.5)	0.350	1.389	0.698 – 2.763	200(100.0)	-	-	-

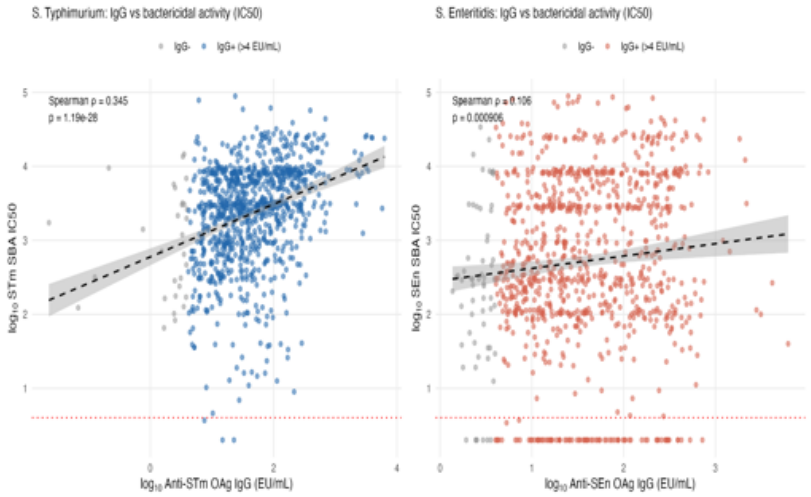

Figure 5. Relationship between anti-O antigen IgG concentrations and SBA against NTS serovars

Scatter plots show the association between $\log_{10}$ IgG levels (EU/mL) and $\log_{10}$ SBA IC50 for S. Typhimurium (left) and S. Enteritidis (right). The individual dots represent seropositivity for each study participant. Dashed regression line with 95% CI shown. Red dotted line represents bactericidal cutoff (IC50 ≤4)

Discussion

This study provides crucial insights into the seroepidemiology of Non-Typhoidal Salmonella (NTS) among children under five years of age in Mukuru informal settlement, Kenya. We report an overall seroprevalence of 96.3%, 93.6% and 90.4% for S. Typhimurium, S. Enteritidis and both serovars combined respectively. The high seroprevalence observed in this study suggests widespread early and frequent exposure to NTS or cross reactivity with other organisms bearing the same O antigens in Mukuru, likely driven by inadequate WASH infrastructure and practice as well unhygienic food handling in homes. According to Mbae et al. (2020) shared sanitation, open defecation, and consumption of street food contributed to enteric pathogen transmission in the informal settlements [17]. Additionally, weaning practices, where children transition from breast milk to potentially contaminated solid foods, could increase early exposure. This findings agrees with the study conducted in healthy Malawian children where by age of 16 months, all had anti-Salmonella IgG antibodies suggesting exposure to NTS [27]. The seroprevalence reported in this study is relatively higher than for a study conducted in Uganda which reported seroprevalence of 82% and 70% for S. Typhimurium and S. Enteritidis respectively [28]. This could be due to differences in environmental settings and age structure among the study participants.

The spatial clustering of seropositivity observed in this study suggests that NTS exposure occurred in localized hotspots. This findings is consistent with recent epidemiological studies demonstrating that transmission of enteric pathogens is shaped by micro-environmental factors, including sanitation infrastructure, water sources, and population density [19,29]. The bivariate

maps further illustrate that zones with the highest seropositivity tend to coincide with relatively lower household wealth and poorer sanitation coverage, reinforcing the role of socioeconomic and structural determinants in shaping the transmission and distribution of NTS.

The findings from this study demonstrates strong association between age and seropositivity which could be attributed to repeated environmental exposure over time driven by increased mobility, dietary changes, and hand-to-mouth behaviour among children, which increase the risk of frequent contact with contaminated food, water, or surfaces. These findings are consistent with findings from a study by Peter et al. (2023) which reported that children in informal settlements exhibit a high seroprevalence of NTS antibodies, with a peak in IgG titers occurring in children under five years [22].

In addition, higher household wealth was associated with significantly lower odds of exposure to both NTS serovars suggesting a protective effect. Income is an indicator of social economic status can impact various aspects of material resources with direct implications for health and can mediate their impacts on enteric disease outcomes [30,31] with variations observed in different population dynamics and geographical regions [32–35]. Male gender was associated with significantly lower odds of exposure to SEn, and this contrasts the findings reported by a study conducted in Netherlands which aimed at understanding the changing epidemiology of invasive non-typhoid Salmonella infection. The study reported that male gender largely influenced increased NTS infection risk [34].

The findings from this study reports that most WASH-related variables, including source of drinking water, water treatment, toilet sharing show no associations with exposure to NTS. This findings are contrary to previous study which attributed unsafe drinking water, sanitation, and hygiene to diarrhoea [36]. Specifically, reports by a studies conducted in the same informal settlement found out that, exposure to NTS was associated with sourcing water from storage containers compared to directly from the taps [17] as well as those who treated their drinking water were less exposed to NTS [22]. On the other hand, the present study identified that the use of pit latrines with wood or soil floors was associated significantly with lower odds of exposure to NTS indicating access to improved sanitation may reflect broader hygiene practice patterns within the home environment, reducing child contact with fecal contaminated surfaces in the immediate living space. This finding contrasts with results from a study conducted in Gondar City, northwest Ethiopia, where increased NTS infection rates were associated with open defecation [37]. These differences may reflect variations in sanitation infrastructure, hygiene practices, and environmental contamination pathways across the study settings.

Children that reported positive malaria test had lower odds of exposure to NTS. Although Nairobi is a non-endemic zone for malaria transmission, participants who reported malaria infection within the past month may have acquired the infection during recent travel to malaria-endemic regions outside the city. This inverse association may be confounded by health-seeking behavior and self-medication dynamics, whereby caregivers promptly access formal healthcare or local pharmacies during a febrile illness. This often results in the empirical administration of broad-spectrum antibiotics and/or antimalarials, which could have inadvertently suppressed subclinical Salmonella colonization. This findings contrasts with the previous study that reported malaria as a risk factor to NTS bacteraemia in children in sub-Saharan Africa [38], though those studies examined invasive disease rather than serological exposure.

The present study findings indicate that exposure to HIV was associated significantly with lower odds of exposure to both serovars. This inverse association may also be confounded by health-seeking behaviour, whereby individuals enrolled in HIV care networks have greater access to clinical services and are more likely to receive cotrimoxazole prophylaxis as recommended by WHO[39]. Cotrimoxazole is broadly active against enteric pathogens, including NTS strains, and its continuous use may suppress subclinical gut colonization [40]. Notably, the present study measures serological exposure rather than clinical disease. These findings contrasts with a study conducted by Jonston et al. which indicate that people living with HIV has higher odds of iNTS disease [41]. In addition, findings from a study conducted in Gauteng Province, South Africa, showed that HIV-infected

patients were more likely to present with invasive S. Typhimurium compared to HIV-uninfected individuals. However, the same study also reported that HIV-infected individuals were less likely to be infected with invasive S. Enteritidis [42].

Despite high seroprevance, we observe a weak correlation between IgG levels and Serum bactericidal activity (SBA); we observed a widespread high SBA, (90.96% of children for SEn and 99.70% for STm) with no noticeable differences between different age groups. However, the clinical significance of the bactericidal activity observed in this study remains unclear, reinforcing the need to validate these findings against clinical outcomes. Nyirenda et al. (2014) found that children with sequential acquisition of SBA to NTS LPS demonstrated a decline in its incidence, potentially contributing to protection against recurrent infections [43].. Moreover, our findings contrasts with previous studies showing reduced SBA activity in neonatal sera against Gram-negative bacteria (44). The weak correlation between IgG levels and functional SBA suggests that passively acquired and naturally acquired IgG antibodies may not be protective against iNTS serovars, reinforcing the need for effective vaccination strategies. This is further supported by study conducted in Malawi which found that, the acquisition of anti–STm lipopolysaccharide immunoglobulin G induced by a glycoconjugate vaccine correlated protection against NTS [43]. These findings are relevant for vaccine development in order to enhance long-term immunity, public health interventions including WASH practices aimed at reducing iNTS exposure in young children. Several vaccine candidates are currently in development, including Generalized Modules for Membrane Antigen (GMMA) vaccines [12], live attenuated vaccines [11] and glycoconjugate vaccines [45].

Young children have an immature immune system that renders them more susceptible to infections. Young children may have reduced neutrophil function, which is crucial for killing bacteria, they also exhibit impaired chemotaxis, phagocytosis, and oxidative burst [46,47]. This reduced activity might explain the failure of IgG antibodies to confer effective bacterial clearance. Also, impaired Toll-Like Receptor (TLR) signaling for example the TLR4, which recognizes lipopolysaccharide (LPS) from Gram-negative bacteria, may contribute to reduced immune responsiveness in neonates, leading to weaker inflammatory responses [48]. Moreover, impaired neonatal macrophages and dendritic cell function where the macrophages have a reduced ability to produce reactive oxygen species and nitric oxide, both of which are important for the intracellular killing of pathogens [49]. Dendritic cells are less efficient in antigen presentation and cytokine production, leading to weaker activation of adaptive immunity [49]. Deficiency in complement factors in young children and neonates i.e.C9 and C8 deficiency may result in the lack of effective bactericidal activity [49]. Deficiencies in these factors may impair complement-mediated lysis of NTS bacteria, contributing to weak IgG -SBA activity. The gut microbiota plays a protective role against Salmonella, but in neonates, the microbiome is still developing and lacks sufficient colonization resistance [50]. Since microbiota composition influences mucosal and systemic immunity, an underdeveloped gut microbiome could impair protective immune responses to pathogens.

This study had certain limitations as assessing IgG antibodies as a serological marker may not fully capture other immune mechanisms, such as T-cell responses and mucosal immunity, which also play a crucial role in protection against NTS. Additionally, there is Potential variability in immune responses due to unmeasured factors such as prior subclinical infections and maternal antibody transfer. Furthermore, the high seroprevalence observed may partly reflect antibody cross-reactivity, as conserved O-antigen LPS shared among different Salmonella serovars could elicit IgG responses, potentially leading to overestimation of serovar-specific exposure. This seroprevalence was based on an arbitrary LLoQ (≥4 EU/mL) rather than proven association of NTS enteric exposure with a serological response, or paired samples with fold increases or with any clinical outcome. Similarly, the SBA IC50 cutoff applied (≥4, equivalent to a 1:100 starting dilution) is a relative measure and has not been correlated against clinical protection outcomes. This highlight the need for further assessment of the serological markers, bactericidal activity and linkage to clinical outcomes. Methodological advances in enteric fever seroepidemiology such as those described by Aiemjoy et al. (2023), which used antibody kinetic modelling from culture-confirmed cases to estimate seroincidence from cross-sectional surveys offer a promising framework that could potentially be adapted for NTS seroepidemiology [51].

Conclusion

This study provides evidence that children in Mukuru informal settlements are highly exposed to NTS bacteria from an early age. The spatial clustering highlights localized transmission dynamics. Despite widespread bactericidal activity detected in the majority of children, the weak correlation between IgG antibody levels and serum bactericidal activity suggests that passively acquired and naturally acquired IgG antibodies may not be protective against NTS. Biological significance of the observed bactericidal activity in terms of protection against clinical iNTS disease needs to be further investigated. This highlights a critical vulnerability in young children which underscores the urgent need for introduction of effective vaccines targeting NTS. This supports the potential introduction of an NTS vaccine in infancy or early childhood to induce protective immunity before peak exposure. Promising candidates, including Generalized Modules for Membrane Antigen (GMMA), glycoconjugate, and live attenuated vaccines, could help reduce NTS-related morbidity. Implementing vaccination programs alongside sanitation and hygiene interventions in endemic areas like informal settlements is essential in reducing NTS morbidity and mortality in high-risk populations.

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Author Contributions.

MN: Conceptualization, Methodology, Investigation, Project administration, Data curation, Visualization, Writing – original draft, Writing – review & editing; SKam: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft; HD: Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing; EK: Methodology, Formal analysis, Visualization, Validation, Writing – original draft, Writing – review & editing; OB: Methodology, Formal analysis, Visualization, Validation, Writing – original draft, Writing– review & editing; OR: Methodology, Writing– review & editing; RC: Methodology, Writing– review & editing; NS: Methodology, Investigation, Writing– review & editing; CMkw: Methodology, Investigation, Writing– review & editing; TN: Conceptualization, Methodology, Writing– review & editing; EKib: Methodology, Investigation, Writing– review & editing; DI: Methodology, Investigation, Writing– review & editing; CK: Methodology, Investigation, Writing– review & editing; RT: Methodology, Investigation, Writing– review & editing; CM: Methodology, Project administration, Writing– review & editing; RO: Methodology, Writing– review & editing; MG: Conceptualization, Writing – review & editing, Resources; SKar: Conceptualization, Writing – review & editing, Supervision, Resources, Funding acquisition.

Ethics approval and consent to participate

All procedures involving human participants complied with the ethical guidelines of the institutional and national research committees and adhered to the 1964 Helsinki Declaration including its amendments or equivalent ethical guidelines. Ethical approval for the study was obtained from the Scientific and Ethics Review Unit (SERU) of KEMRI (SERU No. KEMRI/SERU/CMR/P00149/4182 and National Commission for Science, Technology and Innovation (NACOSTI) - NACOSTI/P/21/10162. Permission to ship serum samples to Malawi Liverpool Welcome Trust Clinical Research Programme (MLW), Malawi for Laboratory was sought from SERU, KEMRI, and shipping was performed under the material transfer agreement (MTA) guidelines.

Written informed consent was sought from all parents or guardians of participating children before being recruited into the study.

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Data availability

The datasets used and analyzed in this study are available from the corresponding author upon request.

Vacc-iNTS Consortium Collaborators

Francis Agyapong (Kwame Nkrumah University of Science and Technology Kumasi); Gianluca Breghi (Fondazione Achille Sclavo); John A. Crump (Centre for International Health, University of Otago, Dunedin 9054, New Zealand); Brama Hanumunthadu (University of Oxford); Liselotte Hardy (Institute of Tropical Medicine Antwerp); Stefano Malvolti (MM Global Health Consulting); Carsten Mantel (MM Global Health Consulting); Christian S. Marchello (Centre for International Health, Unbiversity of Otago, Dunedin 9054, New Zealand); Florian Marks (University of Cambridge); Donata Medaglini (University of Siena); Elena Pettini (University of Siena); Ellis Owusu-Dabo (Kwame Nkrumah University of Science and Technology Kumasi); Maheshi N. Ramasamy (University of Oxford), J. Anthony G. Scott (KEMRI-Wellcome Trust Research Programme); Bassiahi Abdramane Soura (University of Ouagadougou); Tiziana Spadafina (Sclavo Vaccines Association); Bieke Tack (Institute of Tropical Medicine Antwerp).

Conflicts of Interest

The authors declare no conflicts of interest.

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