

Evaluation of Intervention Measures on Controlling the Dissemination of Nontyphoidal Salmonella Disease in Mukuru Informal Settlement, Nairobi County, Kenya.

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Abstract

Nontyphoidal *Salmonella* is the most common bacterial pathogen causing gastrointestinal infection worldwide. Data is limited on the effectiveness of the intervention measures put in place to help control the dissemination of nontyphoidal *Salmonella* disease on Mukuru informal settlement in Nairobi County, Kenya. The aim of the study was to determine the effectiveness of the intervention measures put in place to prevent and control the dissemination of nontyphoidal *Salmonella* disease in Mukuru informal settlement in Nairobi County, Kenya. A cross-sectional study was conducted in patients aged 0-5 years. Nontyphoidal *Salmonella* (NTS) bacteria was cultured from blood and stool collected from the recruited children who reported at three health facilities with symptoms of diarrhea, fever or vomiting and antimicrobial susceptibility testing of the recovered NTS isolates from the stool and blood samples was performed. A questionnaire was administered to obtain relevant demographic, socio-economic and healthcare utilization information. Fisher's test was conducted to determine the difference in the susceptibility patterns between the isolated NTS. Out of 191 collected samples, 3 were found to be positive for *Salmonella* Typhimurium and *Salmonella* Enteritidis. Both *S. Typhimurium* and *S. Enteritidis* isolates were resistant to gentamycin. *Salmonella* Typhimurium was found also to be resistant to tetracycline drug. Significant differences existed between some of the drugs susceptibility patterns ($p= 0.0048$). There are still AMR strains circulating in Mukuru informal settlement. There is still need for the Kenyan government to strengthen knowledge and understanding of AMR through supporting surveillance and research.

Keywords: Nontyphoidal *Salmonella*, antimicrobials, intervention, antimicrobial resistance, Mukuru.

INTRODUCTION

Nontyphoid *Salmonella* (NTS) is the most common bacterial pathogen causing gastrointestinal infection worldwide [1]. Gastroenteritis is associated with approximately 3.4 million annual global cases of NTS disease [2]. In sub-Saharan Africa (SSA), NTS disease occurs in risk group

of patients which comprise of the immunosuppressed individuals due to malignancy, HIV infection, chronic liver disease, sickle-cell or diabetes, steroid use and infants and elderly patients. It is estimated that 65% of iNTS cases in SSA occur in children <5 years of age, with 568/100,000 cases of person-years of observation (pyo) [2]. In 2017, 79% of 535,000 global cases of iNTS were estimated to occur in sub-Saharan Africa with 85% of 77,500 estimated deaths worldwide occurring still in sub-Saharan Africa [3].

The most common cause of nontyphoidal Salmonella infections are Salmonella enterica subspecies enterica serovars Enteritidis and S. Typhimurium [4]. Both Salmonella Typhimurium and Salmonella Enteritidis strains circulating in sub-Saharan Africa (SSA) have different genotypes than the ones circulating in high income countries. The SSA strains are more virulent and human adapted and they convey antimicrobial resistant genes [5; 6]. Salmonella is associated with various symptoms which occur 6-72 hours after ingesting the bacteria and the symptoms include; acute fever onset, nausea, vomiting, abdominal pain, myalgia and headache; however, the paramount manifestation is diarrhea. Fever ranging between 38°C to 39°C and chills are also common. At least two thirds of Salmonella patients do complain of abdominal cramps. The illness lasts for 2-7 days and most persons recover without treatment [7; 8]. NTS disease sometimes can present as a febrile invasive disease in the absence of diarrhea, but with meningitis, bacteremia, or focal infections that, if untreated or inappropriately treated, can be fatal [9]. Due to clinical similarities with other febrile illnesses, early and accurate diagnosis and treatment of non-typhoidal salmonella invasive disease can be difficult. Presentation of the non-specific febrile illness makes early and accurate diagnosis and treatment of non-typhoidal Salmonella a challenge especially for the clinician in low resource setting [10; 9].

The Global Action Plan formed by WHO to aid fight against antimicrobial resistance highlights the significance of optimizing antibiotics use and performing surveillance to strengthen the present knowledge [11]. Emergence of antimicrobial resistance (AMR) continues to threaten treatment of common infectious diseases. Salmonella organisms have been reported to be antimicrobial resistant. Establishing disease status prevalence is the first step to effective disease control [12]. The five objectives of National Action Plan (NAP) are aimed at combating and preventing AMR. The five objectives include; improve awareness and understanding of AMR through education, constructive communication and training, strengthen the knowledge and evidence base through surveillance and research, reduce the incidence of infection through effective sanitation, hygiene, and infection prevention measures, optimize the antimicrobials use in animal and human health and fight against AMR through developed economy for sustainable investment [13]. Most healthcare institutions have been found to indirectly contribute to the increased trend in AMR due to the limited laboratory capacity which causes treatment to be done empirically. Also healthcare access is limited in the informal settlements and also people self-prescribe medication. In the informal settlements, several retail shops have over the counter antibiotics which enhance misuse and abuse of drugs hence contributing to the growing of AMR [14 ;15].

Multi-drug resistance (MDR) to first line drugs in iNTS has been reported in several countries including Kenya and Malawi [16; 17], and in other parts of SSA [18 – 19]. Highly invasive sequence type 313 (ST313) of serovar Typhimurium is multidrug resistant (MDR) which has emerged recently and is mostly associated with large epidemics of NTS bacteremia

than gastrointestinal infections. Serovar Typhimurium sequence type 313 mostly causes transmission between humans and not between animal to human [20]. Emergence of MDR ST 313 also resistant to extended spectrum beta lactams (ESBL) (including ceftriaxone which belong to last-line antimicrobials used to treat severe life-threatening bacterial infection) encoded on a CTX-M-15 gene located on a 304kb plasmid has recently been reported in Kenya and in other parts of SSA including Malawi, South Africa (SA) and Democratic Republic of Congo (DRC) [2]. MDR S. Enteritidis ST11, has also emerged in Kenya [21]. Serovar Typhimurium ST19 is common in Northern America and Europe while ST313 isolates are more commonly circulating in Africa [22].

Mukuru informal settlement where this study was conducted is characterized by poor sanitation, dense population and unreliable water supply. These are elements that create an environment favorable for rapid dissemination of enteric infections and other sanitation-related pathogens through contaminated food and water [23]. Intervention measures suggested in the National Action Plan (NAP) have been put in place in Mukuru informal settlement such as reducing the incidence of infection through effective sanitation, hygiene, and infection prevention measures and also optimizing the antimicrobials use in animal and human health. This pilot study was aimed at evaluating intervention measures on controlling the dissemination of nontyphoidal Salmonella disease in Mukuru informal settlement, Nairobi County, Kenya.

MATERIALS AND METHODS

Study Area

Mukuru informal settlement, located about 15 KM East of Nairobi city center is one of the largest slums in Nairobi County. It has a population of around 250,000 people [24]. High incidence of infectious diseases and increased mortality among those under five years is contributed by insufficient and unclean water sources, overcrowding, inadequate sanitation, poor drainage and solid waste disposal due to the use of public pit latrine [2]. Majority of the Mukuru slum dwellers are casual laborers working in the manufacturing industries situated near the slums, others are hawkers of different items while others run small scale business. 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. The study was conducted in community clinics in Mukuru kwa Njenga and Mukuru kwa Ruben villages. The three selected clinics were Mukuru Ruben Centre (MR), Mukuru city council (MCC) clinic and medical missionary of Mary (MMM) clinic.

Study Subjects and Specimen Collection

Cross-sectional study design was used in this study. 191 consecutive patients presenting for care at three community clinics were approached for participation in the study if they were aged five years or below on the presentation day; had symptoms of diarrhea, fever or vomiting, had axillary temperature of at least 37.5°C, had headache, was a resident in the catchment area and their parents or guardians provided an informed consent form. A questionnaire was administered to the parents and guardians of the eligible study participants (S1 Table). The questionnaire helped provide demographic information and explain the clinical manifestation of the recruited study participant and host and environmental factors associated with NTS disease.

Blood was drawn from the study participants based on guidelines given by institutional review board (IRB) which requires blood to be drawn based on age and weight of a child. 1-2 ml of blood was collected for culture and put into culture media in bactec bottles. Stool/rectal swabs were collected and then transferred to Cary-Blair transport media. The collected blood and stool samples were then stored in a cool box and transported to Kenya Medical Research Institute (KEMRI) Center for Microbiology Research (CMR) laboratories.

Laboratory Procedure

Blood Culture:

The cultured blood in bactec bottles were incubated in BACTEC 9050 Blood Culture System (BD, Franklin Lakes, New Jersey, USA), for up to 5 days and subculture was done to samples which were detected to be positive. The detected positive samples were sub-cultured on MacConkey, blood and chocolate agar and incubated at 37°C for 24 hours. Bacterial isolates were identified using biochemical test on API 20E identification kit (API System, Montalieu Vercieu, France) as per the manufacturer's instructions and specific Salmonella serotype was further identified by species - specific serological tests.

Stool Culture:

Stool or rectal swab was cultured in selenite ficoll (Oxoid, Basingstoke, UK) broth and incubated at 37°C for 24 hours. Broth cultures were sub-cultured on Xylose lysine deoxycholate (XLD) agar and MacConkey agar and incubated at 37°C overnight. Suspected Salmonella bacteria were identified by biochemically testing the non-lactose fermenting colonies in triple sugar iron (TSI), simmons citrate agar, Sulfide Indole Motility (SIM) and urea and was sub-cultured in triple sugar iron (TSI) media. From the subculture bacterial isolates were identified using biochemical test on API 20E test strips and specific Salmonella serotype was further identified by species - specific serological tests.

Antimicrobial Susceptibility Testing (AST):

Antimicrobial susceptibility testing was performed on the NTS isolates using the disk diffusion technique on Mueller- Hinton (MH) agar (Oxoid, Basingstoke, UK). Antimicrobial susceptibility test was performed by applying 2×10⁸CFU/ml of bacterial inoculum to the MH agar surface. Paper antibiotic disks which are commercially prepared with fixed concentration were dispensed on the inoculated MH agar surface. Antimicrobial agents tested included the commonly used antimicrobials in Kenya which include; ampicillin (AMP), ceftazidime (CAZ), nalidixic acid (NA), cotrimoxazole (CTX), ceftriaxone (CRO), cefpodoxime (CPD), sulfamethoxazole-trimethoprim (SXT), amoxicillin clavulanate (AMC), azithromycin (AZM), gentamicin (CN), chloramphenicol (C), tetracycline (TE), ciprofloxacin (CIP) and kanamycin (K). The MH agar plates were incubated for 18h at 37°C prior to determination of results. The antibiotic disks' zones of growth inhibition were measured to the nearest millimeter (Figure 1). The susceptibility of the isolate was related to the zone of inhibition. Clinical and Laboratory Standards Institute (CLSI) 31st edition guidelines were used for the test results interpretation.

Ethical Consideration

This study was carried out on a protocol approved by KEMRI Scientific and Ethics Review Unit (SERU) (SERU 4331/2022). Approval was also obtained from the administration of the hospitals where the study was conducted and from National Commission for science, Technology and Innovation (NACOSTI). All parents or guardians of participating children were

informed of the study objectives and voluntary written consent was sought and obtained before inclusion. A copy of the signed consent was filed and stored in locked cabinets at KEMRI.

RESULTS

Demographic Characteristics of the Study Population

Age was tested whether it was normally distributed using Shapiro-Wilk's test and found that age was not normally distributed since p-value was less than 0.05 ($p < 0.05$). Those aged 49-60 months had the highest number of participants in the study (23.04%) while those aged 0-12 months had the least number of study participants (15.18%) Table 1.

Table 1: Age and gender distribution of study participants

Age	Male	Female	Total
0 - 12	16	13	29
	15.38	14.94	15.18
13 - 24	22	19	41
	11.52	21.84	21.47
25 - 36	21	17	38
	20.19	19.54	19.9
37 - 48	19	20	39
	18.27	22.99	20.42
49 - 60	26	18	44
	25.74	20.69	23.04
Total	104	87	191
Proportion	54.45	45.55	100

Antimicrobial Resistance of the *S. Typhimurium* and *S. Enteritidis* Bacterial Isolates Recovered from the Symptomatic Children's Samples

Following incubation of the MacConkey plates with the paper antibiotic disks, zones of inhibition (Figure 1) formed at the end of the incubation period depending on the susceptibility or resistance of the non-typhoidal Salmonella bacteria to a specific antibiotic.

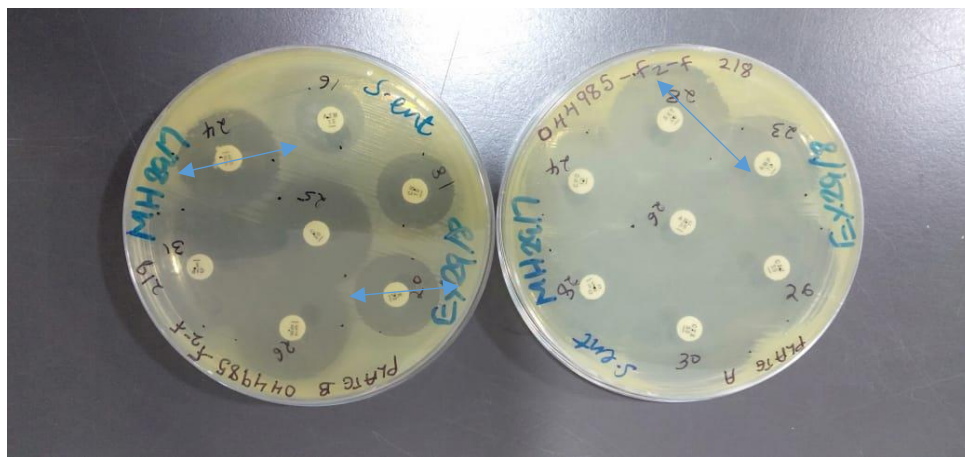


Figure 1: Zone of inhibition by the antibiotics used to test for susceptibility and resistance of the iNTS isolates. The blue arrows indicate the diameters of inhibition zones.

Antimicrobial Susceptibility Profile of the Salmonella Serovars Isolated from the Patient Samples

A total of 3/191 (1.6%) NTS were isolated where *S. Enteritidis* were 2 while *S. Typhimurium* was only 1. Out of the fourteen antibiotics used for antibiotic susceptibility test, *Salmonella Enteritidis* isolates were resistant to gentamycin only while *Salmonella Typhimurium* isolate was found to be resistant to gentamycin and tetracycline drugs (Table 2).

Table 2: Antimicrobial susceptibility profile of the Salmonella serovars isolated from the patients' samples

Antibiotic tested	S. Enteritidis (n=2) No (%) of resistant	S. Typhimurium (n=1) No (%) of	Total No (%) of resistant
Ampicillin	0(0)	0(0)	0(0)
Ceftazidime	0(0)	0(0)	0(0)
Cefotaxime	0(0)	0(0)	0(0)
Ceftriaxone	0(0)	0(0)	0(0)
Cefpodoxime	0(0)	0(0)	0(0)
Trimethoprim + Sulfamethoxazole	0(0)	0(0)	0(0)
Amoxicillin + Clavulanic acid	0(0)	0(0)	0(0)
Ciprofloxacin	0(0)	0(0)	0(0)
Nalidixic Acid	0(0)	0(0)	0(0)
Azithromycin	0(0)	0(0)	0(0)
Gentamycin	2(100)	1(100)	3(100)
Kanamycin	0(0)	0(0)	0(0)
Tetracycline	0(0)	1(100)	1(33.3)
Chloramphenicol	0(0)	0(0)	0(0)

The percentage distribution of the bacterial isolates is illustrated in Table 3

Table 3: Proportion of Salmonella Enteritidis and Salmonella Typhimurium isolates resistance patterns

Study ID	Organism	No. of drugs the organism is resistant to	No. of drugs the organism is susceptible to	% Resistant	% Susceptible
038347	S. Enteritidis	1	13	2.38	30.95
012831	S. Typhimurium	2	12	4.76	28.57
032892	S. Enteritidis	1	13	2.38	30.95

Difference in the Susceptibility Pattern between the Isolated NTS

The difference in the susceptibility patterns between the isolated NTS was not significant as analyzed through Fisher's test ($p > 0.05$). Significant differences existed between some of the drugs susceptibility patterns ($p = 0.0048$). Chi-squared test was performed to determine between which of the drugs the difference existed and found that the difference in both susceptibility and resistance occurred with the gentamycin drug ($p < 0.05$).

DISCUSSION

Among the 14 antimicrobial drugs used belonging to 10 antimicrobial classes, 33% (1/3) of the NTS isolates showed resistance to at least two types of antibiotics used. This finding is lower than the reported 84.1% of Salmonella isolates from patients which were multidrug resistant in Nantong city [4]. The three NTS isolates recovered in the present study displayed resistance to gentamycin which belongs to aminoglycosides class of antibiotics. Salmonella Typhimurium also showed resistance to tetracycline which belongs to class, tetracyclines. The present study findings are in accord with a study done in Tanzania [10] which found that, NTS isolates recovered from stool samples were resistant to tetracycline. The Salmonella Enteritidis isolates recovered in the present study showed same pattern of resistance and susceptibility. The 1.6% NTS prevalence obtained in our present study is almost similar to the findings obtained by a study conducted in the same site which reported NTS prevalence of 1.3% [23].

In Mukuru informal settlement, Nairobi Metropolitan Services (NMS) has contributed towards the prevention and control of NTS and thus AMR by supplying free clean water and controlling buying water which could be contaminated. Also some research centers such as KEMRI has helped supply soap and water guard and have offered education and training to the community on the importance of washing hands with soap after visiting toilets and also treating drinking water using water guard. This prevention and control measures policies and guidelines have been implemented as part of NAP. A lot of surveillance and research has been conducted in Mukuru informal settlement and the researchers have optimized the use of antimicrobial medicine by the people living in the community by discouraging the people from self-prescribing medicine and avoiding over the counter antibiotics which enhance misuse and abuse of drugs hence contributing to the growing of AMR.

CONCLUSION

Salmonella Enteritidis isolates showed resistance to gentamycin only while Salmonella Typhimurium showed resistance to tetracycline and gentamycin. This pilot study therefore concluded that there is still AMR strains circulating in Mukuru informal settlement. The prevalence of NTS reported in this study clearly indicates that even if there is awareness of the intervention measures against the dissemination of NTS, there is still need for the Kenyan government to strengthen knowledge and understanding of AMR through supporting surveillance and research; strengthen the use of antimicrobial agents by making the drugs available at the health facilities; standardize methods of laboratory testing; strengthen hygiene and food safety measures in food value chains and the environment.

Acknowledgement

The authors sincerely acknowledge the field and laboratory staff of the Mukuru kwa Njenga and Mukuru kwa Ruben invasive nontyphoidal Salmonella disease surveillance project for their contribution in this study.

Funding

The authors received no specific funding for this work.

Competing interests

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

S1 Table. Clinical examination data form.

Questionnaire to investigate factors for invasive non-typhoidal Salmonella disease in children living in Mukuru informal settlement

Assessment of exposure to salmonella Enteritidis and Typhimurium O antigen among a population aged 0-5 years attending Mukuru clinics in Nairobi County.

Please answer All questions appropriately

1. Name of the field worker/Nurse _____
2. Date: / / (DD/MM/YYYY)
3. Has parent/guardian provided informed consent for child's participation?
☐ Yes ☐ No
- A. If YES, provide date of Informed consent:
 / / (DD/MM/YYYY)

General Information

3. Who is the respondent?
☐ Mother ☐ Father ☐ Guardian ☐ Other
4. Name of child/participant: _____
5. Phone number where participant /parent/legal guardian can be contacted:
A. First phone number:
B. Second phone number (if necessary):
6. Is the Date of Birth of your child fully or partially known?
☐ Complete (Day-Month-Year) ☐ Partially complete (Year Only)
☐ Partially complete (Month-Year) ☐ Unknown
A. Date of birth of child (if known):
If COMPLETE, provide date: / / (DD/MM/YYYY)
If PARTIALLY COMPLETE, specify month and year: / (MM/YYYY)
If PARTIALLY COMPLETE, specify year: (YYYY)
Age of child __ __ months/years (if DOB is unknown)

Study Inclusion Criteria

	Yes	No
6. Is the participant ≤ 5 years of age?	☐	☐
7. Does the participant reside in this area?	☐	☐
8. Does the parent/guardian agree to have blood samples collected from their child?		

☐ Yes ☐ No

If NO to any of the above, DO NOT proceed with CRF

Study Exclusion Criteria

9. Is the child asymptomatic for invasive non-typhoidal salmonella disease as judged by the study team?

☐ Yes ☐ No

1a) If no in question one above, did you have fever in the past 3 days?

☐ - Yes

☐ 0 - No

☐ 99 - Unknown

1b) Did you take any medication in the last 8 weeks?

0Yes 0No 0not sure

☐ If yes, which medication did you take?

☐ Chloramphenicol	☐ Amoxicillin	☐ Co-Amoxiclav	☐
☐ Ciprofloxacin	☐ Ceftriaxone	☐	☐
☐ Anti-malarial drugs	☐ Others, specify _____	☐ Not	☐ applicable
☐ Don't know			

1c) Does the subject have any past or current symptom of the following in past 4 weeks?

		Yes	No	DK		Yes	No	DK
Respiratory	Cough	☐	☐	☐	Expectoration	☐	☐	☐
	Hemoptysis	☐	☐	☐				
G-I	Vomiting	☐	☐	☐	Diarrhea	☐	☐	☐
	Abdominal Pain	☐	☐	☐	Blood diarrhea	☐	☐	☐
	Distension	☐	☐	☐	Watery diarrhea/colour	☐	☐	☐
Neurology	Headache	☐	☐	☐	Seizures	☐	☐	☐

Socio-Economic Characteristics of the Household

11. Please think about the person who is the head of your household. It maybe you, or it may be someone else. What is the highest educational qualification of the household head? Choose:

- ☐ Never been to school
- ☐ Primary
- ☐ Secondary
- ☐ College/ University/Tertiary
- ☐ Unwilling to answer
- ☐ Unknown

12. Is household head able read and write?

- ☐ Yes
- ☐ No
- ☐ Unwilling to answer
- ☐ Unknown

13. How many people live in your household? Please count all the people who normally live with you and eat meals together. Include yourself when counting. ☐ ☐

13a. How many children less than 5 years of age are there in the household?

14. In the past 7 days, did you worry that your household would not have enough food?

- ☐ Yes ☐ No ☐ Unknown

15. Concerning your housing (structure, form and type), which of the following is true?

☐ It is less than adequate for Household needs ☐ It is more than adequate for Household needs

- ☐ It is just adequate for Household needs ☐ Unknown

16. Concerning your household's clothing, which of the following is true?

☐ It is less than adequate for Household needs ☐ It is more than adequate for Household needs

- ☐ It is just adequate for Household needs ☐ Unknown

17. Imagine six steps, where on the bottom, the first step, stand the poorest people, and on the highest step, the sixth, stand the rich. Show the picture of the steps. On which step are you today?

- ☐ Step 1 ☐ Step 2 ☐ Step 3 ☐ Step 4
☐ Step 5 ☐ Step 6 ☐ Unknown

18. Does your household own a bed?

- ☐ Yes ☐ No ☐ Unknown

19. What does the head of the household sleep on? Training (what is the best bed in the house?)

- ☐ Bed frame and Mattress ☐ Cloth/Sack on floor
☐ Bed frame and Mat (grass) ☐ Floor (nothing else)
☐ Bed frame alone ☐ Other (Specify: _____)
☐ Mattress on floor ☐ Unknown
☐ Mat (grass) on floor

20. Does your household own a table?

- ☐ Yes ☐ No ☐ Unknown

21. Do you, either by yourself or together with another household member or someone outside your household, currently have an account at a bank, credit union, micro finance institution, village savings organization, or another financial institution?

- ☐ Yes ☐ No ☐ Unknown

22. Do you have working electricity in your dwelling?

- ☐ Yes ☐ No ☐ Unknown

23. Does your household own an upholstered chair (arm chair) or sofa set?

- ☐ Yes ☐ No ☐ Unknown

Animal Ownership

24. Does your household keep any animals? ☐ Yes ☐ No

If YES, is it...

A. Companion pet/animal (e.g. Dog, cat etc...): ☐ Yes ☐ No

B. Livestock (e.g. cattle, chicken, goat etc...): ☐ Yes ☐ No

C. If YES to Q22(B), of the following, how many do you have?

☐ Chicken: Number: _____

☐ Cattle: Number: _____

☐ Goat: Number: _____

☐ Pigs: Number: _____

☐ Sheep: Number: _____

☐ Others (Specify): _____

Wash and Sanitation

25. Which source do you use most frequently to get water for cooking?

☐ Public municipal tap ☐ Your own private borehole (pump handle)

☐ Private tap (individual selling water from their house) ☐ Bottled water

☐ Your own tap in your own house ☐ Protected well

☐ Public municipal borehole (pump handle) ☐ Unprotected well

☐ Private borehole (owned by an individual selling water) ☐ River or stream or spring
(unprotected open water source)

26. Which source do you use most frequently to get water for washing clothes?

☐ Public municipal tap ☐ Your own private borehole (pump handle)

☐ Private tap (individual selling water from their house) ☐ Bottled water

☐ Your own tap in your own house ☐ Protected well

☐ Public municipal borehole (pump handle) ☐ Unprotected well

☐ Private borehole (owned by an individual selling water) ☐ River or stream or spring
(unprotected open water source)
☐ Unknown

27. Which source do you use most frequently to get water for bathing?

☐ Public municipal tap ☐ Your own private borehole (pump handle)

☐ Private tap (individual selling water from their house) ☐ Bottled water

☐ Your own tap in your own house ☐ Protected well

☐ Public municipal borehole (pump handle) ☐ Unprotected well

☐ Private borehole (owned by an individual selling water) ☐ River or stream or spring
(unprotected open water source)
☐ Unknown

28. Which source do you use most frequently for drinking?

☐ Public municipal tap ☐ Your own private borehole (pump handle)

☐ Private tap (individual selling water from their house) ☐ Bottled water

☐ Your own tap in your own house ☐ Protected well

☐ Public municipal borehole (pump handle) ☐ Unprotected well

- Private borehole (owned by an individual selling water) ○ River or stream or spring (unprotected open water source)
○ Unknown

29. Do you treat your drinking water before drinking?

- Yes ○ No ○ Unknown

30. If yes to Q30, please specify how the drinking water is treated:

- Use of water guard / bleach/ chlorine tablets ○ Solar disinfection
○ Boiling ○ Purchased water filter
○ Sieve through cloth ○ Others (Specify): _____
○ Unknown

31. What type of toilet is used by your household?

- Flush/pour flush ○ Open defaecation
○ Pit latrine with slab ○ Unwilling to answer
○ Pit latrine with wood/soil floor ○ Unknown

32. Who do you share the toilet facility with?

- Household use only ○ Shared with more than 3 households
○ Shared with 3 households ○ Do not have one (open defecation) ○ Unknown

Past Medical History

33. Medical history taken from (allow multiple select):

- Health card
○ Parental Recall
○ Unknown

Vaccine history	Yes	No	Unknown
BCG	☐	☐	☐
OPV 0	☐	☐	☐
OPV 1, DPT-HepB-Hib1, PCV1 & Rotavirus vaccine 1	☐	☐	☐
OPV 2, DPT-HepB-Hib2, PCV2 & Rotavirus vaccine 2	☐	☐	☐
OPV 3, DPT-HepB-Hib3 & PCV 3	☐	☐	☐
Measles and Rubella 1	☐	☐	☐
Measles and Rubella 2	☐	☐	☐
Additional vaccines? Please Indicate:	☐	☐	

Has your child had fever in the last month?	☐ Yes	☐ No	☐ Unknown
Has your child been tested for malaria in the last month?	☐ Yes	☐ No	☐ Unknown
If yes, what was the result of your child's malaria test?	☐ Positive	☐ Negative	☐ Unknown

If the child tested positive for malaria, did they receive treatment?	☐ Yes	☐ No	☐ Unknown
Is the child HIV exposed? (documentation of the HIV exposure in the H/card)	☐ Yes	☐ No	
Has this child had an HIV test?	☐ Yes	☐ No	☐ Unknown
When the child was last tested for HIV? (if yes to Q above)			
What is the child's HIV status?	☐ Negative		☐ Positive
If positive, is the child on ART treatment?	Yes ☐ No ☐		
Does your child have sickle cell disease?	☐ Yes	☐ No	☐ Unknown
Has your child had anaemia in the last 12 months?	☐ Yes	☐ No	☐ Unknown
Has your child been diagnosed with malnutrition in the last 12 months?	☐ Yes	☐ No	☐ Unknown

General Examination

Axillary Temperature (all age categories)	_____ °C
MUAC	_____ cm

Investigations & Research Samples

SAMPLES COLLECTED FOR RESEARCH					
	Sample	Sample Collection	Sample Volume:	Sample Collection Date (DD/MM/YYYY) And Time (HH:MM)	Lab identifier
53.	Blood	Yes ☐ Yes but Inadequate ☐ No ☐	mls	/ / :	
	Stool	Yes ☐ No ☐			

54. For blood sample, if Yes but inadequate /No to question 53, what is the reason for no sample collection:

- Child has difficult veins
- Unco-operative child
- Sample spilt
- Other (Specify): _____