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Gut Bacteria and Nutritional Status of Nigerian Children at an Internally Displaced Persons' Camp in Benue State, Nigeria: A Pilot Study

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Abstract

This study was conducted to determine the gut bacteria and nutritional status of children (n = 30) aged 2 - 11 in Benue's largest internally displaced persons (IDP) camp since information on this is lacking. Gut bacteria were identified using culture techniques, while Body Mass Index (Kg/m^2), Weight-for-Height (WHZ), and Weight-for-Age (WAZ) z scores were computed from anthropometric measurements. Socio-demographic and economic variables were collected via structured questionnaires. IBM SPSS v25 was used to analyze the data, with $p < 0.05$ considered statistically significant. Children in the camp practiced open defecation, drank from an untreated water source, and suffered from different health conditions. Similarly, the children showed a higher prevalence of the identified bacteria *Salmonella* spp., *Shigella* spp., and *Escherichia coli* compared to children from a nearby private school (n = 10), except for *E. coli*, where the prevalence was equal. The results for BMI revealed that 23 (57.5%) of the children had a healthy weight while 17 (42.5%) were underweight.WAZ z-scores were between (−0.02 - 2.51) with evidence of mildly underweight (20%) and mildly overweight (5%) children. WHZ z-scores were between −0.03 - 2.37, with moderately wasted (30%) and severely wasted (5%) found. To ensure better health outcomes for residents, conditions in the camp must be improved.

Keywords

Nutritional Status, Gut Bacteria, Internally Displaced Persons (IDP), Body Mass Index (BMI), Weight-for-Height (WHZ), Weight-for-Age (WAZ)

1. Introduction

Humans are colonized with a dynamic and marked variation in the population of microbial species, including fungi, bacteria, phages, and viruses, "The Human Microbiome" that inhabits our bodies [1]. Approximately 10^{14} microbial cells colonize the healthy human gut, which is 10-fold more than the total number of cells in the body, with 10^{11} bacteria [2]. While advances in gene sequencing technologies have revealed that the adult microbiota is relatively stable, it is widely recognized that the diversity of human gut microbiota is influenced by dietary habits and nutrition [3] [4] [5] [6] [7]. Similarly, other factors such as antibiotic use [8] [9] mode of delivery at birth [10] [11] [12], and environment [13] can also shape microbiota composition. Several lines of evidence support the key role gut bacteria play in human health. They participate in the *de novo* biosynthesis of essential vitamins that the host cannot synthesize [14]. As an example, Lactic acid bacteria synthesize Vitamin B12 [15]; Bifidobacteria synthesize folate and also contribute to host metabolism [16] [17]. Thus, an imbalance in the gut microbiota is a marker of disease and actively contributes to pathogenesis.

Nutritional status impacts the health, cognition, and academic performance of school-age children [18]. Malnutrition, particularly child under-nutrition is a serious public health issue that persists in low- and middle-income countries [19] such as Nigeria. There are 149.2 million stunted children and 13.6 million wasted children in the world, according to the WHO [20]. It is estimated that 32 percent of under-five children in Nigeria are stunted, accounting for the world's second greatest burden of stunted children [21] [22]. There is also an estimated 2 million children in Nigeria who suffer from severe acute malnutrition (SAM). Studies have found correlations between the gut microbiome and malnutrition in Malawi [23]; Niger and Senegal [24]; and Bangladesh [25]. Children who are undernourished tend to suffer from an altered gut microbiota, decrease in immunity and the emergence of enteropathogenic bacteria [26] [27]. In addition to growth deficits, undernourished children have decreased cognitive and economic potential, and they are more likely to develop chronic diseases later in life [28].

According to UNICEF, 19 million children were internally displaced by conflict and violence in 2019; some of these children were displaced for years [29]. The number of internally displaced people in Nigeria according to the United Nations is approximately three million, ranking first in sub-Saharan Africa and third globally (following Syria with 6.5 million IDPs and Colombia with 5.7 million) [30]. The Agan IDP camp in Benue State is one of the many camps in Nigeria and was set up to cater for individuals that had fled their homes over land disputes for grazing and farming. Poor health and undernutrition are problems experienced by residents in the camp. Until now, there have been limited studies investigating the gut microbiota and nutritional status of individuals in camps in Makurdi, Benue State. The objectives of this study are to assess the nutritional status and gut bacteria composition in thirty children sampled from the Camp and ten children from a selected primary school in Makurdi.

2. Materials and Methods

2.1. Study Design and Area

This cross-sectional study was conducted at the Agan Internally Displaced Persons' (IDP) Camp, located at Agan Ward in Makurdi, Local Government Area of Benue State, North-Central Nigeria. Geographically, the Agan IDP camp is located along the Makurdi-Lafia road, 12 kilometers away from Makurdi, the state capital city of Benue State. The camp is located between the equator's latitudes of 7˚50'4"N and 7˚50'9"N, and the Greenwich meridian's latitudes of 8˚34'40"E and 8˚34'45"E (**Figure 1**). The camp is owned by the Benue State Government and was built primarily to camp individuals displaced by internal conflict. It is the largest in the state. In Makurdi, a rural-urban settlement, the predominant occupations are farming and teaching. The Benue State government feeds residents of the IDP camp. Private individuals and non-governmental organizations also donate food to the camp.

2.2. Ethics Statement

This study was approved by the Ethics Committee of Benue State University Teaching Hospital, Makurdi, Benue State, with approval number (BSUTH/CMAC/HREC/101/V.I/199). The study approval dates are 17th February 2020 and 15th February 2021. Nevertheless, the COVID-19 pandemic-induced lockdown delayed the project start slightly. The parents or legal guardians of the children were approached and educated on the study objectives, and their consent to participate was sought. They were also provided with a written consent form enabling the enrolment and participation of their wards in the study. Confidentiality was maintained throughout the study period.

2.3. Inclusion Criteria

Children were sampled from the Agan IDP camp and a named primary school in Makurdi, and the children were between the ages of 2 and 11 years.

2.4. Exclusion Criteria

Given that antibiotic use can affect gut diversity, children who suffer from long-term health challenges, were ill, or had taken any medication in the month before the study began were excluded from participation.

2.5. Questionnaire

Qualitative data, for example antibiotic use, disease, the sex, and age of each child, food and water sources, family type and household size, parental education level, and toilet facilities in use were collected via a 21-question structured questionnaire (**Appendix**). These were recorded with the help of the caregiver and class teacher (s) for the private primary school children. Also, anthropometric measurements of height and weight were collected. The body weight of each child in light clothing and without shoes was taken using a scale to the nearest

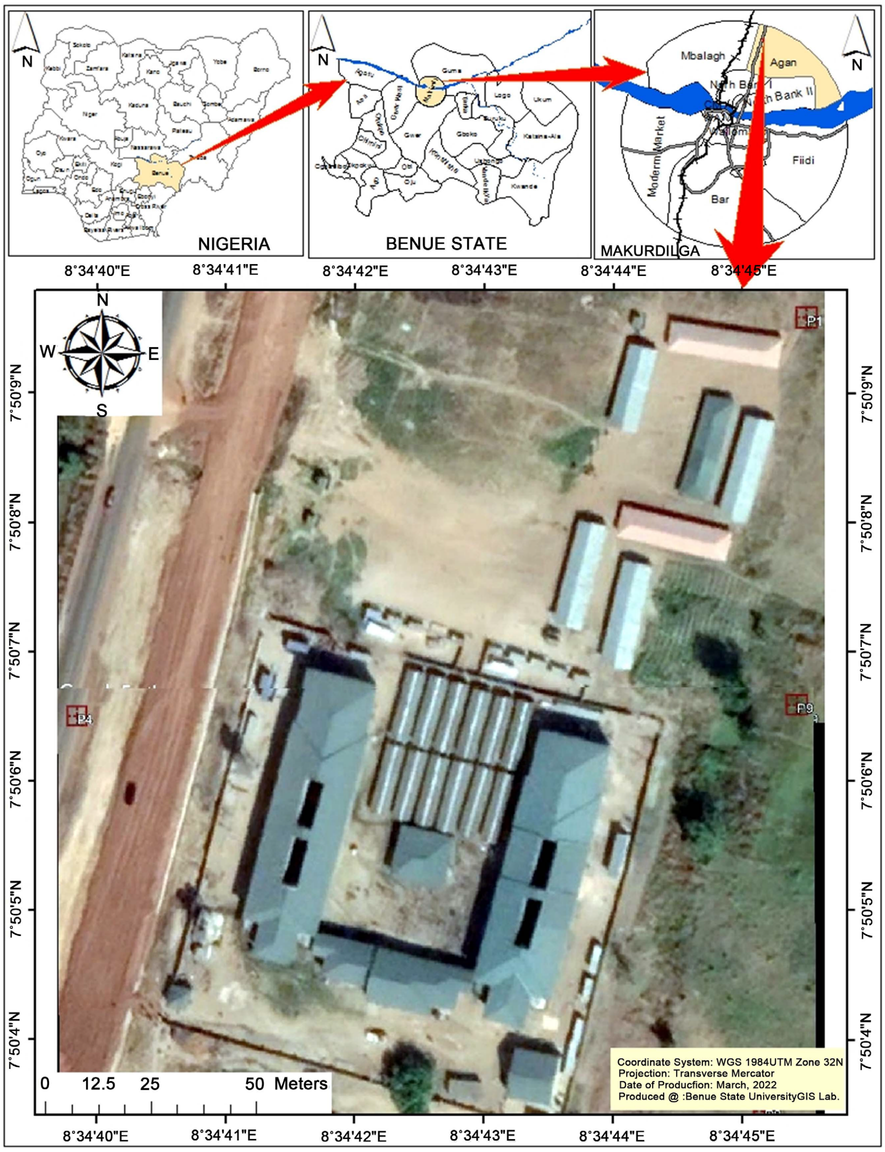

Figure 1. Agan IDP Camp with inset map of Nigeria and Benue State. Source: Benue State University Geographic Information System (GIS) Laboratory.

0.1 kg. Standing height was measured using a constructed vertical wooden rod affixed with graduated tape in cm. Subjects stood barefooted and readings were taken to the nearest 0.1 cm [31].

2.6. Nutritional Status

Nutritional status in the study population was evaluated using body mass index (BMI), weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ). The World Health Organization Child Growth Standards (WHO-CGS) and the WHO Growth Reference 2007 were used to evaluate the nutritional status of young children and schoolchildren respectively [32] [33]. BMI was determined to track the weight status of the population and was calculated as the ratio of each child's weight (kg) divided by his or her height (m^2). The results are expressed in kg/m^2. The weight-for-age (WAZ) classification is as follows: normal weight ($-1 < WAZ < 0$), mildly underweight ($-2 < WAZ < -1$), moderately underweight ($-3 < WAZ < -2$), and severely underweight ($WAZ < -3$). Weight-For-Height (WHZ) is classified as normal ($-1 < WHZ < 0$); marginally wasted ($-2 < WHZ < -1$), moderately wasted ($-3WHZ < -2$), and severely wasted (<-3).

2.7. Faecal Sample Analysis and Bacterial Growth Identification

Faecal samples of volunteers were collected in the morning from 7 - 10 am, using wide-mouthed sterile plastic bottles with tight-fitting lids. The specimen tubes were labelled with the date of collection, sex, and age of each child. On collection, faecal samples were immediately transported in a polystyrene box on ice to the Federal Medical Centre Makurdi laboratory for microbiological analysis within 2 hours. The stool samples were investigated macroscopically for physical appearances (colour, structure: formed, not formed or semi-formed, bloodstains, mucous, etc.), using the Bristol Stool Chart [34]. Blood agar, Salmonella-Shigella agar, and Simmons Citrate agar (HiMedia, Mumbai, India) were prepared according to the manufacturer's instructions. The stool samples of each child were then inoculated on the prepared plates, incubated at 36˚C for 18 - 72 hours, and observed for bacteria growth. Gram stain, motility, catalase, triple sugar iron, indole, and coagulase test were also employed in the identification of bacteria using the procedure by Cheesbrough [35] [36].

2.8. Statistical Analysis

The data was analyzed using IBM® SPSS® v25. An independent sample t-test for means was conducted, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Description of the Study Population

The characteristics of the study population are shown in **Table 1**. In this study, 40 randomly selected children ($n = 40$), corresponding to 30 from the Agan IDP camp and 10 from a selected nursery and primary school (control) in Makurdi metropolis ($t = 2$; $p = 0.295$) were recruited. Twenty-five of the participants (62.5%) were males while fifteen (37.5%) were females ($t = 4$; $p = 0.156$). The age range of the participants was 2 - 11, with the 2 - 4-year-olds (65.0%) dominating the study population, followed by the 5 - 7-year-olds (30.0%), and the 8 - 11-year-old (5.0%) respectively. No statistically significant difference was found ($t = 1.916$, $p = 0.195$). The study populations were from different backgrounds as the camp occupants had been displaced from different villages. Stool consistency was also investigated and the prevalence was 72.5%, 25% and 2.5% for formed, semi-formed, and unformed stool ($t = 1.616$; $p = 0.248$). The weight of the children ranged from 5.6 - 19 kg while their height was between 67 - 119 cm. For parental occupation, the parents of children at the camp were mostly peasant

Table 1. Characteristics of the study population.

Study characteristics	
Sample (IDP Camp)	30
Control (Private School)	10
mean	20
SD	14.14
t	2
p-value	0.295
Sex	
Male	25 (62.5%)
Female	15 (37.5%)
mean	20 (5.0%)
SD	7.07
t	4
p-value	0.156
Age	
2 - 4 years	26 (65.0%)
5 - 7 years	12 (30.0%)
8 - 11 years	2 (5.0%)
mean	13.33
SD	12.06
t	1.916
p-value	0.195
Stool Consistency	
Formed	29 (72.5%)
Semi-formed	10 (25.0%)
Unformed	1 (2.5%)
mean	13.33
SD	14.29
t	1.616
p-value	0.248
Weight	
2 - 4 years	5.6 - 17 kg
5 - 7 years	10.7 - 17 kg
8 - 11 years	16.6 - 19 kg
Height	
2 - 4 years	67 - 114 cm
5 - 7 years	82 - 119 cm
8 - 11 years	118 - 119 cm

Source: Field Survey, 2021.

farmers, while the parents of private school children were civil servants. Due to the lack of previous research on gut bacteria, this population was chosen for this study.

3.2. Questionnaire Survey

Table 2 shows the responses obtained for socio-economic and socio-demographic data from the questionnaires administered to children in the IDP camp and private school. None of the children sampled from the primary school suffered any general health challenges, and had not been on any therapy in the last month prior to sampling. Analysis of the administered questionnaires showed that the children only visited the hospital when they were ill, which was over a month or over a year. The most frequently treated illnesses were malaria, catarrh, and diarrhea. All the children were fed at least three times. The major foods consumed were carbohydrates (bread, yam, and rice), proteins (meat and beans), fruits (watermelon, mangoes, oranges, carrots) and tea. The source of drinking water was sachets or bottled water (90%) or well water (10%). Except for one of the private school participants that practiced open defecation, all the others used water closets. Regarding parents and family type, all the children were from monogamous families. Their parents were literate and educated to a tertiary level. Household size was ≤ 4 and parental occupations were mostly civil servants (50%), teachers (40%), and politicians (10%). Information on combined household income was unavailable for both camp and primary school children. Children in the IDP camps tend to be treated for various health conditions such as typhoid fever, diarrhea, catarrh, ulcers, malaria, and malnutrition. Unlike children from primary school who were fed three times a day, those at the camp ate twice a day, at most. They consumed mostly rice, maize flour, cassava, beans, yam, and sometimes mangoes, and were educated informally in the camp. The drinking water source reported was from a hand-dug well, while pit latrines and open defecation were the most prevalent toilet systems in use. The majority of the children were from polygamous families with family sizes ≥ 5. While a few parents were not educated, the majority had secondary education, while only 10% reported tertiary education. Farming was the predominant parental occupation.

From the nutritional analysis conducted (Table 3), BMI (kg/m^2), weight-for-age (WAZ), and weight-for-height (WHZ) were evaluated. The results for BMI for the total number of children sampled revealed that 23 (57.5%) showed a healthy weight while 17 (42.5%) were underweight. By sex, 56.52% versus 43.48% of males and females respectively were healthy, while for the underweight category, there were 70.59% males and 29.41% females. However, more males than females were sampled in this study. The proportion of mildly underweight and severely underweight children in the population based on WAZ *z scores* is 20% and 5% respectively, whereas WHZ z scores showed that the proportion of children with normal weight for height was 65%, moderately wasted was 30% and severely wasted was 5%.

Table 2. Comparison of questionnaire survey for IDP camp versus private school children.

Criteria	IDP camp	Private school
Frequency of hospital visits	Weekly Monthly	≥1 monthly ≥1 yearly
Common illness treated	Typhoid fever Diarrhea Catarrh Ulcer Malaria Malnutrition	Malaria Catarrh Diarrhea
No. of times fed in a day	≤2	≥3
Major foods consumed	Carbohydrates e.g. rice, yam, maize flour, cassava) Proteins e.g. beans) Fruits e.g. mangoes	Carbohydrates e.g. bread, yam, and rice Protein sources e.g. meat and beans Fruits and vegetables e.g. watermelon, mangoes, oranges, carrot Others tea
Drinking water sources	Hand-dug well	Sachet or bottled water (90%) Well water (10%)
Toilet facility	Pit latrine Open defecation	Water closet (90%) Water closet and open defecation (10%)
Family type	Mostly polygamous	Mostly monogamous
Family size	≥5	≤4
Parental education	Predominantly uneducated or secondary education	Tertiary education
Parental occupation	Peasant farmers	Teachers Civil servants Politician
Combined household income	Unknown	Unknown

Table 3. Nutritional status of the study population.

	IDP Camp	Private School	Population
BMI (kg/m^2)	13.7 ± 1.37	16.12 ± 0.57	14.30 ± 1.61
WAZ	−0.02 - 2.51	−0.09 - 1.06	−0.02 - 2.51
WHZ	−0.09 - 2.36	−0.32 - 0.66	−0.03 - 2.37

BMI values are expressed as Mean and Standard deviation.

Due to the absence of sequencing technologies in Makurdi, the bacteria diversity in the faecal samples of volunteers was assessed by culturing on blood agar, salmonella-shigella agar, and Simmons citrate agar *in vitro*. The results displayed in **Figure 2** show that gut bacteria in the two populations were the same: *E. coli*, *Shigella* spp., and *Salmonella* spp., but *Shigella* spp. was dominant with a prevalence of over 70%.

4. Discussion

This is the first study providing insights into the nutritional status and gut microbiota of children in an IDP camp in Benue State, Nigeria. Although a healthy diet is one of the key predictors of a child's normal growth, the factors that trigger malnutrition are multifaceted. Water, sanitation, hygiene, and also the transmission of diseases such as typhoid and diarrhea have been reported to exacerbate malnutrition. Many families at the camp cannot afford nutritious food and depend on food interventions from the government, non-governmental organizations, and individuals. It was surprising to note that most of the children at the IDP camp had a normal weight, yet 30% of the children in the camp were found to be moderately and severely wasted (5%) when WHZ was assessed. In children, wasting is an indicator of severe undernutrition resulting from poor sanitary conditions, inadequate food consumption or a high prevalence of infections [37]. Consequently, wasted and underweight children are vulnerable and have a higher chance of falling ill with infections or even death due to their low immunity [38]. Similarly, from the WAZ analyses, 20% of the total children sampled were moderately underweight. From this study, the major source of water for children in the IDP camp was from untreated sources. This, together with defecating in the open as well as the use of pit latrines, could result in the variety of illnesses treated, including typhoid, diarrhoea, malaria, ulcers, etc., which could likely explain the proportion of underweight children, reported. There is a correlation between being overweight as a child and having a higher chance of developing obesity in adulthood. Interestingly, 5.0% of the children sampled were overweight. If left unchecked, these children may suffer from chronic non-communicable diseases in later life.

Malnutrition is not always caused by a poor diet but by the gut microflora, which influences nutrient absorption in the gastrointestinal tract [25]. All the bacteria identified, namely *Escherichia*, *Shigella*, and *Salmonella,* belong to the family *Enterobacteriaceae*. Members of the *Enterobacteria* are facultative gram-negative bacteria and are part of the normal human gut resident microflora, though in low numbers [39] [40] [41]. The results of this study appear in line with studies by Ghosh *et al.* [42] that identified *Escherichia* and *Shigella* in Indian children with varying nutritional status. In Bangladeshi children, *Escherichia* and *Salmonella* were found in high numbers in malnourished children [25] by comparison to their healthy counterparts. Contrastingly, Gupta *et al.* [43] reported a higher relative abundance of *Enterobacteria* species in healthy children by comparison to malnourished children. Healthy humans are the major reservoirs of *Shigella* spp. in areas with poor sanitation and overcrowded populations. *Shigella spp.* are the primary pathogens that cause bacillary dysentery in children

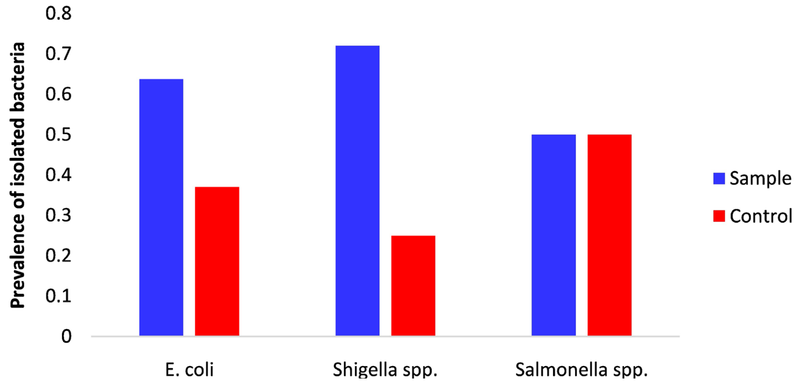

Figure 2. Bacteria identified in the test samples versus control samples.

and are major contributors to stunting [44]. From the results of this study, the prevalence of *Shigella* spp. was nearly three-fold higher in the children at the camp versus their private school counterparts. The growth retardation suffered by stunted children results from nutritional deprivation resulting from the consumption of poor diets or persistent infections that predispose them to illness and mortality. The effects of stunting include reduced cognitive ability, poor academic achievement, and a delay in mental development [45] [46] [47]. Overall, approximately 65.0% of the study population had normal heights from the results of WHZ analyses. But, moderately and severely wasted children were also identified within the population. Though the sampled children appeared healthy, the presence of *Shigella* spp. in our study may partly explain the growth deficit in the children under study. Studies of healthy and malnourished children in Indian children posited that children with a high relative abundance of pathogenic bacteria like *Escherichia* may be associated with subclinical diseases in their gut, with a corresponding inability to absorb enough nutrients from the diet and subsequent diminished health. With the poor sanitary conditions and overcrowding at the IDP camp, it is thus presumable that these could have predisposed the children to suffer malnutrition. Members of the genus *Escherichia* are commensal bacteria and well-established human pathogens with the potential to become opportunistic pathogens, causing gastroenteritis and disease in immune-compromised hosts [48] [49]. Studies by Robertson *et al.* [50] on the human microbiome and child growth in the first three years showed correlation between *E. coli* and severe acute malnutrition.

5. Limitation

Practical financial constraints and the lack of sequencing facilities in Makurdi limited the results obtained from this study. In future, 16s rRNA sequencing targeting the V3 - V4 region will be conducted to reveal the comprehensive gut bacterial repertoire of the study population.

6. Conclusion

The preliminary data presented in this study shed light on the composition of gut bacteria and nutritional status in children living in the largest IDP camp in Benue State, Nigeria. The presence of members of the Enterobacteriaceae family combined with poor diets, overcrowding, and poor sanitation at the IDP camp could be responsible for the myriad of illnesses as well as growth deficits seen in a proportion of the sampled children. Future studies will utilize shotgun metagenomic analysis to identify, to the species level, the microbiota present in their guts and their relationship to the health of the children. We also call on the state government to ensure better nutrition, water sources, education, and sanitation in the camp.

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Author Contributions

IWN designed the study, conducted initial literature and wrote the paper; SNA carried out laboratory work; MEM proofread and carried out initial data analysis.

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Conflicts of Interest

The authors declare that there are no competing interests regarding the publication of this article.

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Appendix

BENUE STATE UNIVERSITY, MAKURDI

Department of Biological Sciences

QUESTIONNAIRE TO OBTAIN PARTICIPANTS INFORMATION

PROJECT TOPIC: GUT BACTERIA AND NUTRITIONAL STATUS OF CHILDREN

STUDY LOCATION: AGAN INTERNALLY DISPLACED CAMP, BENUE STATE, NIGERIA

Dear Respondent, please answer all the questions as honestly and precisely as possible.

SECTION 1: ABOUT THE STUDY

1) Do you have any question to ask the researcher (s)

SECTION 2: ABOUT YOU AND YOUR HEALTH

S/No.	Question	Response				
1	What is your sex?	Male	Female			
2	How old are you?	2 - 5	6 - 8	9 - 11		
3	How often do you visit the hospital monthly?					
4	When last did you visit the hospital/receive treatment?	Within the last 3 days	A week ago	A month ago	Others (Please specify)	
5	What illness did you receive treatment for?	Malaria	Diarrhoea	Typhoid fever	Others (Please specify)	
6	Do you generally suffer any health challenges?	Yes (Please specify)	No			
7	Are you currently on any medication?	Yes (Please specify)	No			
8	Are you currently enrolled in any study? (Formal or Informal)	Yes	No (Give reason)			
9	If yes, where do you study	At the Camp	Public School	Private School	Missionary School	Other (please specify)
10	How many times do you eat in a day?	Once a day	Twice a day	Thrice a day	Others (Please specify)	
11	What type of food is generally served?	Carbohydrates (Please give examples)	Proteins (Please give examples)	Fruits (Please give examples)	Others (Please specify)	
12	What is the source of your drinking water?	Well	Stream	Tap/pipe borne water	Sachet/Bottled water	Others (Please specify)

Continued

13	What kind of family are you from?	Monogamous	Polygamous	Others (Please specify)	
14	Where do you defecate?	Pit toilet	Water closet	Open defecation	Others (Please specify)

SECTION 3: ABOUT YOUR PARENTS

S/NO.				
1	Are your parents literates?	Yes	No	
2	If yes, what is the highest education?	Primary	Secondary	Tertiary
3	What is the main occupation of your parents?	Teachers	Farmers	Others (Please specify)
4	What is the household size?	≤4	≥5	
5	What is the combined household income?			

SECTION 4: DO YOU HAVE ANY MESSAGE FOR THE GOVERNMENT?