CAVENDISH INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY (CIJST)

Open Access

Vol. 1(1): https://journals.cavendish.ac.ug/index.php/cjst/article/view/13/10

August, 2024

RESEARCH ARTICLE

PREVALENCE OF DIARRHEAGENIC BACTERIA AMONG PATIENTS ATTENDING KASHERE GENERAL HOSPITAL, GOMBE STATE

Dahiru, Musa*[1,2]; Aliyu, Habiba Usman[2]

[1*]Department of Public Health, Cavendish University Uganda, Kampala, Uganda.

[2]Department of Biological Sciences, Federal University of Kashere, Gombe State, Nigeria
halibas10@gmail.com (M) or dmusa@cavendish.ac.ug

Abstract

Introduction:
Diarrheal diseases are a major public health concern globally and the leading cause of morbidity and mortality among children in Africa and most other developing countries. Children between the ages of five and below are particularly at high risk of diarrheal diseases. Rural areas in Nigeria are particularly vulnerable to diarrheal diseases due to poor sanitation, unsafe drinking water, poor hygiene practices, and low immunization coverage, among others. Given these, the research wishes to isolate and identify common diarrhea-causing bacteria from stool samples of patients attending Kashere General Hospital in a rural community.

Material and Methods:
Stool samples were collected from patients attending the Hospital and subjected to microbiological analysis using standard procedures for the identification of the targeted bacteria.

Results:
The distribution of age groups of samples collected was high (39.9%) for 0–5 years and 6–10 years (28.6%), across genders, with seven different groups of bacteria identified in male and female samples: *E. coli* sp., *Shigella* sp., *Salmonella* Typhi, *Salmonella* Paratyphi, *Campylobacter* sp., *Vibrios* sp., and *E. coli* O157:H7. While *Campylobacter* sp. was not isolated from any female sample. There was a significant difference in the number of *Shigella* sp. isolates from *Samonella* Paratyphi and *Salmonella* Typhi, *Vibrio* sp., and *E. coli*.

Conclusions:
There is an alarming rate of isolation of *Shigella* among children under five years of age that cuts across genders, and incidence first ever reported prevalence in the study area. This is important in terms of a strategic public health approach to address factors supporting the infection chain for the transmission of diarrheal pathogens in the area. However, further studies may be required to confirm these observations and identify additional risk factors associated with bacterial infections in this population.

Keywords: *Shigellosis, diarrhea, rural areas, Nigeria, Gombe, Kashere*

INTRODUCTION

Diarrheal diseases are a major public health concern globally, and the leading cause of morbidity and mortality among children in Africa and most other developing countries like Nigeria. According to the World Health Organization (WHO), diarrheal diseases are estimated to be responsible for approximately 10% of deaths among children below the age of five in Africa (Manetu et al., 2021; WHO, 2017).

Children between the ages of five and below are particularly at high risk of diarrheal diseases in most low- and middle-income countries, for example Nigeria is reported, among the highest rates of diarrheal disease among children in the world, with an estimated 150,000 deaths annually (Udeh et al., 2021; IVAC, 2020; WHO, 2017). This high rate of diarrheal disease is primarily due to poor sanitation, unsafe drinking water, and poor hygiene practices (Mebrahtom et al., 2022; Bolarinwa et al., 2021; WHO, 2022). Rural areas in Nigeria are particularly vulnerable to diarrheal diseases due to poor sanitation, unsafe drinking water, and poor hygiene practices (Ugochukwu et al., 2020; NBS, 2022)

In many rural areas, people rely on untreated water sources such as rivers, streams, and ponds for drinking, cooking, and cleaning (UNICEF, 2021). These water sources are often contaminated with fecal matter, which can lead to the spread of diarrheal diseases (Holcomb et al., 2020).

Some of the pathogenic bacteria reportedly implicated are Shigella sp., Campylobacter sp., Escherichia coli, Vibrio cholerae, Clostridium botulinum, and Salmonella sp., (Sanyaolu et al., 2020; Ugboko et al., 2020; Kazuhisa et al., 2020). Shigella species and E. coli are among the leading causes of diarrheal diseases in Africa, particularly in developing countries where sanitation and hygiene conditions are poor (Abel et al., 2021; Bilan et al., 2021). Shigella species are a group of bacteria that cause shigellosis, an infectious disease that is characterized by diarrhea, fever, and abdominal cramps (Abel et al., 2021). E. coli, on the other hand, is a type of bacteria that can cause a range of illnesses, including diarrhea (Dela et al., 2022). The prevalence of Shigella species and E. coli as a cause of diarrhea varies across Africa, with some regions reporting higher rates of infection than others (Dela et al., 2022; Hussen et al., 2019).

Regardless of the large burden of diarrheal diseases in Africa, and Nigeria in particular due to continuous poor environmental and water sanitation, overcrowding, and other socioeconomic challenges (NBS, 2022; Ugboko et al., 2020; WHO, 2017) the true nature of diarrheal disease is still underreported, and the few reports available only report research done on urban populations, whose characteristics largely differ from those of the rural population. In fact, to our knowledge, from this research, there is no record of ever reporting the incidence or prevalence of any diarrhea-causing bacteria in the Kashere community and its surroundings. In view of these, the research wished to isolate and identify diarrhea-causing bacteria from stool samples of patients attending Kashere General Hospital, with specific reference to Shigella sp., Vibrio sp., Escherichia coli sp., E. coli O157:H7, and Campylobacter sp. This would complement the existing epidemiological data, which mostly poorly represents the local community, region, and country at large, so that the true picture of the disease can be adequately portrayed.

METHODS AND MATERIALS

Study site and sampling

The study was conducted at Kashere General Hospital located in Kashere town, the town is situated between latitude 9.91230 or 90 54' 44" N and longitude 11.00920 or 110 0' 33" E (Mapcarta. 2023). The target for the research was any patient who reported to the hospital with diarrhea. Patients, parents, or caregivers are informed of the purpose of the research and their consent was sought verbally before the samples were collected. Stool samples were collected from the hospital with the help of laboratory technicians and transported to the Laboratory of Biological Sciences, Department of Federal University, Kashere, through the research focal person for analysis.

Isolation of bacteria

Isolation and identification of unknown bacterial pathogens was conducted using standard bacteriological procedures targeting specific bacterial species or strains capable of causing diarrhea in humans: *Shigella* sp., *Vibrio* sp., *Escherichia coli* sp., *E. coli* O157:H7, *Salmonella* Typhi*, Salmonella* Paratyphi and *Campylobacter* sp. Growth on Sorbitol MacConkey agar (US Biological) supplemented with 0.5% sodium thioglycolate, cefexim, and potassium telurite (CT-SMAC) and a latex agglutination test confirmed the identification of *E. coli* O157:H7. Other tests for the isolation and identification of Campylobacter include the use of an anaerobic jar supplemented with CampyGene (Oxoid), hydrolysis of indoxyl acetate, the hippurate hydrolysis test, and antimicrobial susceptibility testing. For isolation and identification of *Vibrionaceae,* thiosulphate citrate bile sucrose (TCBS) agar (Merck, Germany) was used, and suspected *Vibrio* colonies were subjected to other biochemical tests of salt tolerance, indole test, sugar fermentation, amino acid decarboxylation, the string test, and capsule statin (Koneman *et al*., 1994; Cheesbrough, 2005; Collee, *et al*., 2007).

RESULTS

The results of the number of bacteria isolated from different age groups are presented in the following tables. The distribution of age groups of the samples collected was found to be high (39.9%) for 0–5 years, 6–10 years (28.6%), and 21 years and above (4.2%); however, the distribution of age groups according to gender shows only a small difference of between 1–3% across age groups, with the percentage of males being on the lower side (Table 1). A paired t-test revealed the mean gender difference among age groups at p = 0.05 of the samples collected was (14.8 ± 11.7) for males and (18.8 ± 13.1) for females, t = -5.1, *p = 0.007.* There was a correlation in the distribution of gender of samples collected across age groups of 0.997, *p =.000.*

Table 1: Distribution of gender and age of samples collected from Kashere General Hospital

Age group (years)	Gender		Total (%)
	Male (%)	Female (%)	
0 -5	30 (40.5)	37(39.4)	67 (39.9)
6 -10	22 (29.7)	26 (27.7)	48 (28.6)
11 - 15	15 (20.3)	18 (19.1)	33 (19.6)
16 - 20	5 (6.8)	8 (8.5)	13 (7.7)
≥21	2 (2.7)	5 (5.3)	7 (4.2)
Total	**74 (44.0)**	**94 (55.9)**	**168 (100)**

Table 2: Age distribution of bacterial isolated from Male Diarrhea samples collected from Kashere General Hospital

		No (%) of male samples tested positive for different bacteria from Kashere General Hospital						
Age group (Years)	**Sampled**	***Shigella* sp.**	***Vibrio* sp.**	***E. coli***	***E. coli* O157:H7**	***Campylobacter* sp.**	***Salmonella typhi***	***Samonella paratyphi***
0 -5	30 (40.5)	15 (20.3)	0 (0.0)	22 (29.7)	0 (0.0)	0 (0.0)	12 (15.00)	13 (38.2)
6-10	22 (29.7)	20 (27.0)	0 (0.0)	19 (35.7)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)
11 – 15	15 (20.3)	0 (0.00)	2 (2.7)	13 (17.6)	0 (0.0)	0 (0.0)	7 (8.8)	3 (8.8)
16 – 20	5 (6.8)	3 (4.1)	0 (0.0)	2 (2.7)	0 (0.0)	1 (1.4)	2 (2.5)	0 (0.0)
>21	2 (2.7)	2 (2.7)	0 (0.0)	2 (2.7)	0 (0.0)	0 (0.0)	2 (2.5)	0 (0.0)
	74(44.0)	**40(54.05)**	**2(2.70)**	**58(78.38)**	**2(2.70)**	**1(1.35)**	**23(31.08)**	**16(21.62)**
Mean Standard Deviation		**8±8.99**	**0.4±0.8**	**11.62±9.3**	**0.4±0.8**	**0.2±0.5**	**4.6±4.9**	**3.2±5.6**

Key: sp = species.

Seven different species of bacteria were identified from the male sample, with E. coli sp. (11.6±9.3), Shigella sp. (8±8.99), Salmonella typhi (4.6±4.9), Salmonella paratyphi (3.2±5.6) and Campylobacter sp. (0.2±0.5) respectively. Only two isolates of Vibrios sp. and E. coli O157:H7 were identified in the sample collected between the ages of 6 and 15 years. In general, a high number and percentage of occurrences were observed among samples collected from children between the ages of 0 and 10 years, as was observed in the number of samples per age group that decreased as the age increased (Table 2).

Table 3: Age distribution of bacterial isolated from Female Diarrhea samples collected from Kashere General Hospital

		No (%) of female samples tested positive for different bacteria from Kashere General Hospital						
Age range (Years)	**Sampled**	***Shigella* sp.**	***Vibrio* sp.**	***E. coli***	***E. coli* O157:H7**	***Campylobacter* sp.**	***Salmonella*** Typhi	***Samonella*** **Paratyphi**
0 -5	37(39.4)	30 (31.9)	0 (0.0)	10 (10.6)	0 (0.0)	0 (0.0)	25 (31.3)	10 (29.4)
6 -10	26 (27.7)	22 (23.4)	0 (0.0)	20 (21.3)	1 (33.3)	0 (0.0)	16 (20.0)	8 (23.5)
11 – 15	18 (19.1)	17 (18.1)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	5 (6.3)	0 (0.0)
16 - 20	8 (8.5)	6 (6.4)	0 (0.0)	3 (3.2)	0 (0.0)	0 (0.0)	7 (8.8)	0 (0.0)
>21	5 (5.3)	5 (5.3)	1 (1.1)	2 (2.1)	0 (0.0)	0 (0.0)	4 (5.0)	0 (0.0)
	94 (55.9)	**80(85.11)**	**1(1.06)**	**36(38.29)**	**1(1.06)**	**0(0.0)**	**57(60.63)**	**18(19.15)**
Mean Standard Deviation		**16.0±10.7**	**0.2±0.5**	**7.2±7.9**	**0.2±0.4**	**0.0±0.0**	**11.4±8.9**	**3.6±4.9**

Key: sp = species.

Similar trends were observed among female samples, where six groups of bacteria were identified with *Shigella* sp. (16.0±10.7), *Salmonella typhi* (11.4±8.9) and *E. coli* (7.2±7.9) had the highest percentage occurrence. *Vibrios* sp. and *E. coli* O157:H7 were (0.2±0.5) with only one isolate each, also isolated from sample ages between 6 and 15 years, while *Campylobacter* sp. was not identified from any of the samples. The overall occurrence of bacterial isolates decreases with an increase in the number of ages of samples, with the highest number of occurrences occurring between the ages of 0 and 10 years, as previously observed among male samples (Table 3).The mean difference of the bacterial species and strains identified from the male stool samples collected was conducted using a one-way analysis of variance, and the results show a significant difference at the *p 0.05* level for the seven groups [*F*(2.996, *p = 0.022)]*. A post hoc comparison using (least significant difference) LSD test indicated the mean number of *Shigella* sp. (8.0 ± 8.9) was statistically significant for *Vibrio* sp. (0.4 ± 0.8, p=.043), *E. coli.* (11.6 ± 9.3, p=.043), *E. coli* O157:H7 (0.4 ± 0.9, p=.038), *E. coli.* (11.6 ± 9.3) was also different from *Vibrio* sp. (0.4 ± 0.8, *p*=0.043), *E. coli O157:H7* (0.4 ± 0.9, *p*=0.043), *Campylobacter* sp. (0.2 ± 0.5, *p*=0.004), and *Samonella paratyphi* (3.2 ± 5.6, *p*=0.026) (Table 3). Similarly, the mean difference of the female number of bacterials identified was subjected to one-way analysis of variance, and the results indicate a significant difference at the p = 0.05 level for the seven species of bacteria [*F* (4.298, P = 0.006)]. The post hoc test using LSD indicates the number of *Shigella* sp. (16.0 ± 10.7) identified from five age groups of female samples was statistically significant from *Vibrio* sp. (0.2 ± 0.4, *p = 0.001*), *E. coli* O157:H7 (0.2 ± 0.5, *p* = 0.001), *Samonella paratyphi* (3.6 ± 4.9, *p* = 0.009), and *Vibrio* sp. (0.2 ± 0.4) is statistically different from *Salmonella typhi* (11.4 ± 8.9, *p* = 0.016).

The isolation frequency of different bacterial species was higher among female samples collected with corresponding relative risks for being isolated from females of 94 (RR = 1.27), 80 (RR = 3.08), and 58 (RR = 0.35), as the isolation frequency and relative risk for gender, Shigella sp., Vibrio sp., and E.coli sp., respectively, Salmonella species of Typhi (RR = 1.75), and Paratyphi (RR = 0.9), were also more common among females than males, as shown in the table above. However, Campylobacter was not isolated among the female sample (RR = 0.99), while E. coli O157:H7 was isolated among both genders (Table 4).

Table 4: Kashere General Hospital Gender-wise distribution and occurrence of bacterial Isolation Frequency and Relative Risk

Kashere	Gender	Isolation Frequency		Relative risk
		Positive	Negative	
Isolates	Male	74	94	1.27
	Female	94	74	
Shigella sp.	Male	40	34	3.08
	Female	80	14	
Vibrio sp.	Male	2	72	0.98
	Female	1	93	
E. coli sp.	Male	58	16	0.35
	Female	36	58	
E. coli O157:H7	Male	2	72	0.98
	Female	1	93	
Campylobacter sp.	Male	1	73	0.99
Salmonella typhi				
	Female	0	94	
Salmonella typhi	Male	23	51	1.75
	Female	57	37	
Samonella paratyphi	Male	16	58	0.9
	Female	18	76	

In general, among the bacteria isolated from the samples collected, *Shigella* sp. (DR = 71.4 %) had the highest detection rate and odds rate (OR = 4.86) of no statistical significance, followed by *E. coli* sp. (56.0 %, OR = 0.171), and *Salmonella typhi* (DR = 47.6 %, OD = 3.42). The association of statistical significance between the female samples and the detection of *E. coli* sp. was negative, meaning males have a stronger degree of association with the bacteria (OD =.171, CLL .086, CLU =.342) and *P* = 0.000. Other odds ratios that were observed with *P* values less than 0.05 but were not statistically significant due to high CLs are shown in Table 5 above, except for Campylobacter, where only one isolate was recorded from a male sample.

Table 5: Association and detection rate of bacteria isolated from diarrhea samples collected from Kashere General Hospital

Gender	Sample Number (%)	Frequency (%) of detection of bacteria from Kashere General Hospital						
		***Shigella* sp.**	***Vibrio* sp.**	***E. coli***	***E. coli* O157:H7**	***Campylobacter* sp.**	***Salmonella typhi***	***Samonella paratyphi***
Male	74 (44.0)	40 (54.1)	2 (2.7)	58 (78.4)	2 (2.7)	1 (1.4)	23 (31.1)	16 (21.6)
Female	94 (56.0)	80 (85.1)	1(1.1)	36 (38.3)	1 (1.1)	0 (0.0)	57 (60.6)	18 (19.2)
Detection rate		71.4%	1.8	56.0	1.8	0.6	47.6	20.2

Statistics								
p value	0.029	0.000	0.442	0.000	0.442	00	0.000	0.692
95% CL (lower/upper)	1.049/2.482	2.343/10.069	.034/4.354	.086/.342	.034/4.354	00	1.796/6.498	.403/1.827
Odd ratio	1.614	4.857	.387	.171	.387	00	3.416	.859

DISCUSSION

The results of this study have important public health implications, particularly about the detection rate and prevention of bacterial infections, especially among children and females as well as in providing insight on the high risk of rural population in Gombe state to diarrheogenic bacteria. The high number of bacterial isolates among children under 10 years old suggests that this age group is more susceptible to bacterial infections than older age groups. This finding could be attributed to poor environmental sanitation and body hygiene status of the people in Kashere which is evident in the Multiple Indicator Cluster Survey (MICS) & National Immunization Coverage Survey (NICS) Nigeria 2021 (NBS, 2022). This report observed serious gaps in meeting environmental sanitation, general body hygiene, and provision of portable drinking water as well as low socio-economic status that is further compounded by low educational attainment in Gombe State rural areas. On the other hand, low immunization coverage may have been influence by poor demand creation, which is vital in the acceptability and successful immunization, not only in in rural area but even in urban centers, as well as could be implicated to the high prevalence of these pathogenic bacteria. Therefore, awareness creation through adequate knowledge base among individuals and communities, the advantages, removing vaccine hesitance, understanding the people perception, attitudes, and behaviors on demand, and as well as their beliefs, all of these border on effective communication strategies, which are key to the success of any health program (Bangura *et al.*, 2020; Oku *et al.*, 2017) need to be intensified in the rural areas to increase immunization coverage.

Similarly, health service providers need to be further educated on the importance of breastfeeding to continuously educate nursing mothers and care givers at prenatal and during antenatal visits, on the need for initiation of early breast feeding and continuation of breast feeding as a basis for improving and maintenance of their children immune status, to protect them against childhood killer diseases (Hernández-Cordero *et al.*, 2020). Therefore, there may be a need for targeted public health interventions, such as mop-up or supplementary immunization programs and hygiene education, aimed at protecting under five children and thus reducing the incidence of bacterial infections.

The higher isolation frequency of *Shigella* sp., *E. coli* sp., and *Salmonella* Typhi among female samples indicates that females may be at a higher risk of infection with these bacteria than males. This finding is supported by previous reports that bacteria have been reported to be the main agent causing diarrheal infection in the world, with *Shigella*, *Campylobacter*, *Vibrio cholerae*, *Salmonella* and *Staphylococcus aureus* being the most common among under-five (Sanyaolu *et al.*, 2020; Ugboko *et al.*, 2020). The difference in gender distribution could be due to differences in behavior or exposure to risk factors. Therefore, it may be important to design interventions that are tailored to the specific risk factors and behaviors of females, to reduce the incidence of these infections.

Equally important is the environmental sanitation and hygiene status, which directly influence the chances of child ingestion of contaminated material (food /water/fomites) by fecal matter and subsequently lead to gastrointestinal infection, caused by these bacteria (Ugboko *et al.*, 2020) and going by the characteristic environmental sanitation features of the area of study, a direct correlation can be seen that aid the transmission.

Some of the bacterial identified herein have been implicated as the leading cause of moderate to severe acute diarrhea among children, for example *Shigella*, diarrheogenic *E. coli* and *Salmonella*, this spectrum of bacterial pathogens, although preventable, but poses a challenge in most health facilities for the lack of adequate and proper diagnosis, especially in rural health centers, where state of the art facilities may be lacking or not utilize by the health workers [(Dela *et al.*, 2022; Sanyaolu *et al.*, 2020; Mokomane *et al.*, 2018; Podewils *et al.*, 2004).

The absence of *Campylobacter* sp. and the low rate of isolation of *E. coli* O157:H7 as enterohemorrhagic *E. coli*, in both male and female samples is noteworthy, as these organisms are known to be a common cause of bacterial gastroenteritis. The lack of detection may be due to the small sample size or differences in the study population or the likelihood of regional occurrence. Nonetheless, the results suggest that *Campylobacter* sp. and *E. coli* O157:H7 may not be major contributors to bacterial infections in this population, and further studies may be needed to confirm this observation.

The significant differences observed between the mean of isolates for each bacterial species suggest that some species may be more prevalent in the population than others. For example, *Shigella* sp. and *E. coli* sp. were identified as the most prevalent species in both male and female samples (Dela *et al.*, 2022; Sanyaolu *et al.*, 2020; Mokomane *et al.*, 2018), which suggests that these bacteria may be important contributors to diarrheal diseases in this population. Worthy of noting is the WHO report of 2005, which shows that majority of infections due to *Shigella* occur in developing countries and more than 50% of the cases end up in child death among children under five, ironically less than 1% of these infections due to *Shigella* are treated in hospitals (WHO, 2005; WHO, 2017; WHO, 2022). Going by this report and the isolation rate of *Shigella* sp., shown in this work, it can be declared that there is an outbreak of Shigellosis in Kashere. This finding can be used to design targeted interventions that focus on reducing the transmission of these bacteria.

In summary, the results of this study have important public health implications, particularly in relation to the detection rate and prevention of bacterial infections. The findings suggest that there may be differences in the prevalence of bacterial species between different age and gender groups, which highlights the need for specific targeted interventions to reduce the incidence of diarrhea disease caused by bacteria. Further studies may be needed to confirm these observations and to identify additional risk factors associated with bacterial infections in this population.

DECLARATION

Ethics approval and Consent to participate

This research was approved by research and publications committee of the Federal University of Kashere Gombe FUK/R & D/PRC/4/V1 in accordance with TEFund institutional based research guidelines. For research whose sample are not to be collected within the University, are not required to sought for approval of the University Ethical Committee and therefore this work was only given ethical clearance by Gombe State Ministry for Health MOH/ADM/621/Vol. 1/4178, which is responsible for overseeing all hospitals in the state (where samples were collected from), through the State Health Research Committee, and the same approval was copied to Kashere General Hospital, who do not have Ethical issues committee, for notification and compliance.

Written or verbal participant informed consent, depending on the time given and literacy level, was sought from patients or their parents/guardians/care giver, (in case of minors) who came to the hospital with patient(s), and consent approval was granted in written of verbally, before the sample was collected. Samples were collected in accordance with World Medical Association Declaration/guidelines and processed according to standard laboratory procedures.

Consent for publication

Not applicable

Availability of data and materials

All relevant data are provided in this paper for reference and further guidance.

Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this article in relation to intellectual property right, affiliation and financial support from Nigerian Tertiary Education Trust Funding and Federal University of Kashere for the conduct of this research.

Funding

This research was supported by the Nigerian Tertiary Education Trust Fund and Federal University of Kashere Institutional Based Research Grant. The funding bodies had no role in the designed, collection, analysis, and interpretation of data and in writing this manuscript.

.Limitation

Modern molecular equipment are not available compounded by the lack of constant electric power supply, and the grant was not large enough to source for such services to allow for molecular identification of the biochemically characterized isolates.

REFERENCES

Abel F.N.D.P., Akebe, L. K. A., Daniel, G. A., Rajab, M., Arnfinn, S., Sabiha, Y. E., and Gunnar, S. S. (2021). Burden, Antibiotic Resistance, and Clonality of Shigella sp. Implicated in Community-Acquired Acute Diarrhoea in Lilongwe, Malawi. Trop. Med. Infect. Dis. 2021, 6, 63. https:// doi.org/10.3390/tropicalmed6020063

Bangura, J. B., Shuiyuan, X., Dan, Q., Feiyun, O. and Lei C. (2020). Barriers to childhood immunization in sub Saharan Africa: A systematic review. BMC Public Health (2020) 20:1108 https://doi.org/10.1186/s12889-020-09169-4

Bilan, S. A. N., Nelson, C. M., and Abednego, M. M. (2021). Multidrug-Resistant Shigellosis among Children Aged below Five Years with Diarrhea at Banadir Hospital in Mogadishu, Somalia. Canadian Journal of Infectious Diseases and Medical Microbiology Volume 2021, Article ID 6630272, 7 pages https://doi.org/10.1155/2021/6630272

Bolarinwa, O. A, Zemenu, T. T., James, B. F., Abdul-Aziz, S. and Bright, O. A. (2021). Multi-Level Analysis and Spatial Interpolation of Distributions and Predictors of Childhood Diarrhea in Nigeria. Environmental Health Insights Volume 15: 1–14. https://doi.org/10.1177/117863022110452

Cheesbrough, M. (2005). District Laboratory practice for tropical countries, Part 2, Cambridge University Press, UK, 426 pp.

Collee, J.G., Fraser, A.G., Marmion, B.P. and Simmons, A. (2007). Practical Medical Microbiology. Mackie and McCartney, 4th Edition. Churchill Livingstone, 978 pp.

Dela, H., Beverly, E., Ayodele, O. M., Eric, B., Clara, Y., Dominic, A., Richard, N. A. B., Bassirou, B., Jakob, Z., Langbong, B., and Kennedy, K. A. (2022). Diarrhoeagenic E. coli occurrence and antimicrobial resistance of Extended Spectrum Beta-Lactamases isolated from diarrhoea patients attending health facilities in Accra, Ghana. PLoS ONE 17(5): e0268991. https://doi.org/10.1371/journal.pone.0268991

Hernández-Cordero, S., Ana, L. L., Ana, C. F., Shamah-Levy, T., Matthias, S., Veliz, P. and Izchel, C. (2020). Barriers and facilitators to breastfeeding during the immediate and one month postpartum periods, among Mexican women: a mixed methods approach. International Breastfeeding Journal (2020) 15:87 https://doi.org/10.1186/s13006-020-00327-3

Holcomb, D. A., Jackie, K., Trent, S., Zaida, A., Ellen, de B., Rassul, N., Oliver, C., Joe, B., Jill, R. S. (2020). Human fecal contamination of water, soil, and surfaces in households sharing poor-quality sanitation facilities in Maputo, Mozambique. International Journal of Hygiene and Environmental Health 226 (2020) 113496. https://doi.org/10.1016/j.ijheh.2020.113496

Hussen, S., Getamesay, M. and Zemenu, Y. K. (2019). Prevalence of Shigella species and its drug resistance pattern in Ethiopia: a systematic review and meta-analysis. Ann Clin Microbiol Antimicrob 18:22 https://doi.org/10.1186/s12941-019-0321-1

International Vaccine Access Center (IVAC). Pneumonia & Diarrhea Progress Report 2020: Available from:

https://resourcecentre.savethechildren.net/document/pneumonia-diarrhea-progress-report-2020/

Kazuhisa, O., Warawan, W., Watcharaporn, K., Namfon, S., Piyada, W., Pipat, K., Nuttagarn, C., Witaya, S., Thanee, W., Weerawat, M., Norrathep, A., Patchanee, K., Patpong, U., Chareeya, T., Suwatthiya, K., Lakkana, J., Charoen, J., Supalert, N., Chotipong, S., Pilailuk, A. O., Siriporn, C., Sho, K. and Shigeyuki, H. (2020). Etiologic features of diarrheagenic microbes in stool specimens from patients with acute diarrhea in Thailand. Scientific Reports | (2020) 10:4009 | https://doi.org/10.1038/s41598-020-60711-1

Koneman, W. E., Allen, D. S., Janda, M. W., Schreckenberger, C. P. and Winn, C. J. W. (1994). Introduction to Diagnostic Microbiology. JB Lippincott Company, East Washington Square, Philadiphia. USA, pp 527.

Manetu, W. M., M'masi, S. and Recha, C.W. (2021). Diarrhea Disease among Children under 5 Years of Age: A Global Systematic Review. Open Journal of Epidemiology, 11, 207-221. https://doi.org/10.4236/ojepi.2021.113018

Mapcarta. https://mapcarta.com/ Visited on 5th January, 2023.

Mebrahtom, S., Alemayehu, W. and Daniel, J. G. (2022). The risk of water, sanitation and hygiene on diarrhea-related infant mortality in eastern Ethiopia: a population-based nested case control. BMC Public Health (2022) 22:343 https://doi.org/10.1186/s12889-022-12735-7

Mokomane, M., Kasvosve, I., de Melo, E., Pernica, J. M. and Goldfarb, D. M. (2018). The global problem of childhood diarrhoeal diseases: Emerging strategies in prevention and management. Ther Adv Infect Dis. 5: 29-43.

National Bureau of Statistics (NBS) (2022). Multiple Indicator Cluster Survey (MICS) & National Immunization Coverage Survey (NICS) NIGERIA 2021, Survey Findings Report, August 2022.

Oku, A., Angela, O., Claire, G., Atle, F., Glory, E., Heather, A., Artur, M., Jessica, K., Sophie, H., Julie, C., Yuri, C., Xavier, B., Gabriel, R. and Simon, L. (2017). Factors affecting the implementation of childhood vaccination communication strategies in Nigeria: a qualitative study. BMC Public Health 17:200 DOI 10.1186/s12889-017-4020-6

Podewils, L. J., Mintz, E. D., Nataro, J. P. and Parashar, U. D. (2004). Acute, infectious diarrhea among children in developing countries. Semin Pediatr Infect Dis. 15: 155-168

Sanyaolu, A., Chuku, O., Aleksandra, M., Urooj, J., Stephanie, P., Abdul, J., and Jasmine, M. (2020). Global Epidemiology and Management of Acute Diarrhea in Children from Developing Countries. Ann Pediatr Child Health 2020; 8(8): 1205.

Udeh, E. O., Chineke, H. N., Egenti, N. B, Chizoba, A. F., Igweagu, C. P., Nduka, C., Adogu, P. O. (2021). Sociodemographic Predictors of Diarrhea Disease Among Under 5 Children, Presenting at a Tertiary Health Facility in Nigeria. Hmlyan Jr Comm Pub Hlth. 2: (1).

Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U. and Oyewale, J. O. (2020). Childhood diarrhoeal disease in developing countries. Heliyon. 6: e03690.

Ugochukwu, U., Nwokoro, O. U., Chinemerem, D. O., Izuchukwu, F. O., Murphy-Okpala, N., Chuka, A. (2020). Water, sanitation and hygiene risk factors associated with diarrhoea morbidity in a rural community of Enugu, South East Nigeria. Pan African Medical Journal. 37(115). 10.11604/pamj.2020.37.115.17735

UNICEF Nigeria (2012). 22 March 2021 Press release. Nearly one third of Nigerian children do not have enough water to meet their daily needs. https://www.unicef.org/nigeria/press-releases/

WHO (2017). Diarrhoeal disease. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease

WHO (2022). Drinking water. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/drinking-water

WHO (2005). Guidelines for the control of shigellosis, including epidemics due to Shigella dysenteriae 1. Dysentery, Bacillary - prevention and control 2.Shigella dysenteriae - pathogenicity 3.Disease outbreaks - prevention and control 4.Guidelines I.Title. ISBN 92 4 159233 0 NLM Classification: WC 282) accessed from: https://apps.who.int/iris/bitstream/handle/10665/43252/9241592330.pdf;jsessionid=6BC3E37DEF371BAB8BD77C4989A592AF?sequence=1