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SHIGELLA, E. COLI AND SALMONELLA: A DIARRHEAL PATHOGENS SPECTRUM OF PATIENTS ATTENDING PINDIGA COTTAGE HOSPITAL

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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@gamil.com (M) or dmusa@cavendish.ac.ug

Abstract

Introduction: Bacterial infections significantly contribute to infectious diarrhea, leading to high morbidity and mortality in developing countries. Diarrhea-causing bacteria, including Shigella, Salmonella, and E. coli, are linked to poor sanitation, nutrition, and low immunity, particularly in children. **Methodology:** Patients who reported diarrhea were targeted, and stool samples were collected and analyzed using standard microbiological, cultural, and biochemical techniques for the identification of the targeted bacteria. **Results:** Six different bacteria were identified with a mean number of isolates of 45.5 and 54.5 of the male and female samples, respectively. The most commonly isolated bacteria were: Shigella sp. Mean number of isolates was 7.8±9.5 for males and 13.0±10.3 for females, E. coli sp. The mean number of isolates was 11.0±7.9 and 9.0±4.9 for males and females prospectively. Salmonella typhi were 6.4±4.4 and 6.8±3.6 for males and females, S. paratyphi mean number was 3.8±4.0 males and 3.8±4.9 females, Vibrio sp. was only isolated among males, with an average of 0.2±0.5, and E. coli O157; H7 mean numbers were 0.4±0.9, and 0.2±.4 for males and females. In general, Shigella sp., E. coli sp., and Salmonella typhi were significantly higher than other species, and children aged 0–15 years had a higher detection rate. **Conclusions:** Shigella sp., E. coli and S. typhi were the most frequently isolated bacteria in both male and female samples, especially among children aged 0–15. This emphasizes the need for public health policies and interventions focused on improving sanitation, vaccination, nutrition, addressing gender-specific risk factors, and reducing bacterial infections among children under five.

Keywords: *Public Health, Vibrio, children, gender, Gombe, environmental sanitation.*
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INTRODUCTION

Bacterial infections have remained the biggest contributor to infectious diarrhea associated with high levels of mobility and mortality in developing countries. Diarrheogenic bacteria are usually associated with poor personal and environmental sanitation, poor nutrition, and low immunity, especially among children, whose susceptibility is increased by a lack of immunization and poor breastfeeding (Agegnehu *et al.*, 2019; Baye *et al.*, 2021). *Shigella* is a gram-negative, facultative, rod-shaped member of the family Enterobacteriaceae. *Shigella* has diverse antigenic determinants that comprise four species. *Shigella dysenteriae* with 14 well-established serotypes, *Shigella flexneri* 15 serotypes, *Shigella boydii* 19 serotypes, and *Shigella sonnei* serotype (Kotloff *et al.*, 2017). It is distributed worldwide, and the infection affects people of all ages and genders but is more prevalent and endemic in children under five living in low-income communities. *Shigella* causes acute diarrhea and dysentery, characterized by abdominal cramps, frequent, scanty stools, fever, and prostration (Abdu *et al.*, 2013; Vubil *et al.*. 2018). Several serotypes are multidrug-resistant and spread widely by travelers. Outbreaks are associated with contaminated food and water, poor personal hygiene, overcrowding, nursing homes, and daycare centers (Ngoshe *et al.*, 2017). It is characterized by a very low infective dose of between 10-500 cells, depending on the species (Pakbin *et al*., 2023). *Shigella* is known to secrete Shiga-like toxins, such as verotoxins and verocytotoxins. Diarrheogenic *E. coli*, especially O157, has also been reported to secrete similar toxins (Pakbin *et al*., 2023).

Salmonella bacteria are widely distributed in domestic and wild animals and can be transmitted through contaminated food of animal origin and vegetables through the fecal-oral route (WHO 2018a). The infection is mild, but it can be life-threatening depending on host factors and the serotype involved. Antimicrobial resistance is a global public health concern, and Salmonella is one of the microorganisms in which some resistant serotypes have emerged and is thus very important in food chain monitoring and surveillance (WHO 2018b; WHO 2023). High Salmonellosis-related deaths occur in Sub-Saharan Africa; the estimated actual burden of human Salmonellosis is difficult to determine due to insufficient nation-wide accurate epidemiological data and statistics in Nigeria; the few and sparsely reported among geopolitical zones are mostly serologically dependent (Akinyemi et al 2021). There was a significantly low report of only 3.8 percent Salmonella from the North Eastern region as of 2018, and these were not from Gombe State (Akinyemi *et al*., 2021; Marchello, *et al*., 2021; WHO 2018b).

The WHO has to call for a country-wide strategy to obtain precise disease data regarding Salmonella bacteremia and non-invasive Salmonella diseases (WHO, 2023). Given these growing needs for providing accurate epidemiological information on diarrhea pathogens from rural communities, which constitute the highest percentage of people in Gombe. Little attention is given to diarrheal pathogens in terms of research coverage, notwithstanding their importance in providing and constituting an actual public health information source in the region. It is on this background this work aimed at identifying some of the common bacterial diarrhea pathogens from stool samples of patients attending Pindiga Cottage Hospital. This will serve to fill an epidemiological data gap from this location and population, whose health outcomes were never shared with the outside world for policy guidance or any public health intervention, as was previously observed by the WHO in some reports.

METHODS AND MATERIALS

Study site and sampling

The study was conducted at Pindiga Cottage Hospital, located in Pindiga town, which is situated 45 kilometers away from the Gombe State capital on latitude 9.98330 or 90 59’ N and longitude 10.93330 or 100 56’ E. The study targeted only patients who reported to the hospital with diarrhea.

A total of 143 stool samples were collected from the study site. Patients, their parents, or caretakers are informed of the purpose of the research, and their consent is sought verbally before samples were collected. Stool samples were collected in sterile sample bottles and placed on ice packs while being transported from the hospitals between the months February to April, 2022, with the help of laboratory technicians and transported through the research focal person, to the Laboratory of Biological Sciences Department of Federal University Kashere for analysis. Epidemiological information of the patients was collected including date of visit to the hospital, age, and sex. Participants were discharged after sample collection and could not be identified thereafter.

Isolation of pathogenic bacteria

Isolation and identification of human pathogenic bacteria were carried out using standard bacteriological procedures aimed at specific bacterial species known to cause diarrheal infections: *Shigella* spp., *Vibrio* spp., *Escherichia coli* spp., *E. coli* O157:H7, *Salmonella typhi* and *paratyphi,* and *Campylobacter* spp. Growth on Sorbitol Mac Conkey 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. Specific tests for *Campylobacter* were the use of an anaerobic jar supplemented with CampyGene (Oxoid), the hippurate hydrolysis test, the hydrolysis of indoxyl acetate, and antimicrobial susceptibility testing. Other tests for *Vibrionaceae* were thiosulfate citrate bile sucrose (TCBS) agar growth (Merck, Germany), salt tolerance, the indole test, sugar fermentation, amino acid decarboxylation, the string test, and capsule statin (Koneman *et al*., 1994; Cheesbrough, M. 2005; Collee, *et al*., 2007).

Statistical analysis

Statistical analyses were performed applying SPSS version 15.0 (International Business Machines Corporation (IBN), New York, NY, USA). Descriptive statistics were carried out and means were compared using one-way ANOVA at a 95% confidence limit.

RESULTS

The results of the samples collected and bacterial isolates identified are presented in the following Tables. The percentage distribution of gender among age groups is as follows: 0–59 months (39.2%) are the highest, followed by 60–10 years (29.4%), and 21 years and above (3.5%) are the lowest. The percentage distribution between male and female (45.5%) and (55.5%), respectively, as shown in Table 1.

Table 1: Distribution of gender and age of samples collected from Pindiga Cottage Hospital

Age group	**Gender**		**Total (%)**
	Male (%)	Female (%)	
0 M-59 month	26 (40.0)	30 (38.5)	56 (39.2)
60 M -10 Y	19 (29.2)	23 (29.5)	42 (29.4)
11 Y - 15 Y	15 (23.1)	16 (20.5)	31 (21.7)
16 Y - 20 Y	3 (4.6)	6 (7.7)	9 (6.3)
21 Y – Above	2 (3.1)	3 (3.9)	5 (3.5)
Total	**65 (45.5)**	**78 (55.5)**	**143 (100)**

Table 2: Age distribution of bacterial isolated from Male Diarrhea samples collected from Pindiga Cottage Hospital

		No (%) of male samples tested positive for different bacteria from Pindiga Cottage Hospital						
Age range	**Sampled**	***Shigella* sp.**	***Vibrio* sp.**	***E. coli***	***E. coli O157:H7***	***Campylobacter* sp.**	***Salmonella typhi***	***Samonella paratyphi***
0 M-59 M	26 (40.0)	22 (33.8)	0 (0.0)	17 (26.2)	0 (0.0)	0 (0.0)	13 (19.7)	8 (21.1)
60 M-10 yrs	19 (29.2)	13 (20.0)	0 (0.0)	19 (29.2)	2 (66.7)	0 (0.0)	8 (12.1)	3 (7.9)
11 Y – 15 Y	15 (23.1)	0 (0.0)	1 (1.5)	14 (21.5)	0 (0.0)	0 (0.0)	6 (9.1)	8 (21.1)
16 Y- 20 Y	3 (4.6)	2 (3.1)	0 (0.0)	3 ((4.6))	0(0.0)	0 (0.0)	3 (4.6)	0 (0.0)
21 Y– Above	2 (3.1)	2 (3.1)	0 (0.0)	2 (3.1)	0 (0.0)	0 (0.0)	2 (3.0)	0 (0.0)
	65 (45.5)	**39(58.46)**	**1(1.54)**	**55(84.62)**	**2(3.11)**	**0(0.0)**	**32(49.23)**	**19(29.23)**
Mean Standard Deviation		**7.8±9.5**	**0.2±0.5**	**11.0±7.9**	**0.4±0.9**	**0.0±0.0**	**6.4±4.4**	**3.8±4.0**

Key: M = Month, Y = Year, sp. = species.

The overall distribution of the bacterial isolates was skewed toward smaller age groups of the samples collected from both males and females; thus, the number of isolates was observed to decrease as the ages of the samples decreased. The highest number of bacterial occurrences was equally observed among children of both genders, aged 0–15 years. There was no *Campylobacter* sp. isolated from any sample, while only one (0.2±0.5) isolate of *Vibrio* sp. was identified from the male sample. In the bacterial isolates from the male samples, *E. coli* (11.0±7.9), *Shigella* sp. (7.8±9.5) and *Salmonella typhi* (6.4±4.4) had the highest occurrences, while *Vibrio* sp. was the least (Table 2). The mean difference of the bacterial species and strains identified from the male stool samples collected was conducted using one-way analysis of variance, and the results show a significant difference at *the* p *0.05* level for the five groups [*F (2.899,* $p = 0.035$*)], but Campylobacter* sp. was not detected. A post-hoc comparison using the LSD test revealed that the mean number of *Shigella* sp. (7.8 ± 9.5) was statistically significant compared to the mean number of *Vibrio* sp. (0.2 ± 0.5, p = 0.043), *E. coli* O157:H7) (0.4 ± 0.9, p = 0.048), and *Vibrio* sp. (0.2 ± 0.5) was also statistically different from *Shigella* sp. (7.8 ± 9.5, $p = 0.043$) and *E. coli*. (11.0 ± 7.9, $p = 0.006$), similarly, *E. coli*. (11.0 ± 7.9) was statistically different from *Vibrio* sp. (0.2 ± 0.5, $p = 0.006$) and *E. coli* O157:H7) (0.4 ± 0.9), $p = 0.006$ each.

Table 3: Age distribution of bacterial isolated from Female Diarrhea samples collected from Pindiga Cottage Hospital

		No (%) of female samples tested positive for different bacteria from Pindiga Cottage Hospital						
Age range	**Sampled**	***Shigella* spp.**	***Vibrio* spp.**	***E. coli***	***E. coli O157:H7***	***Campylobacter* spp.**	***Salmonella typhi***	***Samonella paratyphi***
0 M-59 M	30 (38.5)	27 (34.6)	0 (0.0)	10 (12.8)	0 (0.0)0	0 (0.0)	9 (13.6)	11 (28.9)
60 M-10 Y	23 (29.5)	20 (25.6)	0 (0.0)	16 (20.5)	1 (33.3)	0 (0.0)	10 (15.2)	7 (18.4)
11 Y– 15 Y	16 (20.5)	10 (12.8)	0 (0.0)	10 (12.8)	0 (0.0)	0 (0.0)	9 (13.6)	0 (0.0)
16 Y- 20 Y	6 (7.7)	6 (7.7)	0 (0.0)	6 (7.7)	0 (0.0)	0 (0.0)	4 (6.1)	1 (2.6)
21 Y– Above	3 (3.9)	2 (2.6)	0 (0.0)	3 (3.9)	0 (0.0)	0 (0.0)	2 (3.0)	0 (0.0)
	78 (54.5)	**65(83.33)**	**0(0.0)**	**44(56.41)**	**1(1.28)**	**0(0.0)**	**34(43.59)**	**19(24.36)**
Mean Standard Deviation		**13.0±10.3**	**0.0±0.0**	**9.0±4.9**	**0.2±.4**	**0.0±0.0**	**6.8±3.6**	**3.8±4.9**

Key: M = Month, Y = Year, spp = species.

Five groups/species of bacteria were isolated from samples collected: *Shigella* sp. (83.33%, MSD = 13.0 ± 10.3), *E. coli* (56.41%, MSD = 9.0 ± 4.9), *Salmonella typhi* (43.59 %, MSD = 6.8 ± 3.6), *S. paratyphi* (24.36 %, MSD = 3.8 ± 4.9), and lastly *E. coli* 0157:H7 (1.28 %, MSD = 0.2 ± 4) were isolated across age groups, as shown in Table 3. Among the bacteria groups identified from female samples, *Shigella* sp. (13.0±10.3), *E. coli* (9.0±4.9), and *S. typhi* (6.8±3.6) had the highest occurrences, while the least occurrence was *E. coli* 0157:H7 (0.2±.4) (Table 3).

Similarly, the mean difference in the female number of bacteria identified was determined using one-way analysis of variance, and the results indicate a significant difference at the p *0.05* level for the five species of bacteria [*F* (3.563, $p = 0.024$)], excluding *Campylobacter* sp., which was not identified. The post-hoc test using LSD also indicates the number of *Shigella* sp. (13.8 ± 9.5) identified from age groups was statistically significant from the mean number of *E. coli* (O157:H7) (0.2 ± 4) and *S. paratyphi* (3.8 ± 4.9, $p = 0.021$), and *E. coli* (9.0 ± 4.9, $p = 0.026$) was different from *E. coli* (O157:H7) (0.2 ± .4, $p = 0.002$).

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

Pindiga	**Gender**	**Isolation frequency**		**Relative risk**
		Positive	**Negative**	
Isolates	Male	65	78	1.20
	Female	78	65	
Shigella sp.	Male	39	26	2.12
	Female	65	13	
Vibrio sp.	Male	1	64	0.98
	Female	0	78	
E. coli sp.	Male	55	10	0.36
	Female	45	33	
E. coli O157:H7	Male	2	63	0.98

	Female	1	77	
Campylobacter sp.	Male	0	65	-
	Female	0	78	
S. typhi	Male	32	33	0.90
	Female	34	44	
S. paratyphi	Male	19	46	0.94
	Female	19	59	

The isolation frequency of different bacterial species was also higher among female (IF = 78, RR = 1.20) samples collected. Females were relatively more at risk (2.12 compared to their male counterparts) but inversely at less risk of identifying other pathogens (*E. coli.*, *Salmonella typhi*, *Salmonella paratyphi*, and lastly *E. coli* O157:H7), as shown in Table 4. *Campylobacter* was not isolated among both samples, while *Vibrio* was only isolated from the male sample (Table 4).

Table 5: Association and detection rate of bacteria isolated from Diarrhea samples collected from Pindiga Cottage Hospital

Gender	**Sampled number (%)**	**Frequency (%) of detection of bacteria from Pindiga Cottage Hospital**						
		***Shigella* sp.**	***Vibrio* sp.**	***E. coli***	***E. coli* *O157:H7***	***Campylobacter* sp.**	***S. typhi***	***S. paratyphi***
Male	65 (45.5)	39 (60.0)	1 (1.5)	55 (84.6)	2 (3.1)	0 (0.0)	32 (49.2)	19 (29.2)
Female	78 (54.5)	65 (83.3)	0 (0.0)	45 (57.7)	1 (1.3)	0 (0.0)	34 (43.6)	19 (24.4)
Detection rate		72.7	0.7	69.9	2.1	0	46.2	26.6
Statistics								
p value	0.125	0.008	00	0.001	0.470	00	0.501	0.512
95% CL (upper/lower)	.904/2.294	1.315/6.273	00	.110/.557	.036/4.616	00	.412/1.543	.371/1.640
Odd ratio	1.440	2.872	00	.248	.409	00	.797	.780

The detection rate (DR) of bacteria isolated from male and female samples was high for *Shigella* sp. (72.7%), *E. coli* (69.9%), and *S. typhi* (46.2%) and *S. paratyphi* (26.6%) in order of decreasing detection. While an association between female samples and the number of bacteria isolated was observed at $p > 0.05$ for most isolates, it was not statistically significant. However, *E. coli* (OR =.248; CLL =.110; CLU =.557) showed a negative association of female samples with the bacteria with statistical significance (Table 5).

DISCUSSION

The overall distribution of bacterial isolates skewed toward smaller age groups in both male and female samples, indicating that children aged 0–15 years are more vulnerable to bacterial infections (WHO 2017; Mero *et al.*, 2021; Lampo *et al.*, 2021). This finding highlights the need to target preventive measures, such as vaccination and improved personal and food hygiene and sanitation, towards this age group; interventions such as increasing access to safe and adequate drinking water and encouraging mothers to use exclusive breastfeeding (Agegnehu *et al.*, 2019; Baye *et al.*, 2021; WHO 2017) can reduce the risk of transmission of these bacteria.

Shigella sp., *E. coli* sp., and *S. typhi* were the most commonly isolated bacteria from both male and female samples, indicating that these organisms are prevalent in the study population. Vubil and his colleagues had isolated *Shigella* among children less than five years old in southern Mozambique (Vubil *et al.*, 2018), in a study conducted at Manhica district of Maputo province, a rural area that is characteristically similar to Pindiga in terms of warmed climatic condition and annual rainy pattern, reported similar observations as did Lampo and Valera (Lampo *et al.*, 2021; Varela *et al.*, 2015).

The significant difference in mean difference of bacterial isolates among the five groups suggests that each bacterial species may have a different distribution pattern and risk factors; understanding these patterns and risk factors is essential for developing effective preventive measures. Similarly, the higher isolation frequency of different bacterial species among female samples suggests that females may be at higher risk of bacterial infections overall.

The absence of *Campylobacter* sp. and low occurrence of *Vibrio* sp. in both male and female samples suggests that these organisms may not be significant causes of bacterial infections in the study population and the area. Although this could be true based on the zoonotic and foodborne original nature of *Campylobacter,* which is transmitted through consumption of unsafe food animals, resulting in its prevailing as 1 of the 4 causes of diarrhea globally and being implicated in socio-economic factors that make it prevalent in developing countries (WHO, 2020; Bian *et al.*, 2020). Pindiga community members are not used to consuming undercooked meat and are very selective in the types of animal meat they eat, which could have influenced the low isolation of *Campylobacter* in this study. However, further studies are needed to confirm this finding.

Males were significantly associated with the occurrence of *E. coli* and therefore at high risk of diarrhea caused by *E. coli* as well. This finding may be due to gender-related differences in behavior, exposure, or susceptibility to *E. coli* infection, thus male child could be more exposed to the risk of *E. coli* infection, evidence by poor environmental sanitation in the area, since male child were earlier reported to be more physically active than female and therefore more likely to play around refuse waste, contaminated water and have poor personal hygiene, especially in low socio-economic status family (Claire and Joe, 2022; Biadgilign *et al.*, 2022; Telford *et al.*, 2016; He and Qiu, 2022), however this finding was contrary to that of Mapanguy and his colleagues who reported more females with *E. coli* among Congolese students (Mapanguy *et al.*, 2021), and at Mulago hospital Uganda (Odongo *et al.*, 2020). Overall, the results of the study have important implications for public health policy and practice, particularly in the areas of personal and environmental sanitation, vaccination, food sanitation, nutrition, safety, and gender-specific risk factors and interventions to reduce bacterial infections among children under five.

DECLARATION

Ethics approval and Consent to participate

This research was approved by the research and publications committee of the Federal University of Kashere Gombe, FUK/R & D/PRC/4/V1, by TEFund institutional-based research guidelines and the Gombe Ministry of Health, MOH/ADM/621/Vol. 1/4178, which is responsible for overseeing all hospitals in the state through the State Health Research Committee. The same approval was copied to Kashere General Hospital, which does not have an ethical issues committee, for notification and compliance.

Written or verbal participant consent, depending on the time given and literacy level, was sought from patients or their guides who came to the hospital with a diarrhea case, and consent approval was granted before the sample was collected for the research. Samples were collected by World Medical Association guidelines and processed according to standard laboratory procedures.

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 about intellectual property rights, affiliation, and financial support from the Nigerian Tertiary Education Trust Fund and the Federal University of Kashere for the conduct of this research.

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Limitation

Modern molecular equipment is not available, compounded by the lack of a 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.

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