IMCC Journal of Science
2022, 2, 54-62
https://myjournal.imcc.edu.ph/

Studies on Diarrheagenic Bacteria Among Children (<5 Years) from Two Public Health Facilities in Makurdi Metropolis, Benue State, Nigeria

Elum John [1], Dabo Adar Diana [2], Innocent Ishaku Giwa [3], Agbese Joseph Benjamin [4], Musa Usman Halim [3], Olasan Joseph Olalekan [1, *]

[1] Federal University of Agriculture, Makurdi, Benue State, Nigeria; johnelum@gmail.com (E.J.)
[2] College of Health Technology, Jos, Plateau State, Nigeria; adardiana@yahoo.com (D.A.D.)
[3] Federal University of Technology, Owerri, Imo State, Nigeria; giwainnocenti@gmail.com (I.I.G.); halimusman@gmail.com (M.U.H.)
[4] Federal College of Education, Kano, Kano State Nigeria; ajosephbenjamin53@gmail.com (A.J.B.)
* Correspondence: olasan.olalekan@uam.edu.ng

ABSTRACT
Diarrheagenic bacteria are the leading causes of diarrhea or bacterial gastroenteritis, leading to high child morbidity. This work investigated the prevalence of diarrheagenic bacteria among children (< 5 Years) in two public health facilities in Makurdi metropolis, Benue State, Nigeria. A total of 400 children who presented with diarrhea cases were sampled through stool collections. Cultural and biochemical characterizations were carried out following standard practices. Biochemical results identified a total of six bacterial species belonging to five genera that were associated with diarrhea in the 400 stool samples investigated. Bacterial infections associated with diarrheal cases were 20% prevalent with the following distributions: *Escherichia coli* (10.75%), *Proteus mirabilis* (2%), *Proteus vulgaris* (1.5%), *Salmonella typhi* (1.75%), *Shigella dysenteriae* (2%), and *Klebsiella pneumoniae* (2%). The prevalence levels across the five species of bacteria were significantly different ($P<0.05$), attributed to the highest bacterial prevalence of *E. coli* (10.75%) and the low level of other bacteria (≤ 2 %). *E. coli* represented 53.75% of the isolates. Infection was most predominant in the 36-47 months of age, followed by 48-59 months. Thus, a significant association was associated between children's ages and diarrheal infection ($\chi^2 = 81.91$, $p<0.01$). However, infection did not depend on sex status ($P>0.05$). This report is critical to stakeholders in the public health in the control of the rising cases of diarrhea in <5 years old children in Benue State, Nigeria.

KEYWORDS: bacteria; children; control; diarrhea; prevalence

ARTICLE INFO: Received: 08 May 2022; Accepted: 24 June 2022; Volume: 02; Issue: 01; Type: Original Article

1. Introduction

Diarrhea, a leading cause of global infant morbidity, is defined as a daily stool volume higher than 10 g/kg in infants [1-4]. Diarrheagenic bacteria are the leading causes of diarrhea or bacterial gastroenteritis in children [5]. They are attributed to many species of bacteria such as *Salmonella, Shigella,* pathogenic *Escherichia coli, Campylobacter, Clostridium,* and *Vibrio* [4-9]. They are known for causing typhoid and paratyphoid fever as in *Salmonella* [4, 6]; bloody diarrhea and dysentery as in *Shigella* [5]; chronic diarrhea as in *E. coli* and *Clostridium difficile* [5, 7]; bacterial gastroenteritis as in *Campylobacter jejuni* [7]; and cholera as in *Vibrio cholera* [8, 9]. Cholera has proven to be one of the deadliest diseases known in history. Although it is a global disease, Africa has been the worst hit by pandemic cases. It is now said to be endemic in Africa and Nigeria inclusive [5].

Diarrhea has been classified as acute (few days), prolonged (7-14 days), or persistent (above 14 days). Affected children are malnourished due to excessive loss of watery nutrients, and the situation could lead to other complications [4, 5]. This condition is a major challenge in developing countries like Nigeria, aggravated by poor hygienic environment, poor sanitation, illiteracy, high poverty level, and contaminated water sources [5, 10]. Control and prevention measures are crucial to lessen the prevalence of diarrhea among children. To achieve this, a robust database of the disease and the associated aetiological causes are essential in any given area. This work therefore aimed at assessing the range of diarrheagenic bacteria from two public health facilities in Makurdi metropolis, Benue State, Nigeria.

2. Methodology

2.1 Study Area

The study was undertaken in two major public hospitals in Benue State, namely: Benue State University Teaching Hospital (BSUTH) and General Hospital (GH). These are the two public hospitals with orthopedic medical experts who handle health challenges in children in Benue State. Hence, they have high attendance of in-patient children. Serious cases from private hospitals are often given referrals to the two public-based hospitals.

2.2 Sampling Technique and Size

A total of 400 children presented with diarrhea cases were sampled as determined using the sample size calculator of Krejcie and Morgan [11]. Based on the calculation, 180 samples were collected from GH, while 220 samples were collected from BSUTH. Flowchart of methods is given in Figure 1.

2.3 Collection of Stool Samples

Stool samples were obtained from subjects, collected into sterile sample bottles, transported in an ice pack to the laboratory, and investigated [12].

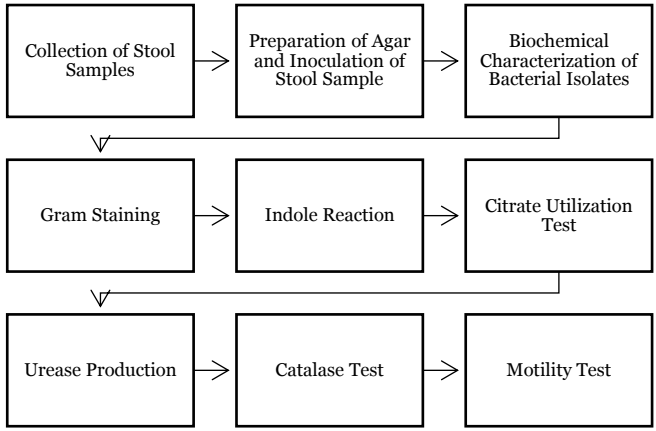

Figure 1. Flowchart of methods.

2.4 Preparation of Agar and Inoculation of Stool Sample

Salmonella and *Shigella* species were isolated on a selective agar medium (deoxycholate citrate agar) following standard procedures described by [13]. MacConkey Agar (MCA), a differential and low selective medium, was used to isolate *E. coli*, *Klebsiella*, and *Proteus spp*. and distinguish lactose fermenting from non-lactose fermenting bacteria. Thiosulfate Citrate Bile Salt (TCBS) agar, a selective medium, was used to isolate *V. cholerae* and other vibrio species from fecal specimens following standard procedures [13]. The surfaces of the media were dried in a hot air oven set at 45°C before inoculation to remove the water from condensation and air bubbles. Stool samples were picked with sterile wire loops and inoculated on *Salmonella-Shigella* agar (SSA), MCA, TCBS agar, and deoxycholate citrate agar (DCA) and incubated at 37°C for 24 h for bacterial growth [13].

2.5 Biochemical Characterization of Bacterial Isolates

All plates were examined, and suspected colonies of enteropathogens were identified by standard biochemical methods [13]. The standard biochemical methods used were gram staining, indole reaction, citrate utilization test, and urease production test.

2.5.1 Gram Staining

A thin smear made on a clean slide was allowed to air-dry and fixed through hot flame and flooded with crystal violet and allowed to stain for 30 s followed by washing in distilled water prior to application of Lugol's iodine. It was then allowed to stand for

20 s. The smear was decolorized with acetone briefly and flooded with safranin to stain for about 20 s. This was then washed with distilled water and allowed to drain dry. The preparation was examined under the microscope with an oil immersion (x100) objective. This technique divides the bacteria into two groups, namely: Gram-positive and Gram-negative bacteria. The structures were then observed whether they were rod-shaped or spherical [13].

2.5.2 Indole Reaction

This differentiates Gram-negative rods, especially *E. coli*. It confirms the breakdown of tryptophan releasing indole. The test was carried out by adding a few drops of commercially prepared Kovac's reagent to an overnight broth culture of the bacterium. A pinkish-red ring appearing on the surface of Kovac's reagent indicated a positive test [13].

2.5.3 Citrate Utilization Test

This test differentiates enterobacteria from other bacteria. The organism was inoculated onto a slant (slope) of Simmons Citrate Agar and incubated overnight at 37^{o}C. A change in color from green to blue indicated a positive result [13].

2.5.4 Urease Production

This test confirms the production of urea enzyme from hydrolysis of urea, releasing ammonia leading to color change. The organism was inoculated onto urea agar and incubated for 24 h at 37^{o}C. A red-pink coloration indicated a positive reaction. It identified *Proteus* species [13].

2.5.5 Catalase Test

This differentiates bacteria that produce the enzyme catalase, such as *Staphylococcus,* from those that do not produce catalase, such as *Streptococcus*. Emulsification of the test organism was done on a glass slide with a drop of hydrogen peroxide. The presence of effervescence confirmed the presence of *Staphylococcus* from *Streptococcus* [13].

2.5.6 Motility Test

This differentiates motile from non-motile organisms in a bottle of peptone water containing the test organism. Incubation was done at 37^{o}C for 24 h. Slide preparation was made and examined under the microscope with x10 and x40 objectives [13]. Motility was then noted.

2.4 Data Analysis

Data analysis was done using the SPSS software (20.0 version) and Minitab (16.0 version). Descriptive and inferential statistical tools were applied. Frequency (f) and

percentage (%) were used to describe the categorical variables. Inferences were drawn using independent t-test, ANOVA, and Chi-square. Post-hoc analysis was also done using the Games-Howell method at 95% and 99% confidence limits.

3. Results

3.1 Biochemical Identification of Bacteria

Biochemical results identified six bacterial species belonging to five genera that were associated with diarrhea in the 400 stool samples investigated, as presented in Table 1. Only genus *Proteus* had two species (*P. vulgaris* and *P. mirabilis*).

3.2 Prevalence, Occurrence, and Distribution of Bacterial Infection

Table 2 presents the prevalence, occurrence, and distribution of bacterial infection across the two health facilities. From the study, bacterial infections associated with diarrheal cases were 20% prevalent (GH=24.44%, BSUTH=16.36%). The isolates associated with diarrhea were *E. coli* (10.75%), *P. mirabilis* (2%), *P. vulgaris* (1.5%), *S. typhi* (1.75%), *S. dysenteriae* (2%), and *K. pneumoniae* (2%). The prevalence levels across the five species of bacteria were significantly different ($F=6.781$, $p=0.019$). The difference was attributed to the highest bacterial prevalence of *E. coli* (10.75%) associated with diarrhea and the low level of other bacteria (≤2%). *E. coli* was the most frequently occurring (10.75%) while other isolated bacteria were ≤2.0%. Thus, *E. coli* represented 53.75% of the 80 isolates while other species were ≤10%.

3.3 Distribution of Bacterial Infection based on Demographic Parameters

The distribution of bacterial infections across the age and sex of the children is presented in Table 3. Bacteria were most predominant in the 36-47 months of age (8%), followed by 48-59 months old children (3.5 %). The lower ages (below 35 months) had low levels of bacterial pathogens associated with diarrhea. Bacterial infection cases depend on the ages of children ($\chi^2=81.91$, $p<0.01$), but are independent of sex status ($p>0.05$).

4. Discussion

The 20% prevalence of diarrheagenic bacteria observed in the study area was higher than the WHO tolerable limit of 5%, therefore a worrisome concern in public health. The bacterial species identified in this report have been previously implicated in the etiology of diarrhea pandemics among children in many parts of the world [14, 15]. In this work, different clinically important bacterial species have been reported. Therefore, the result obtained was consistent with many studies on diarrhea in children [14, 15]. *E. coli* was the most frequently occurring etiologic agent of diarrhea. The present report agrees with earlier reports on *E. coli* as a major cause of chronic diarrhea [4]. According to Fagundes-Neto et al. [7], diarrheagenic types possess virulence properties responsible for their pathogenicity in three possible forms. First, there are possibilities of synergistic interaction between *E. coli* and a wide range of pathogenic

Table 1. Outcome of biochemical characterization.

Identified bacteria	Lactose	Mannitol	Glucose	Indole	Gas production	Motility	Oxidase	Citrate	Urease	H2S	Sucrose
E. coli	+	+	+	+	+	+	-	-	-	-	+
S. dysenteriae	-	+	+	+	-	-	-	-	-	-	-
S. typhi	-	+	+	-	-	+	-	-	-	+	-
K. pneumonia	+	+	+	+	+	-	-	+	+	-	+
P. vulgaris	-	-	+	+	-	+	-	-	+	+	+
P. mirabilis	-	-	+	+	-	+	+	+	+	+	+

Key: "+" = positive reaction; "-" = negative reaction

Table 2. Prevalence of bacterial infection associated with diarrhea across the two health facilities in Makurdi.

Bacterial isolates	Number of subjects infected		Prevalence per species (f and % in N=400)	% per total number of each isolate
	GH (f and % in N=180)	BSUTH (f and % in N=220)		
E. coli	26 (14.4)	17 (7.73)	43 (10.75)	53.75
P. mirabilis	4 (2.22)	4 (1.82)	8 (2)	8.75
P. vulgaris	4 (2.22)	2 (0.91)	6 (1.5)	10.0
S. typhi	2 (1.11)	5 (2.27)	7 (1.75)	7.5
S. dysentriae	3 (1.67)	5 (2.27)	8 (2)	10.0
K. pneumonia	5 (2.7)	3 (1.36)	8 (2)	10.0
Total	44 (24.44)	36 (16.36)	80 (20)	100

Note: WHO tolerable prevalence limit = 5%; bacterial isolates ($F = 6.781$, $p = 0.019$, $p<0.05$).

Table 3. Distribution of bacterial pathogens from stool samples in relation to age and sex of children.

Age group (months)	Sex		Number of bacteria (f and %)		
	Male	Female	GH (N=180)	BSUTH (N=220)	Total
< 6	0	0	0 (0)	0 (0)	0 (0)
6 - 11	1	4	3 (1.67)	2 (0.91)	5 (1.25)
12 - 23	7	6	4 (2.22)	9 (4.09)	13 (3.25)
24 - 35	6	7	4 (2.22)	9 (4.09)	13 (3.25)
36 - 47	17	18	21 (11.67)	14 (6.63)	35 (8.0)
48 - 59	4	10	9 (5.0)	5 (2.27)	14 (3.5)
Total	35	45	41 (22.78)	39 (17.73)	80 (20)

Note: Age: $\chi^2 = 81.91$, $p<0.01$ (association exists); sex: $\chi^2 = 0.09$, $p > 0.05$ (no association)

organisms that cause prolonged diarrhea, such as bacteria, viruses, helminths, and protozoans [16]. Secondly, *E. coli* strains have been reported to possess multi-drug resistance to different types of antibiotics due to resistance genes in the genome. Thirdly, some strains possess toxins to further cause harm to the host [17].

This finding partly deviates from other reports where *V. cholera, S. dysenteriae,* and *S. typhi* were reported as the most dominant bacterial species responsible for high cases of diarrhea in Nigeria [18, 19]. From this work, children develop stronger immunity to infections at lower ages (less than 35 months), possibly due to parental care, attention, and breastfeeding [19]. In the work of Hungs [20], the ages of children were significantly associated with susceptibility to diarrheal infection. However, the older ages (above 35 months) are susceptible to diarrhea. This may be due to inadequate attention given to older ages, carelessness on the part of the mother, malnutrition, and poor hygiene. These factors are also prevalent in the study area, and they have all been previously reported to aggravate diarrheal infections in children in many places [21]. Considering the high prevalence of diarrhea in the study area, there is a need for intervention by the management of the public health care units of Benue State in the aspects of treatments, vaccination, and health education. Non-governmental organizations (NGOs) may also play crucial roles in the control of diarrhea in the study area.

5. Conclusion

The prevalence of diarrhea with bacterial origin was 20% in the study area. *E. coli* was the most frequently occurring. Other diarrheagenic bacteria found in stool samples were *P. mirabilis*, *P. vulgaris, S. typhi*, *S. dysenteria,* and *K. pneumoniae*. Ages of children above 35 months were more susceptible to diarrhea. This report is significant to stakeholders in public health in controlling the rising diarrhea cases in the study area.

Acknowledgment

The authors duly acknowledge the staff and management of the Benue State University Teaching Hospital and General Hospital, Makurdi, Nigeria, for their approval and support.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions: Research design, all authors; sample collection, E.J.; laboratory work, E.J., D.A.D., I.I.G., A.J.B., and M.U.H.; statistical analysis, Olasan Joseph Olalekan (O.J.O.). All authors have read and agreed to the published version of the manuscript.

References

1. Jacob, N.J.; Cohen, M.B. Update on diarrhea. *Paediatr Rev* **2016**, *37*, 313- 322.

2. Kosek, M.; Bern, C.; Guerrant, R.L. The global burden of diarrheal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* **2003**, *81*, 197–204.
3. Zella, G.C.; Israel, E.J. Chronic diarrhea in children. *Paediatr Rev* **2013**, *33*, 207-213.
4. Black, R.E.; Morris, S.S.; Bryce, J. Where and why are 10 million children dying every year? *Lancet* **2003**, *361*, 2226–2234.
5. Abba, K.; Sinfield, R.; Hart, C.A. Garner P. Pathogens associated with persistent diarrhea in children in low and middle income countries: Systematic review. *BMC Infect Dis* **2009**, *9*, 88-97.
6. Binder, H. J. Causes of chronic diarrhea. *Engl J Med* **2006**, *355*, 236–239.
7. Fagundes-Neto, U.; Scaletsky, I.C. The gut at war: The consequences of enteropathogenic *Escherichia coli* infection as a factor of diarrhea and malnutrition. *Sao Paulo Med J* **2000**, *118*, 21–29.
8. Haneef, H.; Nazar, I.; Malik, M.W. Review of trends in cholera. *Air Water Borne Dis* **2015**, *4*, 118-121.
9. Ebob, T.J. Epidemiological distribution of different *Vibrio cholera* strains causing cholera disease in endemic countries: A review. *J Adv MedRes* **2019**, *31*, 1-15.
10. Deen, J.; Mengel, M.A.; Clemens, J.C. Epidemiology of cholera. *Vaccine* **2019**, *38*, A31–A40.
11. Krejcie R.V.; Morgan D.W. Determining sample size for research activities. *Educ Psychol Meas* **1970**, *30*, 607–610.
12. Mulatu, G.; Beyene, G.; Zeynudin, A. Prevalence of *Shigella*, *Salmonella* and *Campylobacter* species and their susceptibility patterns among under five children with diarrhea in Hawassa town, South Ethiopia. *Ethiop J Health Sci* **2015**, *24*, 101-108.
13. Cheesbrough, M. *Medical laboratory manual for tropical countries*, 2nd ed.; Cambridge Press: Cambridge, England, 2016; pp. 292–308.
14. Ifeanyi, C.I.; Isu, R.N.; Akpa, A.C.; Ikeneche. I. Enteric bacterial pathogens associated with diarrhea of children in the federal capital territory Abuja, Nigeria. *New York Sci J* **2009**, *2*, 62-69.
15. Sule, E.I.; Aliyu A.M.; Abdulaziz, B.M. Isolation of diarrheagenic bacteria in children attending some selected hospitals within Kaduna metropolis, Kaduna State, Nigeria. *Cont J Appl Sci* **2011**, *6*, 1 – 6.
16. Gimba, U.N.; Dawan, N.N. Epidemiological status of intestinal parasitic infection rates in children attending Quaqualada township Clinic, FCT Abuja, Nigeria. *Am J Res Commun* **2015**, *3*, 97-110.
17. Ogbu, O.; Agumadu, N.; Uneke, C.J.; Amadi, E.S. Aetiology of acute infantile diarrhea in the south-eastern Nigeria: An assessment of microbiological and antibiotic sensitivity profile. *Internet J Third World Med* **2008**, *7*, 52–85.
18. Adagbada, A.O.; Adesida, S.A.; Nwaokorie, F.O.; Niemogha, M.; Coker, A.O. Cholera epidemiology in Nigeria: An overview. *Pan Afr Med J* **2012**, *12*, 59-71.

19. Reither, K.; Ignatius, R.; Weigtzel, T. S. Acute childhood diarrhea in Northern Ghana: Epidemiological clinical and microbiological characteristics. *BMC Infect Dis* **2007**, *7*, 1-8.
20. Hungs, B.V. The most common causes of and risk factors for diarrhea among children less than five years of age admitted to Dong Anh Hospital, Hanio, and Northern Vietnam. Master's Thesis, University of Oslo, Norway, May 2006.
21. Abdullahi, M.; Olonitola, S.O.; Inabo, I.H. Isolation of bacteria associated with diarrhea among children attending some hospitals in Kano Metropolis, Kano State, Nigeria. *Bayero J Pure App Sci* **2010**, *3*, 10–15.