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Patterns and prevalence of gastroenteritis among children aged 0 – 15 years

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Abstract

The aim of this study is to determine the prevalence of bacterial gastroenteritis among children aged 0-15 years, the bacterial types involved, the age-range mostly affected and the bacterial load in the children sampled. This study was carried out in selected primary schools in Ekpoma, Edo State, Nigeria. The study population comprises of one hundred and fifty (150) Primary School Children within the age range of 0-15 years. Stool samples were examined using standard bacteriological method. Results obtained showed the rate of bacterial isolates from the stool of the children from the various age groups for the population under study is 44%. Out of the samples collected from each of the age range, 9(41%) were positive for age range 0-3, 19(46%) were for 4-7 years, 17(49%) for 8-11 years, and 21(40%) for 12-15. Age 12-15 years have the highest bacterial isolates followed by age 4-7 years, 8-11 years and 0-3 years. *Escherichia coli, Salmonella typhi Pseudomonas aeruginosa, Klebsiella pneumonia and Streptococcus faecalis* were isolated from the study population. The bacterial load of bacteria gastroenteritis is high so also the individual loads of the bacterial types apart from *Streptococcus faecalis.* Therefore, the isolation was enough to cause problem to the children.

Keywords: Bacterial, Children, Gastroenteritis, School, Stool, Stomach.

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Contents

Contribution of this paper to the literature: The study aim to determine the prevalence of bacterial gastroenteritis among children aged 0-15 years. *Escherichia coli*, *Salmonella typhi Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Streptococcus faecalis* were isolated from the study population. Children aged 12-15 years have the highest bacterial isolates. Cleanliness is required by parents and children to avoid these infections.

1. Introduction

The stomach flu, also known as gastroenteritis, is an inflammation of the stomach and intestines that usually results in vomiting and diarrhea. It is typically brought on by a virus, although it can also be brought on by bacteria, parasites, or specific chemicals [1]. An estimated 100 million cases of gastroenteritis occur each year worldwide, making it a significant contributor to illness morbidity in babies [2]. A major pathway for the spread of infectious pathogens between gastroenteritis and pneumonia is interpersonal interaction. Moreover, environmental variables like water and animals can potentially spread gastroenteritis, which could account for the increased morbidity from the illness [3]. It is further described as characterized by abrupt diarrhea and vomiting due to significant inflammation of the gastrointestinal tract, affecting both the stomach and small intestine [4, 5].

Coming into contact with tainted food or water can spread it. The most common causes of inflammation are infections with certain viruses or, less frequently, bacteria, their toxins, parasites, or an unfavorable reaction to a food or drug. Consuming foods that have been inadequately prepared, reheated meat dishes, shellfish, and dairy and bread items are risk factors. Although the symptoms of each organism vary slightly, they invariably lead to diarrhea [4]. Antibiotics can be used to treat parasites like Giardia and bacteria like Shigella and Campylobacter. According to Azzazy, et al. [6] rotavirus, norovirus, adenovisus, and astrovitrus are among the viruses that cause gastroenteritis. Antibiotics don't work on viruses, and children who get infected typically recover completely in a few days. The most frequent cause of pediatric gastroenteritis is rotavirus. Iyevhobu, et al. [7] and Azzazy, et al. [6]. According to Vecchio, et al. [8] the signs and symptoms include diarrhea, vomiting, and occasionally a fever. In more extreme situations, dehydration symptoms could show up. According to Nino-Serna, et al. [9] inadequate baby feeding is one of the primary contributing factors. Vomiting frequently follows diarrhea. Bile may occasionally be thrown up when the stomach is empty. *Salmonella*, *Shigella*, *Staphylococcus*, *Camphylobacter jejuni*, *Clostridium*, *Escherichia coli*, *Yersinia*, *Vibrio cholera*, and other pathogenic bacterial species can all cause gastroenteritis.

In order to lower childhood morbidity and mortality, the WHO and UNICEF collaborated to develop and implement integrated management of childhood illness (IMCI) more than ten years ago [10-12]. The five leading causes of childhood mortality—malnutrition, measles, acute respiratory infections, and diarrhea—are the core emphasis of IMCI [11-13]. While some studies praised the approach, others failed to scale up the interventions across the country, which resulted in a limited scope and the intended national impact [14, 15]. Despite high vaccination rates and the implementation of IMCI, it has been reported that Eritrea's babies are seeing an increase in the annual incidence rates of acute respiratory tract infections (ARI) [16, 17]. In addition, the disease burden from diarrhoea continued to be high.

Numerous pathogenic bacterial species, including *Salmonella*, *Shigela*, *Staphylococcus*, *Camphylobacter jejuni*, *Clostridium*, *Escherichia coli*, *Yersinia*, *Vibrio cholera*, and others, can cause gastroenteritis [9]. This claim made it necessary to conduct a study on the prevalence of bacterial gastroenteritis in children ages 0 to 15.

2. Materials and Methods

2.1. Area of Study

This study was carried out in selected primary schools in Ekpoma, the administrative headquarters of Esan West Local Government Area, Ekpoma, Edo State, Nigeria. The area proper lies between latitudes $6^{0}43'$ and $6^{0}45'$ North of the Equator and longitudes $6^{0}6'$ and $6^{0}8'$ East of the Greenwich Meridian [18]. Ekpoma is made up of many quarters including Eguare, Iruekpen, Emaudo, Ujoelen, Ihumudumu, Illeh, Uke, Uhiele, Ujemen, Ukpenu, Idoa, Ukhun, Egoro, Emuhi, Igor and Idumebo. The following quarters, Ihumudumu, Idumebo and Ujoelen are considered in this study. Ekpoma has a population of 127,718 [19] majority of which are civil servants, traders, businessmen/women, transporters, farmers, teachers/lecturers and students by occupation [18].

2.2. Study Population

The study population of this study comprises of Primary School Children within the age range of 0-15 years. The School Children comprise of 80 males and 70 females. The Primary Schools used were Ujoelen Primary School, Ihumudumu Primary School and Idumebo Primary School.

2.3. Sample Size

A total of one hundred and fifty (150) stool samples from children in the selected schools were used in the study area. A sample size of 150 was calculated based on the assumption of 5% expected margins of error and 95% confidence interval according to Creative Research System.

Formulae: sample size = Z^{2}x (P) x (1- P)

$$\frac{}{C^2}$$

Z = Z value (1.96).
P = Percentage picking a choice (0.5).
C= Confidence interval (0.05).

$$\frac{(1.96)^2 \times (0.5) \times (0.5)}{(0.05)^2}$$

Sample size (ss) = 384.
Correction for finite population.

$$\text{New sample size} = \frac{\mathbf{ss}}{1 + \frac{Ss - 1}{\text{Population}}}$$

$$\frac{384}{1 + \frac{384 - 1}{200}}$$

New sample size = 114 approximated to 120 to the nearest whole number of 150.

2.4. Ethical Approval

This was obtained from the Health Research and Ethics Committee of Ambrose Alli University, Ekpoma, Edo State with approval number HREC/2023/04740. Also, permission and consent was sought from headmasters/ headmistress and from parent/ guardian of children in selected primary schools in Esan West Local Government Area, presenting letter of recommendation from my department. The aim and objectives, economic importance and benefit of the study to the subjects and society were well stated.

3. Methods of Analysis

Sterilization: Already properly washed petri-dishes, Durham tubes, test-tubes, conical flasks, beakers, pipettes wire loop, cork borer, some capacity bottles ware sterilized in hot air oven at 180^{0}C for one hours (in their respective canisters) and stored at 4^{0}C

Collection of Samples: One hundred and fifty (150) different stools samples were collected from the different schools in a clean, dry containers. All specimens were properly labeled alphabetically to avoid repeated examination. Freshly collected stool sampled were examined of the day of collection but those the time limit could not meet were preserved in a closed container at (2 – 5^{0}C) in the refrigerator.

Preparation of Culture Media: The media for culturing were aseptically prepared as when necessary, according to the manufacture's institution and autoclave at 121^{0}C for 15mins. Remaining media in flasks were stored at 4^{0}C.

Preparation of Sample: A loopful of the stool sample were taken using a wire loop and put in a bottle. Distilled water was added with the use of pipette. Then 1 ml of the sample was poured into nutrient agar, Macconckey agar and blood agar respectively and mixed in clockwise and anticlockwise direction. Inverted and incubated at 28^{0}C for 24 hours.

3.1. Laboratory Analysis

This involves macroscopy, microscopy and culture analysis.

(i) Macroscopy Examination: By looking at the feces samples' physical characteristics, including color and consistency, as well as whether they were formed, semi-forward, or not, the participants' stools were analyzed macroscopically.

(ii) Microscopy Examination: From the growth plate, a colony was removed using a wire loop, cleaned, plated in a drop of potassium peroxide on a glass slide, covered with a slip, and examined under a microscope to investigate the bacteria.

(iii) Culturing Using Plating Technique (Streak Plating): On the surface of the solidified agar media, 0.1 ml of the last two sample dilutions were applied using a pipette and dispersed with a hockey stick. After covering and inverting the petri dishes, they were incubated at 370C for 48 hours. Observations and examinations were conducted on the formed colonies.

3.2. Bacteria Counting

After incubation, the numbers of colonies on the petri-dishes were counted using the colony counting chamber. The average total and different standard plate counts (SPC) were taken. The colonies were then placed into groups based on pigmentation, colonial morphologies and gram reactions.

3.3. Gram Staining and Microscopic Work

A thin smear was prepared on a clean slide form each representative colony and heat fixed. The slide containing each smear was placed on a staining rack and flooded with crystal violet and allowed to act for 30 seconds. Excess stains was washed off with Lugol's iodine and later flooded with Lugol's iodine to act for 30 seconds. The smear was decolourized with acetone-alcohol until the blue colour ceased to come off. This was done within 3 seconds. The smear was washed quickly with water, the smear was counter stained with safranin to at act for 60 seconds. Then washed with water and blot dried. Then the smear was observed under oil immersion objective lens of the microscope. In this technique, a gram-positive organism was characterized by purple colour while gram-negative cell-stained red.

3.4. Biochemical Test for Identification of Isolates

(i) Catalase Test: A clean, sterile platinium wire loop was used to remove a tiny portion of the culture off the agar slopes. This was placed on a sterile microscope slide in droplets of H_2O_2. A good reaction was shown by the formation of gas bubbles.

(ii) Coagulase Test:

(a) Slide Coagulate Test: On a spotless slide, a drop of regular saline was put. Using a sterile wire, one or two colonies of the test organisms were selected, and they were then emulsified in the saline drop to create a smooth suspension. Stains that were unable to create a smooth suspension were thrown away. One diluted plasma was used to dip the wire loop. A successful outcome was indicated by coarse clumping that became visible to the unaided eye in 5–10 seconds. The tube approach was used to confirm the suspected negative results for those who reacted more slowly.

(b) Tube Coagulase Test: A 1 in 10 dilution of the plasma in a saline solution was prepared of which 1ml of diluted plasma was inverted. Durham tubes into each of the bottles and tubes of the broth. This presumptive test was done twice for each sample using the broth separately and the incubation was left for another 24 hours.

(iii) Urease Test: The tubes were inspected and re-examined following an overnight incubation period in which the street Christensen area medium was injected and incubated at 37^{0}C for four hours. It was not until after incubation that any tubes were reported negative.

(iv) Indole Test – The inoculation process lasted 48 hours at 37^{0}C in the peptone water medium. To allow the nutrients to accumulate, the tubes were left in the incubator for a another 48 hours. When 0.5 ml of Kovac's reagent was applied to each tube separately after the period, the emergence of a red color in the alcohol layer signified a successful reaction.

(v) Methly Red Test: Five drops of the methyl red reagents were added to each tube after the sterile glucose-phosphate peptone water medium was silently inoculated from a young slope culture and incubated for 48 hours at 37^{0}C. The contents were then mixed and read right away, yielding bright red and negative yellow results.

(vi) Voges-Proskauer Test: The sterile medium was inoculated and incubated for 48 hours at 37^{0}C. 0.5 ml of Omeares reagent was added, and the existence of aril space at both of the sealed end of the inverted Durham tubes indicated the formation of gas, while a color shift from orange to yellow indicated the production of acid.

(vii)Motility Test: Using a loop, two to three drops of peptone water containing the organism's growth were put on a sanitized slide. The slide was covered with the cover slide. The slide was left for a while before being seen under a light power objective. You would see motile critters swimming around.

(viii) Citrate Utilization Test: Simmon's citrate agar. The sterile medium was cultured for 96 hours at 37^{0}C after being inoculated with a saline solution of the test organisms. A favorable response was shown by a blue color and a growth streak, whereas a negative reaction was represented by the initial green color and no growth.

(ix) Hydrogen Sulphide Test: The isolated organism was infected with 5 milliliters of sterile peptone water, and a sterile filter paper soaked in lead acetate solution was fastened at the inoculated organism using a cork. It was incubated for two hours at 37^{0}C. Results: A positive result is shown by a black stain at the filter paper's tip.

(x) Sugar Fermentation Test: The following sugars were used in this test: sucrose, lactose, and glucose. Three drops of phenol red were added and shook after 1.5g of each sugar was separately dissolved in 10ml of peptone water in test tubes and labeled appropriately. Durham tubes were sealed with aluminum foil, filled with non-absorbent cotton wool, and autoclave sterilized for 30 minutes at 121^{0}C. Following sterilizing, the test tubes were invested to eliminate any remaining air in the Durham tubes. A sterile wire loop was then used to aseptically inoculate the tubes with a little bacterial culture. The fluid in the tubes changed color from orange (alkaline) to yellow (acid) after being incubated for 24 hours at 37^{0}C. At the sealed end or bottom of the inverted Durham tubes, gas production bubbled.

3.5. Data Analysis

The percentage prevalence was calculated in each case. Comparative analysis of the results was done using Chi-square. A p-value less than 0.05 ($p<0.05$) was considered statistically significant using Statistical Package for Social Science (SPSS) software version 21.

4. Results

The results of the study were based on the microbiological examination of stool samples for the isolation of bacterial gastroenteritis among children between the age range of 0-15 years. A total of 150 stool samples were collected and 80 stool samples were from male children, out of which 31 were positive for bacterial enteritis. While the remaining 70 stool samples were from female children out of which 34 were positive for Bacterial gastroenteritis. Table 1 shows the distribution of positive bacterial isolates and their percentages according to age range, with the age range 12-15 years having the highest bacterial isolated. Table 2 shows the age distribution of samples collected; positive and negative cases. Table 3 shows the age distribution of the number of bacterial isolated and their percentages with *E. coli* being the highest bacterial isolated. Table 4 shows the M±SD of each bacterial isolated from the children samples. Table 5 shows the M±SD of the bacterial isolates of each Age range. Table 6 shows the Biochemical test on the isolates in the study. It was observed that all the organisms isolated were catalase, urease and coagulase negative but showed varied differences amongst other biochemical tests. These are illustrated in the table below.

Table 1. Prevalence of positive isolates and their percentages according to age range in the study.

Age range	No sampled	No positive	No negative	X^2 (p-value)
0 – 3	22	9	13	27.27(0.105)
4 – 7	41	19	22	
8 – 11	35	17	18	
12 – 15	52	21	31	
Total	**150**	**66**	**84**	

Table 2. Age distribution of samples collected, positive and negative cases in the study.

Age range (Years)	No examined			Positive			Negative		
	Male	Females	Total	Male	Females	Total	Male	Females	Total
0 – 3	14	8	22	6	4	9	8	5	13
4 – 7	20	21	41	8	10	19	13	11	24
8 – 11	19	16	35	8	9	17	11	7	18
12 – 15	27	25	52	9	11	21	17	14	31
Total	**80**	70	150	31	34	66	49	36	86

Table 3. Age distribution of number of bacterial isolated and their percentages in the study.

Age range (Years)	*E. coli*		*S. typhi*		*K. pnemoniae*		*P. aeroginosa*		*S. facealis*	
	N	%	N	%	N	%	N	%	N	%
0-3	5	55.6	2	22.2	4	44.4	5	55.6	6	66.7
4-7	13	68.4	8	94.7	4	21.0	6	31.6	2	10.5
8-11	14	82.4	7	41.2	8	47.1	6	35.3	2	11.8
12-15	17	80.9	10	42.9	6	28.6	8	38.1	3	14.3
Total	49		27		22		25		13	

Table 4. M±SD of each bacterium isolated from the children sampled in the study.

Bacterial isolated	Total No	M±SD	P value
E. coli	49	12.3±5.12	<0.05
S. typhi	27	6.8±3.40	<0.05
K. pneumoniae	22	5.5±1.92	<0.05
P. aeruginosa	25	6.3±1.26	<0.05
S. faecalis	13	3.3±1.89	<0.05

Table 5. M±SD of bacterial load of each age range in the study.

Age (Years)	*E. coli*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*	*S. faecalis*	M±SD	p-value
0-3	5	2	4	5	6	4.4±1.52	<0.05
4-7	13	18	4	6	2	6.6±4.22	<0.05
8-11	14	7	8	6	2	7.4±4.34	<0.05
12-15	17	9	6	8	3	8.8±5.26	<0.05

Table 6. Biochemical test on the isolates in the study.

Identified organisms	Biochemical tests										
	Catalase	Oxidase	Indole	Methyl red	Urease	Coagulase	Citrate	H2S	Glucose	Sucrose	Lactose
Escherichia coli.	-	-	+	+	-	-	-	-	AG	AG	AG
Klebsiella pneumonia	-	+	-	-	-	-	+	-	AG	AG	AG
Pseudomonas aeruginosa	-	+	-	-	-	-	-	-	A	-	-
Salmonella typhi.	-	-	-	+	-	-	-	+	AG	-	-
Streptococcus faecalis	-	+	-	+	-	-	-	-	AG	AG	-

Note: **Key:** A = Acid production during sugar fermentation; G = Gas production during sugar fermentation
+ = Positive; - = Negative

5. Discussion

The study provides the assessment of microbiological examination of stool samples for the prevalence of the bacterial gastroenteritis among children aged 0-15 years in Ekpoma, Edo State. Microorganisms isolated include *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Streptococcus faecalis*. From the results obtained, it showed the rate of bacterial isolates from the stool of the children from the various age groups for the population under study is 44% which is similar to those of several previous studies by (citation needed). Out of the samples collected from each of the age range, 9(41%) were positive for age range 0-3, 19(46%) were for 4-7 years, 17(49%) for 8-11 years, and 21(40%) for 12-15. Age range 12-15 years appeared to have the highest bacterial isolates followed by age range 4-7 years, 8-11 years and 0-3 years. This work is in agreement with the study of Nino-Serna, et al. [9] on bacterial gastroenteritis in terms of pathogenic bacterial organisms isolated.

It was also shown that *E. coli* was the highest bacterial isolated with a total number of 49 for all the age groups followed by *S. typhi*, with a total number of 27 and *S. faecalis* having the lowest bacteria isolated with a total number of 13. The distribution of isolates according to occupation and the Mean±SD of the bacteria isolated from each age groups were 4.4±1.52 for age range 0-3 years, 6.6±4.22 for age range 4-7 years, 7.4±4.34 for age range 8-11 and 8.8±5.26 for age range 12-15. E. coli found in highest number because it's found in the mucosa of the lining of the stomach and the causative agent of *E. coli* strain possess virulence factors that allow them to be intestinal pathogens.

The results obtained in this work had shown bacterial isolation of *Escherichia coli*, *Salmonella typhi Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Streptococcus faecalis*. The results equally shows that the bacterial load of bacteria gastroenteritis is high so also the individual loads of the bacterial types apart from *Streptococcus faecalis*. Therefore, the isolation was enough to cause problem to the children.

6. Conclusion

In conclusion, the isolation of the bacterial organisms might be due to improper hygienic practices by parents and children right from the point of child bearing. Therefore, cleanliness is required by parents and children to

avoid these diseases. It is recommended that parents should teach their children the importance of hygienic practices. Also, schools should also introduce hygienic practices in their syllabus in order to teach the young ones living in the dormitory the importance of observing personal hygiene and also enforce it on them.

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