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Types of Microorganisms Isolated from Children with Diarrhoea in Akure, Ondo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors QCO and TTA designed the study. Author QCO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors QCO and BAO managed the analyses of the study. Author QCO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Diarrhoea continues to be the scourge of children across the world. Despite efforts by the World Health Organisation and other agencies, much still remains to be done in combating diarrhoeal diseases in the developing world, including Nigeria. This study was conducted to determine the types of microorganisms responsible for diarrhoea in children in Akure, Ondo State, Nigeria. A total of 125 children with symptoms of diarrhoea that visited the Mother and Child Hospital, Akure, Ondo State, Nigeria was recruited for the investigation. Stool samples were obtained from these children and assayed using standard microbiological methods to isolate and identify the types of microorganisms present. The investigation showed the types and the percentages of the different microorganisms isolated from the examined stools as follows: *Escherichia coli* (89.6%), *Enterobacter aerogenes* (59.2%), *Klebsiella pnuemoniae* (47.2%), *Shigella flexneri* (37.6%),

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Salmonella typhimurium (32.8%), *Vibrio cholerae* (28.8%), *Salmonella typhi* (28.0%), *Shigella sonnei* (25.6%), rotavirus (24.8%), and *Vibrio paraheamolyticus* (21.6%). The significance of this result is discussed.

Keywords: Microorganisms; infantile diarrhoea.

1. INTRODUCTION

Diarrhoea is a medical condition of having at least three loose/watery bowel movement each day. In Nigeria, it is encountered both in urban and rural areas [1].

It is a sudden onset of watery stool lasting for less than four weeks (acute) or persistent (chronic). It affects people of all ages but it is more common and more severe in babies and young children who are more susceptible to dehydration and nutritional losses during an episode of acute diarrhoea. Diarrhoea is an important cause of under nutrition because patients eat less during diarrhoea and their ability to absorb nutrients is reduced. Moreover, nutrient requirement is increased as a result of infection [2].

It can be very contagious when one eats or drinks contaminated food and water, or touch contaminated surfaces or objects [3]. It is usually due to acute infection by viruses, bacteria, food poisoning toxins.

The illness usually lasts for 3 to 5 days. In severe cases, dehydration can result which causes approximately 1.8 million deaths every year [4]. Many of the risk factors for contracting diarrhoea are lack of access to safe water, poor hygiene practices and unsafe human waste disposal [5]. Diarrhoea is characterized by nausea, vomiting, stomach cramps, fever, headache, blood or pus in the faeces, loss of appetite, bloating, lethargy and body aches. The following viruses are implicated in diarrhoea; norovirus, calicivirus, rotavirus, astrovirus and adenovirus while the following bacteria; *Salmonella* species, *Shigella* species, *Yersinia*, Enteropathogenic *Escherichia coli* (EPEC), Entero aggregative *Escherichia coli* (EAEC) and *Vibrio cholerae* can also cause the illness [6]. Parasites such as *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* have also been reported as aetiologic agent of diarrhoea. This study is to know the types of microorganisms implicated in infantile diarrhoeal and because they are of medical importance associated with diarrhoeal diseases.

2. MATERIALS AND METHODS

A total number of 125 children passing unformed stool between the ages of 0-5 years that sought medical help at the Mother and Child Hospital Akure, Ondo State, Nigeria was recruited for this investigation between March and September, 2015.

2.1 Data Collection and Ethical Consideration

Permission was sought from the Hospital's Ethical Committee before the commencement of this investigation. A standardized questionnaire was administered to the mothers of the infected children whose stool samples were collected and were analysed based on the different socio - demographic variables considered in its design.

2.2 Specimen Collection and Assay

Stool specimens were collected into sterile plastic containers and transported immediately to the Microbiology Laboratory, Federal University of Technology, Akure, Ondo State, Nigeria where they were analyzed. The stool samples were cultured in an appropriate selective media such as, Eosin methylene blue agar (EMB agar), Salmonella - Shigella agar (SS agar) and Thiosulphate citrate bile sucrose salt agar (TCBS agar) at 37℃ for 24 hours. Each of these agars was prepared and sterilised according to the manufacturers' specifications. Immunochromatographic assay was used to detect the presence of rotavirus. The kit used was manufactured by R-biopharm AG, An der Neuen BergstraBe 17, D-64297 Darmstadt Germany. Test devices and extracted samples were allowed to attain room temperature prior to testing for rotavirus antigens in the stool samples. A disposable dropper was used to add 1 ml of sample diluent into a sample collection tube. Portions of the stool specimen 100 µl (about 50 mg) were aspirated from the stool sample using a disposable micropipette. The pipetted stool samples were introduced into the tube which contained 1 ml of sample diluent and homogenised to make a mixture.

Table 1. Biochemical characteristics of the bacteria isolated from the stools of infected children

CELL shape	Gram reaction	Catalase	Oxidase	Citrate	H_2S	MR	VP	Maltose	Mannitol	Lactose	Sucrose	Galactose	Glucose/dextrose	Probable organisms
SR	-	+	-	-	-	+	-	+	+	+	+	-	+	*Escherichia coli*
SR	-	+	-	+	-	-	+	+	+	+	+	-	+	*Enterobacter aerogenes*
SR	-	+	-	+	-	-	+	+	+	+	+	-	+	*Klebsiella pnuemoniae*
SR	-	+	-	-	-	+	-	+	+	-	-	-	+	*Shigella flexneri*
SR	-	+	-	-	-	+	-	+	+	+	-	-	+	*Shigella sonnei*
SR	-	+	-	+	+	+	-	+	+	-	-	-	+	*Salmonella typhimurium*
CR	-	+	+	+	-	+	+	+	+	-	+	+	+	*Vibrio cholerae*
CR	-	-	+	+	-	+	-	+	+	-	-	+	+	*Vibrio parahaemolytucus*
SR	-	-	-	+	+	-	-	+	+	+	+	-	+	*Salmonella typhi*

Keys: SR-Short rods, CR- Curved Rods, MR- Methyl Red, VP-VogesProskaur.

The homogenised solution was allowed to precipitate for at least 3 minutes until a clear supernatant was formed from which at least 200 µl and at most 500 µl was transferred into another clean tube. The test strip was then inserted into the prepared sample to reach the marked line on the strip for 5 minutes. If one color red band (test band) and one color blue (control band) are seen it shows that the stool contains rotavirus antigen. However, if the blue control band is missing, the test is invalid and not evaluated.

3. RESULTS

Rotavirus and nine bacterial species were isolated and identified from the stools examined. The bacterial species are *Enterobacter aerogenes, Klebsiella pneumoniae, Shigella flexneri, Shigella sonnei, Salmonella typhimurium, Salmonella typhi, Vibrio cholerae, Vibrio paraheamolyticus* and *Escherichia coli*. The biochemical characteristics of the bacterial isolates can be seen in Table 1.

4. DISCUSSION

The isolated bacterial species from the stool of diarrhoeic children examined in this study showed that most of the bacterial pathogens isolated from the stools are known to cause diarrhoea. Generally, the aetiology of diarrhoea in young children could be attributed to wide range of factors, but one of the main aetiology of the diarrhoea is related to bacteria Acute diarrhoea due to bacterial infections is an important cause of morbidity and mortality in infants and young children in most developing countries including Nigeria [1]. Clarification of the enteropathogens involved in diarrhoeal disease in the country is an essential step towards the implementation of effective primary health care activities against the disease [7]. and the presence of *Salmonella* species is of great significance. Most diarrhoea episodes occur during the first 2 years of life due to combined effects of declining levels of maternally acquired antibodies, the lack of active immunity in the infant, the introduction of food that may be contaminated with faecal bacteria and direct contact with human or animal faeces when the infant start to grow. Most enteric pathogens stimulate at least partial immunity against repeated infection or illness, which helps to explain the declining incidence of disease in older children and adults [8]. A couple of researchers have associated bacterial diarrhoea to *Salmonella* species; for example, [9,10] and [11]. The percentage incidence of rotavirus infection among children with diarrhoea in the community sampled was found to be 24.8%. Most of the infected children in our study were observed to be between the age group 0-12 months (31.0%). This age distribution is comparable to that of previous reports in Ile-Ife, Nigeria [12], in India [13], in Iran [14] and in Nepal [15]. Among all the bacterial isolates, *Escherichia coli* was found in 112 (89.6%), *Enterobacter aerogenes* in 74 (59.2%), *Vibrio cholerae* in 36 (28.8%), *Klebsiella pnuemoniae* in 59 (47.2%), *Salmonella typhi* in 35 (28%), *Salmonella typhimurium* in 41 *(*32.8%), *Shigella flexneri* in 47 (37.6%), *Shigella sonnei* in 32 *(*25.6%) and *Vibrio paraheamolyticus* in 27 (21.6%). Overall, the most frequently encountered microorganism was *Escherichia coli* (89.6%) while the least frequently encountered was *Vibrio paraheamolyticus* (21.6%).

Table 2. Frequency of occurrence of microorganisms isolated from the infected stools (n=125)

Organisms	Number infected (%)
Rotavirus	31 (24.8)
Escherichia coli	112 (89.6)
Enterobacter aerogenes	74 (59.2)
Klebsiella pnuemoniae	59 (47.2)
Shigella flexneri	47(37.6)
Shigella sonnei	32 (25.6)
Salmonella typhi	35 (28)
Salmonella typhimurium	41 (32.8)
Vibrio cholera	36 (28.8)
Vibrio paraheamolyticus	27 (21.6)

Legends: n = number of stool samples

5. CONCLUSION

This study has shown that *Escherichia coli* is the most frequently encountered microorganism in cases of infantile diarrhoea in the community studied.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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