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Research Article

Prevalence of Enterohaemorrhagic Escherichia coli (EHEC) and Enteric Parasites in Children Under 5 Years of Age Suffering from Acute Diarrhea in Ibadan, South West Nigeria F.C

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Abstract:

Diarrhea remains one of the diseases with high infant mortality in developing countries. Enteric viruses, bacteria and parasites have been implicated in diarrhea among children under five years of age. This study evaluates the prevalence of enterohaemorrhagic bacteria and parasites with interest on Entero-Haemorrhagic Escherichia coli (EHEC) among children under 5 years of age in Ibadan, Nigeria. Two hundred and thirty-five (235) samples were collected from four different hospitals in Ibadan metropolis. Presence of E. coli O157:H7 in the samples, was screened on Sorbitol MacConkey Agar and its identification was carried out with Analytical Profile Index (API) 20E. Presence of other isolates in the samples was confirmed using latex agglutination test. Antibiotic susceptibility test was carried out on bacterial isolates using disc diffusion method. Altogether, 208 entero-pathogens were isolated, among them were bacteria including 70 Escherichia coli isolates with two being EHEC strains; 2 Vibrio cholerae and 2 Salmonella spp. Pathogenic parasites were 134 altogether, out which 76 were Ascaris lumbricoides, 17 Taenia spp, 3 Enterobius vamicularis, 2 Trichuris trichiuria and 34 Entamoeba histolytica. Highest prevalence of bacterial and parasitic infections was among 11 and 22 months old children, with the males having higher prevalence. All bacteria tested exhibited resistance to tetracycline and ampicillin. The results from this study showed that, EHEC 0157:H7 is gradually becoming one of the causes of diarrhea among children in Ibadan metropolis. Also, of utmost public health concern is the resistance of all the bacterial isolates to ampicillin and tetracycline.

Keywords: enterohaemorrhagic escherichia coli (ehec); infantile diarrhea; enteropathogens; children; antibiotic resistance; 'ibadan'

Introduction

Diarrhea has been reported to be the second most common cause of mortality in children who are less than five years of age throughout the world and especially in developing countries [43] where mortality in children aged 0-5 years is one of the major indicators of child health [3]. It is characterized by an increase in frequency, consistency or volume of feaces, a result of water malabsorption or secretion in the bowel and may be a symptom of infection of the digestive system. Sometimes, it may be accompanied with vomiting and fever. [36] Diarrhea is a disease caused by a wide range of pathogens, including, viruses, protozoa and bacteria. [1] *Rotavirus*, is the leading cause of acute diarrhea and it is responsible for about 40 per cent of diarrhea among children under five years in hospital admissions worldwide [3]. Enteric bacterial pathogens commonly causing diarrhoea include *E. coli*, *Shigella*, *Campylobacter* and *Salmonella spp*. [26]. As a result of increasing morbidity and mortality rate, enteric pathogens have been implicated as one of the principal causes of infantile diarrhea, a major public health concern in developing countries and a huge impediment to socioeconomic development worldwide. [43] These pathogens have also been reported to develop resistance against different antibiotics especially Tetracycline and Ampicillin. Antibiotics resistance in many human pathogens of public health interest is on the increase as a global health issue and scientists around the world continue to work and make effort to combat this menace. Cattle and other ruminants can be asymptomatic carriers of *E. coli* O157:H7 and through exposure to antimicrobial drugs in the livestock production system, may serve as a source of antimicrobial-resistant bacteria. [35] Diarrhoea caused by EHEC is a potentially fatal infection and has remained frequent in Nigeria, particularly among children under the age of five years for many decades. [30] On an average, there can be an occurrence of 5-6 episodes of diarrhea in a child in the developing countries before he/she arrives the first year of birth (Rice *et al.*, 2000). In Africa, Asia, and South America, morbidity rate as a result of diarrhoea has been reported to be as high as 37% (Degebasa *et al*., 2018). The etiology of infantile diarrhea stems from poor living conditions like; lack of portable water, poor environmental hygiene and

sanitation coupled with high level of illiteracy and unawareness, overpopulation, underfeeding and poor standard of living (WHO, 2017; Gyang *et al*., 2019). Etiological agents implicated in diarrheal syndromes can either be single or multiple and they include bacteria, viruses and parasites (Bhutta and Syed, 2016). Of huge importance among the causative organisms of diarrhoea in Nigeria is *Escherichia coli* (Elimian, 2019), a common facultative anaerobe found in the colon of mammals. Many *E. coli* strains are harmless; therefore, they are rarely responsible for disease in individuals that are healthy, however some strains that produce biotoxins and as such potentially pathogenic can lead to intestinal and extra-intestinal diseases in healthy and immunocompromised individuals (CDC, 2021). Strains that are implicated in diarrheal diseases are important etiological agents and have acquired characteristics that make them to persist in the host through horizontal gene transfer (Ugboko *et al.,* 2020). Many of the diseases caused by intestinal parasites such as worms and protozoans have been classified among Neglected Tropical Diseases (NTDs), which can be prevented with low-cost medications except for economic challenges (Corell, 2020). Though humans of diverse age groups are affected by enteric parasites, the age group that are mostly vulnerable are the children and this may be a direct result of their poor hygiene practices and immature immune system [4] The common symptoms which include diarrhea, nausea or vomiting, gas or bloating, dysentery, itching around the rectum or vulva, stomach pain or tenderness, tiredness, weight loss passing of worms along with stool; have been linked with deficiency in absorption, loss of weight, anemia, stunted growth, poor assimilation, mental and intellectual impedance (Gyang *et al*., 2019; Nemeth and Pfelegaar, 2021). Both infections with EHEC and enteric parasites result in the damage of the intestinal tract which makes it impossible for absorption of digested food and water hence the purging. The damage to blood vessels lining the guts also contributes to the blood watery stool because the epithelial cells of the gut are unable to absorb water from the intestinal lumen. This is typical of infections caused by both pathogens. [36] Routine laboratory diagnosis of diarrhea in Nigeria does not usually involve screening for enteric parasites. Therefore, this study attempted to provide data on the prevalence of EHEC, other *Escherichia coli* pathotypes and enteric parasites present in diarrhea cases in infants and children under five years. Also, considering the lack of sufficient information on co-infection of enteric parasites and EHEC, this study provided data on the correlation between these two groups of infective agents.

Materials and Methods

Study Area and Population

The study was carried out within some selected hospitals in Ibadan, South west Nigeria. These hospitals include; University College Hospital, (UCH); Adeoyo Maternity Teaching Hospital, (AMTH) Yemetu; Our Lady of Apostles Catholic Teaching Hospital, (OLA) Oluyoro Oke-Offa and Kola Daisi Foundation Primary Health Care Centre, (KDFPC) Yemetu, all in Ibadan metropolis. Samples were collected between the periods of December, 2020 to August, 2021. Participants included in this research were children, both male and female, under the ages of 5 years, presenting with diarrhea, indicated by the passage of three or more loose stools within a 24 hour time frame, were not on any medications and visited any of the selected hospitals during the period of study. A total of two hundred and thirty-five (235) children whose parent/guardian consented to participate in the study were recruited for the study. Approval to conduct the study was obtained from the Oyo State Health Research Ethics Committee, Ministry of Health, Secretariat, Ibadan.

Sample Collection

Fecal samples were collected from 235 patients who attended the selected hospitals during the period of the study. Information on the age, sex, history of diarrheal illness, and clinical diagnosis were collected and documented accordingly. The specimens were collected in sterile, easy to open, leak proof, universal bottles that were free from traces of detergents. Samples were immediately placed in the refrigerator before processing or were transported to the laboratory within few hours of collection. Rectal swabs were used only in cases when it was not possible to obtain fecal samples especially in neonates. The swab specimens were transported to the laboratory and all processed within 2 hours of collection. Aseptic techniques were employed in order to avoid contamination by bacteria on the anal skin, outside of the containers and contamination with urine.

Media and Assay Kits

Bacteriological media used in this study include Alkaline Peptone Water (APW), Selenite-F broth, Sheep Blood Agar, Xylose Lysine Deoxycholate (XLD). Sorbitol MacConkey Agar (SMAC) and Mueller Hilton Agar all from Oxoid (U.K). Peptone water was purchased from Fluka (Spain). Analytical Profile Index (API) 20E is a biochemical panel (by BioMerieux Inc, Lyon, France) for identification and differentiation of members of the family *Enterobacteriaceae* 20 and this was used to identify the organisms isolated. Antisera for serotyping of *Salmonellae*, Shigella, Vibrio cholerae and *E. coli* (Mast Co, UK) were used to serotype the isolates. All media were prepared according to manufacturers' guidelines. Prepared plates were subjected to quality control (QC) before use. All media and supplies used passed the required QC tests and were used before their expiry dates.

Isolation of Pathogens

One gram of the stool specimens was inoculated into an enrichment broth of Tryptic Soy Broth at 37°C. After enrichment, the sample was streaked unto Sorbitol MacConkey Agar (SMAC) supplemented with cefixime and potassium tellurite. *E. coli* O157:H7 strains do not ferment sorbitol, so they appear as colorless or pale colonies on SMAC, while other E. coli strains ferment sorbitol and produced pink colonies on SMAC.One gramme of the stool specimen were also inoculated onto Salmonella-Shigella Agar (SSA) and Xylose Lysine Deoxycholate (XLD) plates and streaking was done to obtain discrete colonies. The sample was emulsified heavily in Selenite F broth and sub cultured onto SSA and XLD after 6-8 hours (Tadesse, 2017). This was used to isolate salmonella, shigella and other enterobacteriaceae.

Gram Staining

The Gram stain reaction was carried out on the isolates is based on the difference in the chemical composition of bacterial cell walls (Helmenstine, 2021).

Biochemical Test

A battery of biochemical tests were conducted which included the indole tests, Methyl red tetst, Voges- proskauer test, triple sugar iron tests, lysine decarboxylase test and catalase test to use to identify all the isolates. (Aryal, 2022; Tankeshwar, 2022). The tests was carried out in a lamina flow hood.

Antimicrobial Susceptibility Testing- AST

Antimicrobial Susceptibility testing was carried out using antibiotic discs obtained from Oxoid (UK). The discs were impregnated with: Ampicillin (AMP) 10µg, Cefotaxime (CXM) 30µg, Cefoxitin (FOX) 30µg, Tetracycline (TE) 30µg, Ceftriaxone (CRO) 30µg, Ciprofloxacin (CIP) 5µg, Meropenem (MEM) 25µg, Gentamicin (CN) 10µg and Amoxicillin-Clavulanic Acid (AMC) 30µg. Disk diffusion method was used to carry out this tests and the results were interpreted based on the Clinical and laboratory standard institute manuals (2019).

Identification of Parasites

In this study, microscopic examination method was used to identify parasites which included *Ascaris, Taenia, Enterobius, Trichuris,* and *Entamoeba* from the stool samples. Direct wet mount examination was

done with a small amount of the stool sample mixed in saline solution on a microscope slide. Staining to enhance the visibility and contrast of the parasites, was carried out to make parasites easier to identify with morphological features. Iodine stain was used for the detection of *Ascaris Taenia* and *Trichuris* eggs. Modified acid-fast stain, Kinyoun stain was used for the identification of *Entamoeba* and *Enterobius*. The slides were then mounted and observed under a light microscope at x40 objective. Morphological characteristic structures of the parasites, such as eggs, larvae, cysts, or trophozoites, were visualized and used to identify and record these parasites.

Data Analysis

All data generated from the study were expressed as percentages, statistical analysis of data was performed using SPSS version 22. Descriptive statistics were used to summarize the socio-demographic characteristics of the study participants. Bivariate and multivariable analyses were conducted to assess the risk factors associated with EHEC and enteric parasites among the participating children. P-values <0.05 were considered significant.

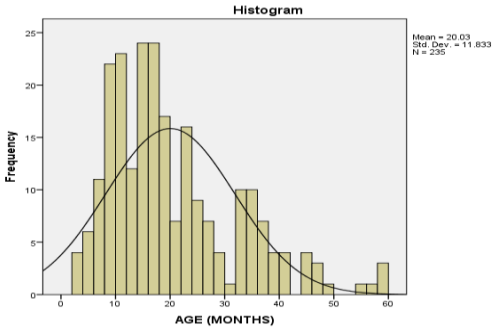


Figure 1: Age distribution of children under 5 years

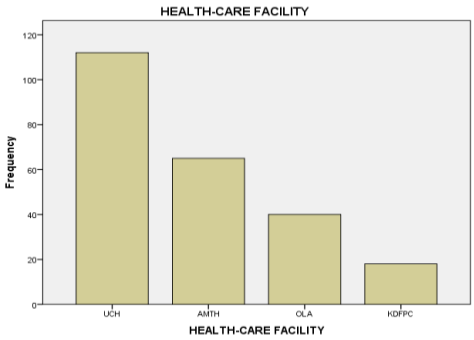
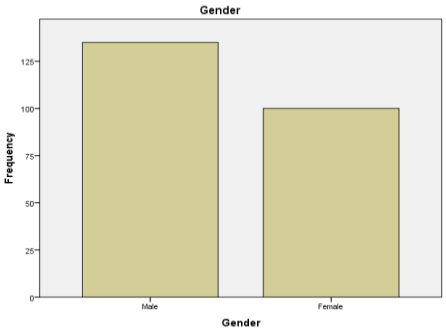


Figure 2: Gender distribution of children under 5 years



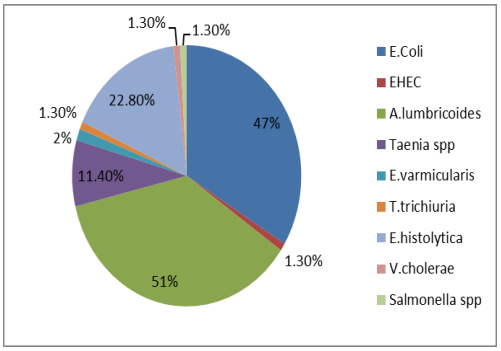


Figure 3: Frequency of the participants from selected Health- care centers. Figure 4: Percentage cases of Causative Agents

Correlation	EHEC	Ascaris	Taenia	Enterobius	Trichuris	Entamoeba
EHEC						
Ascaris	-0.064					
Taenia	-0.026	-0.053				
Enterobius	-0.009	-0.064	-0.026			
Trichuris	-0.009	-0.064	-0.026	-0.009		
Entamoeba	-00038	-0.010	0.025	-0.038	-0.038	

Table 1: Correlation coefficient between EHEC and Enteric parasites

Isolates	CRO		AMC		AMP		CXM		CIP		CN		TE		MEM	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Enterohaemorrhagic *E. coli (EHEC)* (n = 2)	1	1	-	2	-	2	-	2	2	-	-	2	-	2	2	-
Other Escherichia coli Pathotypes (n=70)	63	7	53	17	29	41	32	38	68	2	55	15	23	47	70	-
Salmonella spp (n= 2)	2	-	1	1	-	2	1	1	2	-	1	1	-	2	2	-
Vibrio cholerae (n= 2)	-	2	2	-	-	2	-	2	2	-	-	2	-	2	2	-

CRO: ceftriaxone, AMC: amoxicillin-clavulanic acid, AMP: ampicillin, CXM: Cefuroxime, CIP: ciprofloxacin, CN: gentamycin. TE: tetracycline, MEM: meropenem

Table 2: Antibiotic Susceptibility Pattern of Bacterial Isolates

Discussion

Diarrheal diseases are one of the major health problems in the world. The World Health Organization defined diarrhea as the passage of three or more loose or liquid stools within a period of 24 hours (or more frequent passage than usual in a healthy individual (Degebasa *et al*., 2018). Every year more than two million deaths from diarrhea are recorded in children under the age of five (accounting for one in ten child deaths worldwide annually) in many developing countries with Nigeria ranking second globally having about 151,700 deaths annually according to the World Health Organization. [37] The results of the study confirmed the presence of enteric pathogens and parasites in fecal samples from children suffering from diarrhea thereby confirming and implicating these organisms as the cause for the diarrhea being suffered by these children. This aligns with Peter and Umar (2018) which suggested that causative organisms of diarrhea may include bacteria, virus and parasitic organisms. The identification of *Escherichia coli* in 47.0% of the samples as the most predominant bacteria in infected children is in agreement with some studies that identified *E. coli* as a bacterium that colonizes the intestinal track of animals and humans [15,41] and is implicated in many of the reported cases of diarrhea in children under 5 years of age (Bhutta and Syed, 2016; Giannattasio *et al*., 2016). The result of this study showed that neither age nor gender of the children predisposes them to diarrhea. This finding corroborates with the report of [24] who also concluded in their study that age and gender are not significant risk factors for diarrhea infection in children. Other reports have also identified other causes (other than age and gender) as predisposing factors to diarrhea [17]. The prevalence of Enterohaemorrhagic *Escherichia coli* O157:H7 in this present study is 0.9% and this is in contrast with a study conducted by Okeke *et al.*, 2000, that reported a lower prevalence rate of 0.6%. A few studies have also reported higher prevalence of EHEC as 2.7% [24] These variations could be related to differences in sample size, age, study population or area, and specificity of methods used for identification [17]. The low prevalence of EHEC O157/H7 in this research work may be associated with the washing of hands with soap and water by most mothers or caretakers and general household sanitation as suggested by some literature [4,7,43] Studies in Spain and Mexico have also reported similar low prevalence. [9,10] In Nigeria and many developing countries Enterohaemorrhagic *Escherichia coli* O157/H7 have been isolated from different food sources which include vegetables, water, meat, cattle, sheep, goat and abattoirs (Yilgwan and Okolo, 2012). Therefore, the transmission of Enterohaemorrhagic *Escherichia coli* O157/H7 and associated diseases involve multiple reservoirs which are potential health hazards to human [41] Antibiotic resistance remains a significant threat worldwide to the control of bacterial infections [2] this stems from many factors including the administration of antimicrobials or antibiotics to animals and humans without proper prescription and this has led to the development of antimicrobial/antibiotic-resistant microorganisms. Very high antibiotic resistance rates were observed in all the *Escherichia coli* including the Enterohaemorrhagic *Escherichia coli*. EHEC O157:H7 isolated which showed multi-drug resistance to at least 4 of the antibiotics tested. This corresponds to the research carried out by Ahmadi *et al.* (2012) who reported that *E. coli* isolates from meat and meat product were resistant to ampicillin, tetracycline etc. The resistance pattern might be related the broad use of tetracycline [16] and ampicillin in management of various infections in livestock, indiscriminate over the counter sale of antibiotics and other factors [14] In addition, antibiotic multi-resistance to ampicillin, tetracycline, gentamicin, cefuroxime, ceftriaxone, and cefotaxime have also been reported by other researchers [1] The implication of this is that the use of these antibiotics for treatment may therefore be ineffective in patients with diarrhea from samples used within this study. More enteric pathogens were isolated in patients within the age group range of 11-22 months, with the least isolated from 0-9 months and also from 55 months of age, this suggests an association between age and bacterial diarrhea as well as breastfeeding and dairrhea. Those whose ages are below 11 months are essentially under their mother's care, and may feed mainly on breast milk thereby reducing their susceptibility to these pathogens [40] The predisposing factors that enhance spread and increase the risk of diarrhea in young children include failure/inadequate exclusive breast feeding for the first 6 months of life [11]. This is because the risk of developing diarrhea is greater in non-breastfed infants who are more susceptible to malnutrition than those exclusively breast fed. Breast feeding until at least one year of age or prolonged breast feeding reduces incidence and severity of diarrhea disease [29] The use of infant feeding bottle which may be contaminated with enteric pathogens; under nutrition, immunodeficiency or immune suppression, current or recurrent measles attack are among the risk factors which predispose young children to diarrhea infection (Giannattasio *et al*., 2016). The spread of intestinal parasites in various parts of the world, especially the tropics and subtropics can also be a result of population density in areas with a lack of basic amenities for the health of the public. It is also believed that the use of human feces as fertilizer is an organic factor that increase the chances of infection and spread of these parasites [17], Evidence supported that (70%) of cases of diarrhea in developing countries are due to contamination of foods; lack of health awareness and lack of hygiene. [23] Most diarrhea episodes occur during the first 2 years of life due to combined effects of declining levels of maternally acquired antibodies, lack of active immunity in the infant, the introduction of food that may be contaminated with faecal pathogens and direct contact with human or animals (domestic animals/pets) facecs when the infant starts to grow. Most enteric pathogens stimulate at least partial immunity against repeated infection or illness, which helps to explain the declining incidence of the disease in children 55 months and older [7,11]. The reason for high incidence of enteric pathogen isolates in children between 11 and 22 months could also be due to the fact that children within this age group on their own cannot differentiate between what to eat and what not to eat; they have not learnt the rudiments of adherence to aseptic or hygienic practice [3] this is also in line with research that have documented high incidence of diarrhea in children lesss than 2 years of age [25]. The result from the study also showed isolation of *Vibrio cholerae* (0.9%) from children suffering from diarrhea. [27] reported that this organism is widely distributed; and they isolated it from rivers, estuaries, sewage, cockles, oysters, lobsters, a bird that died of a cholera-like disease; and also from human facecs. This bacteria has been implicated in incidences of human diarrhea. This has serious implication for the health of children and can lead to fatality. The results showed that the total infection with enteric parasites is high (52.9%), this implies that there are many young children suffering from parasitic infestations which could be due to poor sanitation; poor public health practices, increasing of vectors and malnutrition states [3,17]. There is certainly the need for campaigns and collaborations by academics and concerned authorities to lower this prevalence and increase sanitation and hygiene practices in homes. The study results showed the highest percentage of parasitic infestation with *Ascaris lumbricoides* (32.6%) followed by *Entamoeba histolytica* (14.5%) among study subjects. This result agrees with the result of other studies in Nigeria [12].

Conclusion

This study affirms that diarrhea caused by EHEC 0157:H7, a potentially life-threatening pathogen, and is becoming particularly common among the children under the age of five years in Ibadan along with other Escherichia coli pathotypes, salmonella and Shigella species. This

research study also reveals that non-O157:H7 *E. coli* continues to constitute a major cause of pediatric diarrhea infections in Ibadan

Recommendations

Hygiene is very important to keep infants and their mothers healthy and this can be achieved by encouraging regular hand washing practices among mothers and children. Exclusive breastfeeding for the first six months of life as recommended by the WHO is also an important method of safeguarding the health of babies and infants. This practice will reduce or almost eliminate the exposure of infants to pathogens and enteric parasites. Academics and researchers must also provide information and engage in health campaigns to the general public either individually or in collaboration with government agencies and non-governmental institutions. This will help to increase the level of awareness at local, state and national scales in other to prevent deaths from childhood diseases and lower childhood and infant mortality to the barest minimum.

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