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Prevalence of *Entamoeba histolytica* Among Children using Different Methods in Port Harcourt, Rivers State

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Abstract

The prevalence of *Entamoeba histolytica* using different methods in detecting the causative pathogens causing amoebic dysentery among children in Rivers State was carried out using standard laboratory procedures. This research was carried out in some selected health establishments in Port Harcourt City, the capital of Rivers State, Nigeria. The aim of the study was to evaluate the prevalence of *E. histolytica* among children in Port Harcourt, Rivers State. The study which involved about one thousand stool samples were collected from consented subjects in Rivers State hospitals, of which 533 fecal specimens from male and 467 from female children were collected. Stool sample examined using the three diagnostic methods which were microscopic methods using normal saline and lugol's iodine, formol-ether sedimentation technique and Entamoeba culture methods. The results showed that, out of the 1000 stool samples examined, 54 (5.4%) were positive for cyst and or trophozoites of *E. histolytica* when examined by direct saline and lugol's iodine bright light microscopy. Twenty-one (2.1%) showed presence of *E. histolytica* when processed by sedimentation method, whereas 87 (8.7%) showed presence of *E. histolytica* in all the samples processed by culture method. These results implementing 89%, 81% and 95% specificity for microscopy, sedimentation and culture method respectively. Furthermore, culture method, microscopy method and sedimentation method showed sensitivity of 95% 62% and 40% respectively. Data analysis of kappa statistics showed culture method have the highest value of (0.75) followed by microscopy (0.55) and sedimentation (0.33). Finally, culture method which is the most reliable test method could be a very useful tool in the routine assessment as well as surveillance of *E. histolytica* in a developing country like Nigeria. Therefore, public and personal health hygiene should be taken seriously to avoid and control the infection in Rivers State.

Keywords: *Entamoeba histolytica, prevalence, formol-ether, Amoebiasis, Stool, Dysentery*

INTRODUCTION

Laboratory methods are the most paramount tools in the definitive diagnosis of disease in a population. In Africa and the world at large, parasitic disease is a common feature due to socio and environmental factor, among these parasites is *Entamoeba histolytica* [1]. *E. histolytica* is a parasitic protozoan that causes liver abscess and amoebic dysentery. Amoebiasis is a household name in most regions of the World and has been elaborately studied around considering its negative impact to human health [2]. *E. histolytica* localizes in the large intestine and can also spread to various visceral organs such as pleura, lungs, liver, spleen, the skin, the genitor-urinary tract, and the brain. The medical effect of intestinal Amoebiasis is alarming. The World Health Organization had reported that amoebiasis is the most aggressive pathogenic protozoa disease that affects the human bowel and as such, it is regarded as the second foremost cause of morbidity and mortality amongst

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the various parasitic diseases, exceeded by malaria and schistosomiasis. About 50 million people infected per annum causing close to 100,000 mortalities [3].

Amoebiasis is prone in areas of meager hygiene and malnutrition, predominantly in the tropics [3]. The prevalence and presentation of symptoms of amoebiasis varies from different geographical location. The Clinical symptoms associated with intestinal amoebiasis include abdominal discomfort, weakness, malaise, constipation that may interchange with diarrhea, dysentery with the passage of exudates, blood, and mucus as well as flatulent abdominal pain. Systemic amoebiasis signs include fever, rigors, and polymorph nuclear leukocytes while the liver abscess results from infection through the intra-hepatic portal vessels [4].

Prevalently, among children are mostly affected by intestinal amoebiasis owing to unhygienic lifestyle and this triggers their high-rate hospitalization in endemic areas of developing countries [5]. It has been evaluated to cause up to 19% of the annual 10 million under-five mortality in the developing world [1]. Records have shown that the incidence of gastroenteritis caused by *E. histolytica* in Nigeria is more significant among children than adults, as reported by some researchers. Epidemiologically, it has been affirmed in some quarters that there is a high prevalence rate of intestinal parasitic infections among children in Nigeria [6].

Infection is characterized by the cysts and trophozoites in the stool (either formed stool, semi-formed stool, or loose stool) and aspirates in the case of extra-intestinal *E. histolytica* infection [7]. Other laboratory diagnostic procedures of amoebiasis are usually based serological methods. Nevertheless, molecular diagnosis is the best test for accuracy and precision for detecting the pathogen in symptomatic and asymptomatic patients [4], particularly in developing countries which have poor hygienic circumstances and insufficient water treatment systems including Nigeria and its states.

RESEARCH METHOD

Study design

Comparative study with simple random sampling method was used to carry out this research among one thousand selected stool samples from consented subjects in Rivers state hospitals and the duration of the study was for eleven months. About 533 male and 467 female children stool samples was collected.

Sample collection

About 533 male and 467 female children stool samples was collected using wide mouthed clean grease free and detergent free universal bottle with a tight cork. Information relating to biological and demography of the patients were recorded on submission of samples using a questionnaire. To estimate sample size of 0.426 proportion (42.6%) at a confidence level of 95% with an acceptable difference/Error Margin of 0.05 (5%). Required sample size = 418 if the proportion is 0.426, inflated to compensate for loss of 10% of subjects. Assumed proportion of 0.426 (42.6%) is the prevalence of E. histolytic among Children (8). Sample Size Calculator used was Winpepi version 11. 44, under describe program version 2.72 "K Sample size (to estimate proportion/rate/mean or find cases" [9]. The sample size was increase to 1000 in order to increase the power of the study.

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Informed consent from parents/guardians of the wards and children before samples were collected from them either orally or written.

Sample analysis

Macroscopic examination of all stool samples received from subjects were observed for consistency, color, the absence or presence of mucus or blood and microscopic examination was also carried out with the aid of an applicator stick, a representative of each stool sample was collected and emulsified on normal saline and lugol's iodine drops placed on a clean grease free dry slide placed on each separate ends of the slide and viewed under the microscope using X10 and X40 objective lens [10].

Concentration of cyst using sedimentation method was also employed on the stool samples by formol-ether concentration technique on each sample. About 4ml of formol water was added to a screw capped test-tube and with the aid of an applicator stick, about 1 gram of a representative fecal sample was emulsified in the formol-water in the test tube. The tubes were corked and spun at 1500rpm for 10 minutes. Thereafter, the supernatants were decanted while a small mixed portion of the sediment was collected into a clean glass slide, covered with cover slip and viewed microscopically using X10 and X40 objective lens [10]. Cultural method using Entamoeba medium was employed but was supplemented with proteose peptone and infusion from liver which provide amino acids, Sodium chloride, Sodium a-glycerophosphate and other nitrogenous substances that enhances the growth of *E. histolytica*. The prepared media were added to sterilized glass tubes, autoclaved at 121^{o}C allowed to solidify in a slant position and covered with fresh sterile horse-serum-saline mixture in a ratio (1:6). Then, a 5mm diameter l00 cupful of sterilized rice powder heated at 160°C for one hour was added [10].

FINDINGS AND DISCUSSION

Findings

The comparison of different laboratory methods used for the detection of E histolytica shown culture method was more reliable with (K = 0.75, x^2=599.4,) followed by microscopy (K=0.55, x^2=375.78) while sedimentation was the least reliable (k=0.37, x^2 =225.11) (Table 1). This observation was significant with the p-value of p<0.0001.

Table 1. Comparison of Laboratory Methods

Variables	Classification	Total(number)/ Frequency (%)	Df	x^2	Kappa	P – value
Microscopy	Negative	946/94.6				
	Positive	54/5.4	1	375.78	0.55	0.000
Sedimentation	Negative	979/97.9				
	Positive	21/2.1	1	225.11	0.37	0.000

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Culture	Negative	913/91.3				
	Positive	87/8.7	1	599.04	0.75	0.000

The comparison of different laboratory methods used in the detection of E histolytica using diagnostic testing are shown on the Table 2. Culture method was more sensitive and reliable (sensitivity 95%, specificity 95%, positive predictive value 64%, negative predictive value 99%, positive likelihood ratio 4.5%, negative likelihood ratio 0.5% and rank first in order of validity). Microscopy results showed (sensitivity 62%, specificity 89%, positive predictive value 24%, negative predictive value 87%, positive likelihood ratio 3.2%, negative likelihood ratio 1.6% and rank second in order of validity). Sedimentation results showed (sensitivity 40%, specificity 81%, positive predictive value 4.3%, negative predictive value 36%, positive likelihood ratio 1.8%, negative likelihood ratio 2.4% and rank third in order of validity.

Table 2. Comparative Indices for the Different Laboratory Methods

				Diagnostic Testing Indices (%)						
Lab Methods	Pos	Neg	% Prev	Sens	Spec	PPV	NPV	LR+	LR-	Order of Validity
Microscopy	54	936	5.4	62	89	24	83	3.2	1.6	2nd
Sedimentation	21	979	2.1	40	81	4.3	36	1.8	2.4	3rd
Culture	87	913	8.7	95	95	64	97	4.5	0.5	1st

Note: POS => Positive; Neg => Negative; Prev => Prevalence; Sens => Sensitivity; Spec => Specificity; PPV = Positive Predictive Value; NPV = Negative Predictive Value; LR+ = Positive Likelihood Ratio; LR- = Negative Likelihood Ratio.

The distribution of Children with *E. histolytica* infection based on age group shown on the table 3. The age range 6 to 10 years had the highest prevalence of 109 (10.9%) of *E. histolytica* infection, followed by 0 to 5 years with prevalence of 43 (4.3%) and the least with prevalence of 12 (1.2%) was group 11 to 15 years.

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Table 3. Age Distribution of Amoebiasis among Children in Port Harcourt, Rivers State

Grouping Criteria	No. Examined	No. Positive	% Prevalence
1-5 yrs	420	43	4.3
6-10 yrs	320	109	10.9
11-15 yrs	240	12	1.2

Table 4 showed the distribution of Children with *E. histolytica* infection based on gender. Females showed higher prevalence of 83 (8.3%), while males showed lower prevalence of 81 (8.1%) of *E. histolytica* infection.

Table 4. Gender Distribution of Amoebiasis among Children in Port Harcourt, Rivers State

Gender	No. Examined	No. Positive	% Prevalence
Female	467	83	8.3
Male	533	81	8.1

Discussion

Out of the 1000 stool samples of children examined, 54 (5.4%) were positive for cysts and/or trophozoites of *E. histolytica* when examined by direct saline and lugol's iodine bright light microscopy. 21 (2.1%) of the 1000 stool samples showed the presence of cyst of *E. histolytica*, when processed by sedimentation method whereas 87 (8.7%) showed presence of *E. histolytica* in all samples processed by cultural method. These results were in consonance with other research on amoebiasis among children; 8.6% prevalence reported in a study carried out in Pakistan [7] and 11.0% in Degema [11].

There was no significant difference between the proportion of male and female children that were positive for amoebiasis in this study. Therefore, gender was however a minor attribute that predisposes or influences the prevalence of amoebiasis amongst children which was contrary by a study carried out by Pinheiro et al. [12] who in their research found out that gender was significant.

The diagnosis of *E. histolytica* in fecal specimen was mainly by the observation of *E. histolytica* trophozoites or cysts in several fresh stool samples sent for stool analysis using microscopic and cultural method of isolation of the causative agent, which are reliable methods for detection of parasites and protozoa. Nevertheless, this was buttressed by the calculation of specificity and sensitivity of these methods in detection of the presence of *E. histolytica* in fresh stool samples used in this study. It showed that culture method had the highest specificity of 95%, which indicates that there was a possibility of 0.95 chance of all the stool samples without the causative agent of amoebiasis to have a negative result. This was not the case for microscopy and

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sedimentation methods that showed 89% and 81% specificity respectively, although, these children had clinical manifestations of gastroenteritis.

The Kappa statistics of the three methods of detection of *E. histolytica* were sedimentation (0.37), microscopy (0.55), and culture method (0.75). The cultural method significantly detects the presence of *E. histolytica* in the stool samples examined more than the other methods ($p<0.0001$). This research also revealed the age dependent distribution of Amoebiasis along age groups in children whose result was not surprising because children between ages 6-10 were given minimal supervision as compared to toddlers, their unhygienic lifestyle, poor sanitation especially in rural areas, overcrowding and excessive playing habit predisposed them greatly to contamination and infection of the Entamoeba parasite. The children below age 5 (creche and nursery school stage) were also prone to Amoeba infection; though with more supervision by parents and caregivers, their weak immunity, fondness for putting substances in their mouth and poor nutrition predisposes them to infection [10]. Furthermore, children between ages 11-15 (secondary school stage) are the least prove to amoebiasis because at this stage they are fully aware of the importance of proper personal hygiene, good sanitation and source of water and good contamination. However, sheer carelessness on their part alongside poverty could expose them to contracting the infection. Thus, with a seldom occurrence of positive samples (8.7%), the values speak for themselves.

Based on the culture method, the prevalence of *E. histolytica* is 8.7%. This is different from a previous work by Ochei et al. [10] who reported a prevalence of 11% in Degema Local Government Area of Rivers State in Nigeria. It may therefore be posted that *E. histolytica* infection occurs more in the rural areas such Degema than Port Harcourt metropolis of Rivers State. This is probably due to low socio-economic factors that are prevalent in rural settings in Nigeria [13]. Children between the age range of 6 to 10 years (10.9%) was observed to have the highest rate of *E. histolytica* infection in this study.

Gastroenteritis likely to be associated with *E. histolytica* infection was found to be more prevalent as detected by the culture method. This may explain why children presenting signs and symptoms suggestive of the infection within the study area at various hospitals. This invariably reflects deterioration in both public and individual hygienic standards, besides unimproved drinking water plays a contributing role in such increase in *E. histolytica* infection. Conclusively, this study is a contribution to the epidemiological survey of amoebiasis in Rivers State of Nigeria especially in children. The findings of this study depict that culture method which is not a routine procedure in our immediate environment stands out to be the most reliable mode of isolation of *E. histolytica*.

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