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Prevalence of Isolated Agent in Diarrheal Infections of Children O-3 Years in Anambra State in Relation to Sex: A Survey of Five Rural Communities

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Abstract

A random sample of a population size of 600 was utilized in the study; comprising of 300 males and 300 females. There were no problems in collecting samples from the hospitals and clinics in Enugu. It was conveniently carried out with the permission of the Health-workers in charge. Examination of samples were carried out in the department of Parasitology Laboratory, Anambra State University of Technology, Awka Campus. Stool and blood samples were collected. Disposable sterile containers were used for collection of stool samples. Samples were collected in such a way as to ensure non contamination with urine, so as to avoid lysis of the trophozoites on contact with water. The prevalence of the isolated agent does not show any significant difference in relation to sex. From the observed 611 isolated agents, 258 (42.2%) was recorded for males while 353 (57.8%) was for females. Also, for organism like E. coli, it was observed that 33 or 5.4% and 42 or 6.9% were for males and females respectively. Generally, therefore, it was established that the prevalence of isolated agents is independent of or not associated with sex of patients.

Keywords: Prevalence; Diarrhea; Children 0-3 years; Sex rural communities

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Introduction

Gastroenteritis is the inflammation of the gastrointestinal tract lining, which involves both the stomach ("gastro") and the intestines (entero) and this results in sudden onset of diarrhoea and vomiting [1]. Gastroenteritis remains a major global problem in children around the world. Children in sub-Saharan Africa are 15 times more prone to death from diarrhoeal diseases before they attain the age of 5 years than children living in countries that are developed [2]. Infection due to gastroenteritis has been known to be caused by microorganisms such as: *Salmonella species*, *Shigella species*, *Campylobacter species*, *E. coli* O157:H7, *Yersinia enterocolitica*, *Vibrio cholera*, Rotavirus, *Cryptosporidium species*, *Entamoebahistolytica*, *Listeria monocytogenes* and *Giardia lamblia* [1]. Other causes are by ingestion of some food items, chemical toxins or drugs.

A change in bowel habit from normal with an increase in stool volume and/or fluidity resulting in an increase in stool frequency is referred to as diarrhoea. It is also defined as a form of gastrointestinal infection caused by a variety of bacterial, parasitic and viral organisms or via contaminated drinking water, food or from person-person as a result of poor hygienic practices. If not untreated, diarrhoea can typically last several days [3].World Health Organization (WHO) regards a disease to be diarrhoea if there is a passage or excretion of watery stools in about two-three times within 24 h period [3]. However, factors such as; stool frequency, stool consistency, and the usefulness of parental discernment in determining whether children have diarrhoea or not is clearly important to ascertain if diarrhoea has occurred or not. Acute diarrhoeal illnesses or dysentery is often easily characterized by bloody appearance of stool, irrespective of frequency or consistency [3].

Diarrhoeal episode is usually divided into acute, persistent and chronic. The most common of diarrhoea disorders, acute

diarrhoea often begins abruptly, are as a result of infections and are subdue/resolved within 14 days. Persistent diarrhoea arises as a result of secondary infections in the presence of complications like malnutrition while chronic diarrhoea is majorly a product of congenital defects of digestion, absorption in the body system and it lasts for a minimum of 14 days [3]. Among children below the age of 5 years old, diarrhoea- related diseases account for the second highest cause of death [4]. Although the diarrhoea mortality rate has reduced globally, morbidity rate is still high in Sub-Sahara Africa because the region is experiencing increased population growth, practices, lack of proper hygiene conditions and resources for surveillance, diagnosis, treatment and prevention of the disease is scarce in the region [4].

In sub-Saharan Africa, there are more than one billion diarrhoeal cases and an estimated 606,024 deaths of diarrhoea yearly with nearly half of the deaths occurring in children lesser than five years of age [5]. In Nigeria, there are an estimated 151,700 yearly child mortality as a result of diarrhea [6] with the prevalence rate of diarrhoea ranging between 10% and 18.8% [6] and 80,968 deaths as a result of unsafe water and unhygienic sanitation thus making Nigeria one of the leading contributors to diarrhoeal morbidity and mortality worldwide [6].

However, resistance has emerged even to newer, more effective antimicrobial agents [7]. Among the factors leading to increased risk of diarrhoea among children are: failure to adequately breast-feed a child for the first 4-6 months of life. Diarrhoea has been observed to be much greater in non-breastfed than adequately breastfed infants. Susceptibility of host to infection is assessed by the child's age, presence of protective maternal factors (trans-placental antibodies), immunological status, nutritional status, and prior exposure to foreign harmful entities, acquired immunity and genetic susceptibility [7].

Material and Methods

Sample population size

A random sample of a population size of 600 was utilized in the study; comprising of 300 males and 300 females. There were no problems in collecting samples from the hospitals and clinics in Enugu. It was conveniently carried out with the permission of the Health-workers in charge.

The rural Health Clinics and general hospital of the study areas in the Enugu environs were also visited for collection of samples since it was not easy to get enough cases of diarrhoea in the individual homes in those areas, it was noted that most diarrhoea cases in those areas go to health Clinics and general hospitals where available, for treatment. Sampling of individuals was done purely on the basis of availability and chance.

Procedure for field work

In the study areas of the rural dwellers, young boys and girls were employed and trained to scan for cases and collect fresh samples. These were made available at specific days and times for mass transportation to appropriate quarters for investigation. These assistants were also trained to record answers obtained from the interview-administered questionnaires given to illiterate parents, and to distribute, collect and return self-administered questionnaire forms from literate parents. They co-operated and did the job judiciously. The health workers were incorporated to do the same in most of the health centres. That made data collection very easy. The village heads in most cases helped in inviting the young boys and girls who were employed for the job, and in enlisting the co-operation of parents and guardians. A mobile laboratory which consisted of 70% alcohol, lancetten, cotton wool slides, empty sterile containers, tapes, bathroom scale and a big padded slide box was quite useful for the field work.

Physical examination

The patients were examined physically to detect diarrhoea signs such as skin's elasticity which also occur in malnutrition. This is measured by the pinch of the skin retracting immediately, slowly or very slowly in more than 2 seconds. The state of the eye were noted, whether it is normal, sunken or deeply sunken. Dryness of the mucous membrane of the mouth was checked.

The child's anterior fontanelle was checked to know if it is normal sunken or very sunken. The parents answered few questions associated with their personal observation of their sick children which include.

(a) Does the child vomit?

(b) Does the child produce tears?

(c) How many times does the child stool in a day?

(d) And, how is the consistency of stool? If hard, soft or watery.

Use of questionnaires

Two types of questionnaires were used. The interview-administered questionnaires for illiterate parents and self-administered questionnaire forms were given to literate parents to fill and return.

The information needed include the following: Name of patient, age, sex, residential address and occupation of parent. Date of onset of illness, symptoms, frequency in stooling, and quality of diet (breast milk, artificial milk or solid foods). If artificial milk, the type of sterilization unit method employed and tolerance of milk formula. Frequency of vomiting and types of drugs taken. The questionnaire forms were made available to the parents through the assistants in various collecting centres, they were written in English language as seen in appendix. In this way, important data for each patient was obtained at the time of visit to the clinic or homes. The economic status of the patient's parents was also noted.

Surveys

The study areas were visited repeatedly to scan for diarrhoeal cases. The environmental and households surveys were undertaken to establish the fact that poor socio-economic and environmental conditions predisposes to high incidence of diarrhoeal infection in children. In order to establish these facts, on the spot assessments were made on the degree of cleanliness of the environments. Socio-economic status of the people was recorded with the use of questionnaires. Three

hundred households in the Enugu Environs were surveyed; sixty households were visited in each of the environs.

Laboratory examinations

Examination of samples were carried out in the Department of Parasitology Laboratory, Anambra State University of Technology, Awka Campus.

Sample collection

Stool and blood samples were collected. Disposable sterile containers were used for collection of stool samples. Samples were collected in such a way as to ensure non contamination with urine, so as to avoid lysis of the trophozoites on contact with water. It was noted if the child had been on antibiotics or antidiarrhoeal compounds containing kaolin, pectin, bismuth or magnesium hydroxide, as these could also suppress the growth of the micro-organisms. The diarrhoea cases wore chosen on the basis of, 'Three or more soft or liquid stools within 12 hours or a single soft or liquid stool-containing blood, pus or mucus.'

Blood samples were taken from patients with violent vomiting to screen for presence of malaria parasites. These were collected by ear-lobe or finger pricking technique using Lancetten. Thick films were usually made immediately on the microscopic slides, allowed to dry.

All the samples were clearly labeled and packed for transportation. They were preserved in the refrigerator at 4ºC if the tests were not carried out 2 hours after collection.

After each sample collecting session, a group talk was given to the mothers (especially in the rural areas) by way of advice on oral rehydration therapy (ORT) as shown in the plates of the appendix 2 and also enlightened them on ways of protecting their babies against diarrhoea. The talk was usually delivered in the native language for better comprehension.

Microscopic examination

Macroscopic examination of stool samples gave useful information. In profuse watery stools (rice water stool) sometimes flecked with mucus, enteropathogenic *E. coli* was suspected. Lesser quantities of soft stool containing blood and mucus was suspected of amoebiasis bacillary dysentery, shigellosis or campylobacter infections.

Bacteriological examinations

Loopfuls of each specimen of stool were directly inoculated onto deoxycholate citrate agar, plates and into tubes of selenite F. The stools were emulsified in tubes of peptone water before MacConkey agar plates and blood agar plates were inoculated; all were incubated at 37ºC for 24 hours. The following day the plates were examined for non-lactose fermenting colonies. The selenite F cultures were plated out onto MacConkey plates and incubated at 37ºC overnight. Non-lactose fermenting colonies were identified. Blood agar culture plates were used for serological identifications.

Examination of Blood Samples for the presence of malaria parasites

My assistants were trained to make only the thick films. A drop of blood on a microscopic slide, was rotated with a stick to about 2cm in diameter, and allowed to air dry Staining was done using one in ten dilution of Giemsa solution for 10 minutes. The water content of the solution lyses the red blood cells exposing the parasites. The presence of malaria parasites was determined by microscopic examination.

Identification of organisms isolated

Identification of the different bacteria was carried out using a battery of biochemical tests and serotyping. About 5 to 10 non-lactose fermenting colonies from each plate transferred to triple sugar iron agar slopes and incubated at 37ºC for 24 hours. Christensen's Urea slopes were inoculated to test for proteus, while Pseudomonas was tested by oxidase reaction. Agglutination of the non-lactose fermenting organisms from DCA Agar plates with polyvalent and type specific antisera identified shigella and salmonella For identification of *E. coli* a lactose fermenting organism, colonies were subcultared from the MacConkey plates into peptone water for indole test, dextrose phosphate medium for methyl red and vogues-Proskauer tests and finally into Citrate medium for citrate utilization tests.

Serological typing for Salmonella was done first by agglutination tests with polyvalent O group serum (Wellcome) Later, the o group was identified by using individual group sera (Wellcome). Finally, the antigenic pattern was determined by using various monospecific sera. For determining the H antigens, agglutination was first done against polyvalent H serum and subsequently with monospecific sera of both phases.

Strains of *E. coli* were classified according to their specific serological types. Colonies from Blood agar plates were used for attaining an accurate serological test. Slide agglutination tests with polyvalent OB antisera (Welcome) was done. Those found positive were tested with individual anti OB sera. The strains were confirmed biochemically using sugar fermentations according (Stokes 1970).

Strains of shigella were similarly tested using slide agglutination techniques with antisera for Shiqella (Welcome). The sera used were polyvalent flexner, polyvalent boydii, Shigellasonnei and Shigelladysenteriae antisera. Confirmation of the identification of all strains was done by carrying out detailed biochemical tests. Attempt was not made to isolate viruses because, in the absence electron-microscope and other facilities, detection of rotovirus in stools cannot be undertaken.

Areas of study

The areas of study which include the environs of Enugu, notably Amechi, Ugwuaji-Awkunanaw, Abakpa-Nike, Emene and Agbani were chosen for comparative studies on the incidence of diarrhoeal infection.

Table 1. Investigative cases of diarrhoeal infections in community according to sex.

Community	Hospitals/Health Centres/Private/Clinics/ Homes		Total
	Male	Female	
Enugu	150	150	300
Urban	30	30	60
Abakpa Nike	30	30	60
Amechi	30	30	60
Ugwuaji	30	30	60
Emene	30	30	60
Agbani			

Results

Prevalence of isolated Agents in relation to sex

The prevalence of the isolated agent does not show any significant difference in relation to sex. From the observed 611 isolated agents, 258 (42.2%) was recorded for males while 353 (57.8%) was for females. Also, for organism like *E. coli*, it was observed that 33 or 5.4% and 42 or 6.9% were for males and females respectively,

Generally, therefore, it was established by Chi square (X^2)test that the prevalence of isolated agents is independent of or not associated with sex of patients (Table 1).

(x^2c = 12.072; X^2 Table = 15.507), P > 05.

Discussion

The most affected babies in the rural areas were babies whose age range were from 2 ½ to 3 years, and were mostly weaned, babies, while in the urban town of Enugu, diarrhoeal cases occurred as early as from the fifth month to the third year of life. This confirms the finding that people in rural areas breast-feed their children longer than those of the Urban dwellers who introduce milk formulas to their children at the early age.

It is noted that weaned babies of the rural areas contract some parasitic infections such as ova of hookworm which are out during diarrhoea. This may occur as a result of these babies accompanying their parents to the farmlands where the soil is rampantly polluted with faecal matters. Other parasitic infections causing diarrhoea include Giardiasis, Trichuriasis, Ascariasis and other cystic infections.

Serotypes of EEC 0111, and 055 were- readily recovered from water supplies associated with outbreaks of gastroenteritis in the United States and France respectively [8].

Entamoeba histolytica was incriminated in 15 (2.5%) of diarrhoeal cases. This may occupy as a result of faecal contamination of drinking water or water supplies. The causative agents of diarrhoeal infection may be ingested through drinking such contaminated water if it is not boiled and filtered as experienced in most families in the rural and the urban areas. Vegetative state of E histolytica was found not to be much in number in the rural dwellers in relation to the consumption of unboiled and unfiltered contaminated water in those areas. It is envisaged that these trophozoites die in the cause of transportation of faecal samples to the appropriate quarters for investigation [8].

Conclusion

The prevalence of the isolated agent does not show any significant difference in relation to sex. Generally, therefore, it was established that the prevalence of isolated agents is independent of or not associated with sex of patients.

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