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PREVALENCE OF DIARRHOEIC AGENTS AMONG HOSPITALIZED CHILDREN IN SOME HOSPITALS IN PARTS OF SOUTH EAST NIGERIA

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ABSTRACT: Studies were carried out to determine the prevalence of diarrhoeic agents among the hospitalized children in Uyo, Akwa Ibom State, Nigerian. One hundred stool samples were analysed using standard microbiological methods. All the one hundred samples screened contained one or more organisms. This gave a prevalence rate of 100%. Seventy two stool samples were infected with bacterial agents giving the prevalence rate of (72%). The different prevalence rate of bacterial agents from hospital samples were obtained but there was no significant difference ($p \geq 0.05$).The prevalence rate of (28%) were observed with parasitic agents. The prevalence of parasitic agents of diarrhea in each hospital screened was determined and it did not differ statistically ($p \geq 0.05$).The percentage frequency of occurrences of each bacterial isolates revealed *Escherichia coli* (31.1%) *Vibrio cholera* (22.2%) *Salmonella typhi* (15.6%), *Salmonella paratyphi* (4.5%) while *Shigella* species and *Staphylococcus aureus* were with 13.3% of the isolates respectively. The percentage frequency of occurrence of parasitic agents showed *Gardia lamblia* (50%) *Entamoeba histolytica* (25.0%), *Ascaris lumbricoids* (20%) and *Taenia* species had the frequency of occurrence of 5%. The sensitivity test on the bacterial isolates showed high sensitivity of the bacterial isolates to some antimicrobial drugs such as Streptomycin, Nalidixic acid, Augumentin, Ciprofloxacin etc and high resistance patterns of some isolates to these drugs was also observed. The results suggest improved hygienic practices to children, mothers and caregivers as well as administration of appropriate antibiotic to children with diarrhoea so as to prevent the outbreak of this infection in the study area.

INTRODUCTION

Diarrhoea is an illness in which waste water is emptied from the bowel frequently and in a liquid form. In clinical practice, the term "diarrhoea" is used to describe increased liquidity of stool usually associated with increased weight and frequency of more than three times per day (Soffer, 2001).

Diarrhoeal diseases rank high as a major cause of illnesses and deaths among infants, young children and elderly especially in developing countries. According to Oquike (1997) diarrhea is one of the leading causes of children mortality and morbidity in the tropics especially among children between ages of zero to ten years.

Diarrhoea in newborns or growing children may indicate systemic infection, gastroenteristic or improper feeding. Generally, children are known to be more susceptible to gastrointestinal problems due to their eating and playing habits as they handle contaminated soil and seldom

wash their hands thoroughly before and after meals (Oquike 1997, warren and Bishop 1996). Non-observation of personal hygiene by some babysitters, guardians and parents especially after visiting the toilets also contribute greatly to diarrhoeic conditions in newborns and young children. Consumption of contaminated food and water has also been implicated as one of the possible factors leading to diarrhoeic infections.

A number of microorganisms and parasite agents have been implicated in cases of paediatric diarrhoea (Itah *et al*, 2005). Bacteria such as *Shigella* species, *Salmonella* species, *Escherichia coli, Vibrio* species, *Clostridium* species, *Yersinia* species and many others are known to be causatic agent of diarrhoea. Viruses include rotavirus, adenovirus and Norwalk virus and parasitic agents such as *Entamoeba histolytica, Gardia lamblia, Trictus trichuria, Ascaris lunbricoides, Ancyclostoma duodenale,* just to mention a few.

The aim of this research was to determine the prevalence of bacterial and parasitic agents among hospitalized children in some hospitals in parts of south East Nigeria particularly Akwa Ibom State. (Fig. 1).

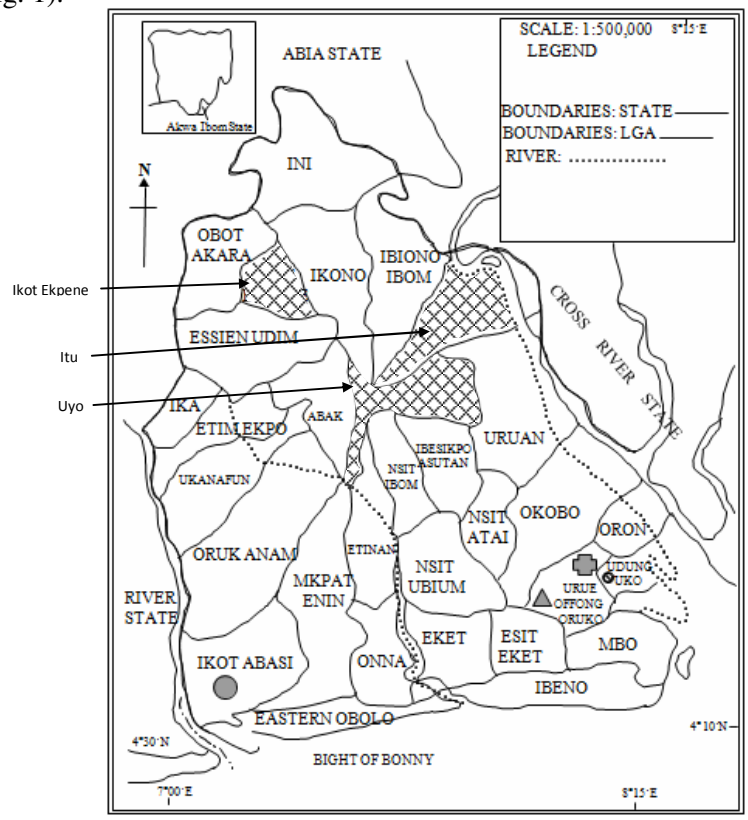

Fig. 1: Map of the study area (pointed arrows)

MATERIAL AND METHODS

Collection of Samples

Stool samples were collected from children of 0-1 years of age in 4 hospitals in Akwa Ibom State of Nigeria. The hospitals were University of Uyo Teaching Hospital (UUTH) Uyo, General Hospital, Ikot Ekpene, Health centre, Ibiaku Itam, Itu and Saint Luke's Hospital, Anua. The stool samples were aseptically collected into universal bottle containing freshly prepared peptone water to keep the organisms viable. The samples were taken to Microbiology laboratory, University of Uyo, Uyo for analysis.

PROCESSING OF THE SAMPLES

(a) **Microscopy of the stool Samples**

The consistency and the nature of each stool sample were observed. Before cultivating of each sample, microscopic examination was carried out by smearing of loopful of the specimen on a clean degreased slide. A cover slip was then placed over the preparation. The slide was placed under the microscope to examine the presence of cyst and ova of some parasitic agents. This was viewed using x40 objective of the microscope.

(b) **Inoculation of Samples**

A loopful of each diarrhoeal stool sample was transferred from the alkaline peptone water and streaked on freshly prepared plates of Thiosulphate-Citrate-Bulesalt-Sucrose (TCBS) agar. *Salmonella-Shigella* agar, and MacConkey agar. The plates were incubated at 37^0C for 24 hours after which the observations for growth were made.

Maintenance of Pure Culture

Isolation of organisms from the cultured plates were carried out based on the techniques described by (Holt *et al* 1994, Cheesbrough, 2002). These include colonial, morphology and biochemical characteristics. Subcultures were done on Nutrient agar and representatives of discreet colonies after subculture were maintained in Nutrient agar slant in the refrigerator at 4^0C for further analysis.

Antibiotic Sensitivity Tests on Bacterial Isolates

Antibiotic sensitivity tests were carried out on the bacteria obtained in the study using agar-disc diffusion method (Cheesbrough, 2002). Eighteen hour culture of each test isolates were carefully spread uniform over Muellar-Hinton plate using a spreader. A sterile forcep was used to pick each antibiotic multi-disc and placed at the centre of the plate. The Gram-positive discs were used for Gram-positive isolates while the Gram-negative discs were placed on plates containing Gram-negative isolates. The discs were pressed carefully onto the agar to ensure direct contact with the test organisms. All plates were incubated at 37^0C for 24 hours before observations for zone of inhibitions.

STATISTICAL ANALYSIS

Difference in the prevalence of bacterial agents of diarrhoea as well as parasitic agents of diarrhoea obtained from each of the hospital screened were analyzed using chi-square method.

RESULTS AND DISCUSSIONS

A total of 100 stool samples were screened from children in the paediatric wards with diarrhoeic cases. All the stool samples screened contained diarrhoeic agents giving the prevalence rate of 100%. Seventy two stool samples came out with one or more bacteria responsible for diarrhea. The prevalence rate of 72% was observed in this case. The prevalence rate from each of the hospitals sampled is as presented on Table 1.

Table 1: Prevalence of Positive Samples with Bacterial Isolates Responsible for Diarrhoea in Children from each of the Hospitals Sampled

Name of Hospital	No. of children screened	No. of Positive Samples with Bacterial Isolates / prevalence (%)
UUTH	25	20 (80)
General Hospitals, Ikot Ekpene	25	19 (76)
Health Centre, Ibiaku Itam	25	15 (60)
Saint Luke's Hospital, Anua	25	18 (72)
Total	100	72

Although different prevalence rate of positive cases with bacterial isolates were obtained from each hospital sampled there was no significant different when analysed statistically ($p \geq 0.05$).

The isolates percentage frequency of occurrences obtained revealed *Escherichia coli* with the highest frequency of occurrence of 28 (31.1%) followed by *Vibrio cholerae* with 20 (22.2%), *Shigella* species had 12 (13.3%), *Staphylococcus aureus* had 12 (13.3%), *Salmonella typhi,* 14 (15.6%) while *Salmonella paratyphi* had 4 (4.45%) of the isolates (Table 2).

Table 2: Isolates and Their Percentage Frequency of Occurrences

Isoaltes	Frequency of occurrence	Percentage
E. coli	28	31.1
Vibrio cholerae	20	22.2
Shigella species	12	13.3
Staphylococcus aureus	12	13.3
Salmonella typhi	14	15.6
Salmonella paratyphi	2	4.5
	88	100

Prevalence of parasitic agents responsible for diarrhoeic cases of the hospitalized children were also determined. Out of the 100 stool samples examined, 28 of the samples yielded parasitic agents giving the prevalence rate of 28% of the cases. The prevalence of parasitic agents from each hospital sampled showed Health Centre, Ibiaku Itam, Itu with the highest prevalence rate of 40% while the least came from University of Uyo Teaching Hospital, Uyo (Table 3). But statistically there was no significant difference ($p \geq 0.05$) in the prevalence .

Table 3: Prevalence of Parasitic Agents Responsible for Diarrhoea in Hospitalized Children in Each Hospital Sampled

Hospitals Sampled	No. of children screened	No. of positive cases with Parasitic Agents / prevalence (%)
UUTH	25	5(20)
General Hospitals, Ikot Ekpene	25	6(24)
Health Centre, Ibiaku Itam	25	10(40)
Saint Luke's Hospital, Anua	25	7 (28)
Total	100	28

The percentage frequency of each parasitic agents obtained from the wet mount miscroscopic examination of the stool samples revealed *Gardia lamblia* with the highest percentage frequency of occurrence of 50%, *Entamoeba histolytica* had 25%, 20% and 5% were observed with *Ascaris lumbricoides* and Taenia species respectively (Table 4).

Table 4: Parasite Agents and Their Percentage Frequency of Occurrences

Parasites	Frequency of occurrence	Percentage
Gardia lamblia	20	50
Entamoeba histolytica	100	25
Ascaris lumbricoides	8	20
Taenia species	2	5
	40	100

Antimicrobial susceptibililty tests on the bacteria isolates revealed the sensitivity as well as the resistance patterns of these isolates. Some Gram-negative organisms such as *E. coli* obtained in the study was sensitive to Ciprofloxacin with 15mm zones of inhibitions, 20mm zone of inhibition was observed with Nalidixic acid (Table 5). Staphlococcus aureus the only Gram-positive isolates in the study was sensitive to Gentamycin (16mm), Erythromycin (20mm) and Ampiclox (22mm) (Table 6).

Table 5: Antimicrobial Susceptibility Patterns of some Gram-Negative Isolates and their Zones of Inhibitions (mm)

Isolates	Drugs used and zones of inhibitions (mm)									
	CPX	OFX	CN	AU	S	PEF	NA	CEP	PN	SXT
E. coli	15	12	8	15	16	14	20	12	22	15
	S	1	R	3	S	S	S	I	S	S
Salmonella typhi	8	12	16	13	19	8	12	23	14	16
	R	I	S	I	S	R	I	S	S	S
Salmonella paratyphi	10	8	12	15	18	10	17	20	18	12
	R	R	I	S	S	R	S	S	S	R
Vibrio cholerae	8	15	16	8	16	12	20	17	19	12
	R	S	S	R	S	I	S	S	S	S
Shigella species	8	8	14	16	20	8	12	22	20	14
	R	R	S	S	S	R	I	S	S	S

Keys and Interpretations:
S = 14mm and above = Sensitive
I = 11mm – 13mm = Intermediately sensitive
R = ≤ 10mm = Resistance

CPX	=	Ciprofloxacin (10mcg)	PET	=	Peflucine (10mcg)
OFX	=	Tarivid (10mg)	NA	=	Nalidixic acid (30mcg)
CN	=	Gentamycin (10mcg)	CEP	=	Ceporex (10mcg)
AU	=	Augumentin (30mcg)	PN	=	Ampicilin (30mcg)
S	=	Streptomycin (30mcg)	SXT	=	Septrin (30mcg)

Table 6: Antimicrobial Susceptibility Pattern of Gram-Positive Isolates obtained and Its zone of inhibitions (mm)

Isolates	Drugs used and zone of inhibitions (mm)									
	CPX	NB	CN	LC	S	RD	ERY	CH	APX	FLX
Staphylococcus aureus	12	8	16	10	8	8	20	10	22	12
	I	R	S	R	R	R	S	R	S	I

Key:
S = 14mm and above = Sensitive
I = 11mm – 13mm = Intermediate
R = ≤ 10mm = Resistance

CPX	=	Ciprofloxacin (10mcg)	RD	=	Rifampicin (30mcg)
NB	=	Norfloxacin (10mcg)	ERY	=	Erythromycin (30mcg)
CN	=	Gentamycin (10mcg)	CH	=	Chloramphenicol (30mcg)
LC	=	Lincocin (20mcg)	APX	=	Ampiclox (20mcg)
S	=	Streptomycin (30mcg)	FLX	=	Floxapen (20mcg)

The results of this research work revealed that both bacterial and parasitic agents were the major cause of diarrhoea among hospitalized children in Akwa Ibom State . The prevalence rate of 100% was observed. Isolation of several species of bacterial isolates from these children showed some endemicities of diarrhoeal disease in the area studied.It was also observed that diarrhoea is a major cause of sickness and death in children of 0-10 years of age in the area.This agreed with earlier study by (Okolocha and Umoh 2002) who screened the children of this age range and isolated both bacterial and parasitic agents of diarrhoea which led to their conclusion that diarrhoea still remains a major cause of morbidity and mortality in children in Nigeria.

The highest prevalence of 72% was observed as diarrhoea of bacterial aetiology while 28% prevalence was observed with parasitic agents. The higher prevalence of bacterial agents may be due to poor hygienic condition of mothers when breast feeding the infants. Bacteria could also come from the variety of different food carrying various organisms which often produce toxic substances that do not disturb the adult but cause diarrhoea or other gastrointestinal problems in children when present in large number (Itah, 1999).

Greater number of *E. coli* (31.1%) was isolated from the infantile stools. This results confirmed earlier research done by Gorbach (1989) who isolated a greater number of *E. coli* from infantile diarrhoeal stools and he observed that *E. coli* produces toxic substances that cause diarrhoea in children. The isolation of *Vibrio cholerae, Salmonella* species, *Shigella* species and *Staphylococcus aureus* could be due to contaminated food and water (Nester *et al*, 1995 and Steingart and Megran 2000).

Among the parasites obtained, *Gardia lamblia* had the highest prevalence of 50%, followed by *Entamoeba histolytcia* with 25%. These findings agreed with the reports of similar studies by (Itah *et al*, 2005 and Change *et al*, 2004). The parasites may be introduced through the infant's foods, feeding bottles that are not properly sterilized and oral-route contamination. Since children walk and exploit the environment picking anything of interest and put inside their mouth, there is every possibility that these objects picked may contained eggs of these parasites which when entered into the body, undergo their normal cycles.

The sensitivity patterns of some Gram-negative isolates showed that majority of these organisms were highly resistance to Ciprofloxacin, Peflucine and Tarivid with (8mm) zones of inhibitions but highly sensitive to Ceporex, Streptomycin and Ampicilin with zones of inhibitions ranging from 20mm to 23mm respectively. *Staphylococcus aureus* showed resistance to Norfloxacin, Streptomycin with (8mm) zones of inhibitions and highly susceptible to Ampiclox, Erythromycin and Gentamycin with zones of inhibition of 22mm, 20mm and 16mm respectively. The resistance patterns of the isolates to routine antibiotics observed in the study showed indiscriminate use of antibiotics on these children.

Despite developments in the last decades in understanding the aetiology, pathogenesis of diarrhoeal diseases and the discovery of effective rehydration solution to treat the young children with diarrhoea, the illness still remains the significant cause of death. Therefore, in the treatment of this bacterial infection, there is need to monitor the susceptibility patterns of these organisms in different hospitals (population) to their commonly used antimicrobial agents. Proper handling of children utensils, proper faecal disposal, good hygienic conditions for the children as well as caregivers and a good source of drinking water are necessary to eliminate diarrhoea caused by these bacterial and parasitic agents in children.

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