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Original Research Article

Prevalence of diarrhoea and antibiotic susceptibility test in children below 5 years at University of Benin Teaching Hospital, Nigeria

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Diarhoea disease is among of leading public health significance among children of below five years where that more than 315,000 deaths of preschool age children are recorded annually in Nigeria. A survey to determine the significance of bacterial species as possible pathogenic microorganisms that cause diarrhoea was carried out in the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The age, sex, prevalence and maternal level of education in relation to diarrhoea in children younger than five years of age were determined. A questionnaire was used for collecting information on socio-economic characteristics, environmental hygiene and behavioural practices of patients and their care givers. Faecal specimen from 50 children, less than 60 months (5 years) old, were collected and assessed for microbiological profile of enteric pathogens. Antimicrobial susceptibility tests were performed on all identified isolates using the Kirby-Bauer disc diffusion method. The prevalence of infectious diarrhoea was age specific being highest at the age of 7 - 12 months and lowest at the age of 37-48 months. *Escherichia coli* was the most frequently isolated bacteria in all age groups (62.58%). However, the isolation rate of *Staphylococcus aureus* and *Klebsiella* sp. in this study showed these bacteria as veritable aetiologic pathogens of infectious childhood diarrhoea. Antibiotics susceptibility test conducted revealed that the isolates were most resistant to doxycycline followed by ciprofloxacin while the isolates were most susceptible to amoxicillin. Proper education and awareness are keys to combat this public health problem caused by these pathogens.

Key words: Diarrhoea, prevalence, susceptibility, antibiotic disc, watery stool.

INTRODUCTION

Diarrhoea is the passage of usually loose or watery stools, usually at least three times within 24 hour period (Sinclair et al., 2003). It is one of the leading causes of illness in young children in developing countries (Parashar et al., 2003). Nearly half of deaths from diarrhoea among young children occur in Africa where diarrhoea is the largest cause of death among children under 5 years old and a major cause of childhood illness (WHO, 2007; Bezatu et al., 2013). The public health significance of diarrhoeal diseases cannot be overemphasized. Available reports in Nigeria indicate that more than 315,000 deaths of preschool age children are recorded annually as a result of diarrhoea disease (Babaniyi, 1991; Alabi et al.,1998). Diarrhoea is frequent in poor populations and in immunocompromised individuals.

The main aetiology of the diarrhoea disease is related to a wide range of bacteria, enteroparasites and viruses (Vargas et al., 2004). Among the causative agents, the following bacteria have been reported: *Escherichia coli*, *Shigella, Salmonella* and *Campylobacter* (Black et al., 1981; Suan et al., 1985). Among the viruses, rotavirus seems to be the most common (Afroza et al., 2013). Viral infections such as

rotavirus, cytomegalovirus, Norwalk, hepatitis, and herpes simplex viruses also play a major role in diarrhoeal problems among children and adult (Fetety, 1997; Dupont, 1997). In developing countries, diarrhoeal infections in under 5years old children are generally associated with rotavirus often at the time of weaning (Afroza et al., 2013). Emerging diarrhoeagenic enteric parasites have been reported (Gianotti, 1990; Hoge et al., 1995; Sherchand et al., 1996; Ono et al., 2001).

The contribution of the various pathogens of diarrhoea may differ substantially between regions depending on local meteorological, geographic and socio-economic conditions (Ifeanyi, et al., 2010). Food intolerance, reactions to medicines such as antibiotics and antacids containing magnesium may also contribute to diarrhoea. Chronic diarrhoea can be caused by chronic ethanol ingestion, though this kind of diarrhoea is typical among children under 5 year old (Longstreth et al., 2006; Mwambete and Joseph, 2010). The main cause of death from acute diarrhoea is dehydration, which results from loss of fluid and electrolyte in stool (Akingbade et al., 2013).

There is the current problem of emergence of strains of microorganisms implicated in food contamination that are resistant to most of the present antibiotics (Akpomie and Akpan., 2013). These antibiotics has been used in medicine and veterinary medicine (Soulsby et al., 2005). Antibiotics resistance have been attributed to abuse and misuse of antibiotics (Prescott et al., 2008). *Staphylococcus* sp, *Enterococcus* sp, *Streptococcus pneumoniae* and *Shigella dysenteriae* were found to exhibit resistance to antibiotics (Ash et al., 2002). The main mechanism of drug resistance are drug in activation or modification, alteration of target site, alteration of metabolic pathway and reduced drug accumulation by decreasing drug permeability and/or increasing active efflux of the drugs across the cell surface (Li and Noixado, 2009).

The current study, therefore, was to assess the prevalence of diarrhoea and associated factors among children of age under
five years in UBTH, also to determine the antibiotic susceptibility pattern of the isolates.

MATERIALS AND METHODS

Sample collection

The study was carried out from the months of April, 2014 to January 2015. A single faecal sample was collected from each child with diarrhoea on presenting at Teaching Hospital, University of Benin. This was done for 50 children under 60 months old with diarrhoea. All samples were analyzed immediately after collection in the laboratory. Standard questionnaires were used to investigate the socio-economic, environmental and behavioral risk factors of diarrhoea in the children. The respondents were primarily mothers of eligible children, from whom informed consent were obtained for each participating child. All collected records were kept confidential.

Isolation and Identification of isolates

The samples were examined with the naked eye for consistency, colour and atypical components such as mucous, blood and parasites. Samples were also examined by light microscopy for the presence of red blood cells, parasitic ova and cysts.

The samples were cultured on differential and selective media for bacterial cultivation in order to isolate bacterial enteropathogens. Selenite-F broth, Alkaline peptone water, MacConkey, Eosin methylene blue agar, Xylose lysine deoxycholate agar (XLD), thiosulphate citrate bile salt sucrose (TCBS) and *Campylobacter* blood-free selective agar were used for the isolation of bacterial pathogens. The bacterial isolates were identified by standard biochemical test and by comparing their characteristics with those of known taxa, as described by Jolt et al., 1994; Cheesbrough, (2006), and Oyeleke and Manga, (2008).

Antibiotic susceptibility test

Antibiotic susceptibility test was performed by the Bauer-Kirby disc diffusion method (Bauer et al., 1996). The results were expressed s susceptible\resistant according to the criteria developed by National Committee for Clinical Laboratory standards (NCCLS, 2002), and manual of antimicrobial susceptibility testing guidelines (Coyle, 2005; Cheesbroug, 2006; Okonko et al., 2009 a, b). The antibiotics used were: Doxycycline (10 µg), Gentamicin (10µg), Ciprofloxacin (10 µg), Amoxycillin (10 µg), Chloramphenicol (10 µg), Nalidixic acid (10 µg), Tetracycline (30 µg). Multidrug resistance was defined as resistance to ≥ 3 of the antimicrobial agents tested (Oteo et al., 2005).

Statistical analysis

The Statistical Package for Social Scientists (SPSS, version 16.0) was used for the analysis of the data obtained. Two way ANOVA test was used to determine the level of significance of the test organisms at 95% confidence limits or 5% level of significance.

RESULTS

Table 1 shows the distribution of diarrhoeal children by age. It shows that diarrhoea is associated with age and majority of cases occurring in children between 7 months and 24 months of age.

Table 2 shows the maternal educational level of patients out of which 25(50%) mothers without formal education and 4(8.0%) with tertiary education.

Table 3 shows the sex of patients. Out of the fifty children used in this study, 29 (58.0%) were male while 21 (42.0%) were female.

The breastfeeding type of diarrhoeal children if exclusive

Table 1. Prevalence of diarrhoea diseases among underfive children by age, and sex

Age group (months)	Number	Percentage (%)
0 – 6	9	18.0
7 – 12	17	34.0
13 – 24	11	22.0
25 – 36	6	12.0
37 – 48	2	4.0
49 – 60	5	10.0
Total	50	100

Table 2. Maternal educational level and feeding type of patients with diarrhoea

Parameter	Frequency	Percentage (%)
None	25	50
Primary	15	30
Secondary	6	12
Tertiary	4	8.0

Table 3. Sex of patients

Sex	Frequency	Percentage (%)
Male	29	58.0
Female	21	42.0

Table 4. Feeding type of patients

Parameter	Frequency	Percentage (%)
Exclusive	16	32..0
Not exclusive	34	68.0

Table 5. Distribution of bacteria isolated

Species	Number of isolates	Percentages (%) of bacteria isolated
Escherichia coli	87	62.58
Salmonella sp.	4	2.88
Klebsiella pneumonia	12	8.63
Staphylococcus aureus	8	5.76
Enterobacter sp.	3	2.16
Pseudomonas sp.	6	4.32
Shigella sp.	2	1.44
Proteus sp.	17	12.23
Total	139	100

or not exclusive is shown in Table 4 out of which 16 (32.0%) were exclusive and 34 (68.0%) were non-exclusive.

Table 5 shows the distribution of bacteria isolated out of which 87 (62.58%) were strains of *Escherichia coli* others included *Salmonella* sp. 4 (2.88%), *Staphylococcus aureus* 8 (5.76%) and other Enterobacteriaceae.

The total viable bacterial count as well as total coliform count is shown in Table 6.

Table 7 shows antibiotic sensitivity pattern of the isolated bacteria in resistance or susceptible format.

DISCUSSION

In this study our results showed that eight bacterial species (*Escherichia coli, Salmonella* sp., *Klebsiella pneumoniae, Staphylococcus aureus Enterobacter* sp, *Pseudomonas* sp.,

Table 6: Total bacterial count

Organisms	Total **count (cfu/ml)**
Escherichia coli	87×10^2
Salmonella sp.	4×10^2
Klebsiella pneumoniae	12×10^2
Staphylococcus aureus	8×10^2
Enterobacter sp.	3×10^2
Pseudomonas sp.	6×10^2
Shigella sp.	2×10^2
Proteus sp.	17×10^2

Table 7. Antibiotic sensitivity of isolates

	Antibiotics						
Isolates	**DX**	**GET**	**CIP**	**C**	**AM**	**NA**	**T**
Escherichia coli	R	S	R	R	S	S	R
Salmonella sp.	S	R	S	S	S	S	R
Klebsiella pneumoniae	R	R	R	R	S	R	R
Staphylococcus aureus	R	R	R	R	S	S	S
Enterobacter sp.	R	R	R	R	R	R	S
Pseudomonas sp.	R	S	S	S	R	R	S
Shigella sp.	S	S	R	S	S	S	S
Proteus sp.	R	S	S	S	S	R	S

KEY:

R - RESISTANT
S - SUSCEPTIBLE

DX – DOXYCYCLINE.
GET – GENTAMICIN.
CIP – CIPROFLOXACIN.
C – CHLORAMPHENICOL.
AM – AMOXYCILLIN.
NA – NALIDIXIC ACID.
T – TETRACYCLINE

Shigella sp. *and Proteus* sp*)* were isolated from diarrhoeal children. Although there are geographical differences in the spectrum of bacteria incriminated in childhood diarrhoea, *E. coli, Proteus* sp. and *K. pneumoniae* were isolated at a relatively high rate with *E. coli* being the most predominant. This is similar to the findings of Ifeanyi et al*.,* 2010. There appear to be conflicting reports about the association of *Salmonella* species with diarrhoea (Seung-Hak et al., 2006). Conversely, the occurrence of *Salmonella* species in this study is in conformity with the findings from Abakaliki, south –eastern Nigeria (Ogbu et al., 2008), Sao Paulo Brazil (Ethelberg et al.,2006) , Bissau, Guinea Bissau (Valetiner-Branth et al., 2003) and Hong Kong (Nelson et al., 2004). In addition, the report of *Salmonella* sp. (3.2%) and 8.0% is more inclined to former reports from similar studies in Bangladesh (Albert et al., 1999) and Korea (Seung-Hak et al., 2006).

From this study, bacterial isolation age-wise diminished between the ages of 25-36 months, 37- 48 months and 49 - 60 months and is in consonance with past reports from Brazil, Denmark and Turkey (Olesen et al., 2005, Diniz-Santos et al., 2005 and Karadag et al., 2005). The low morbidity of children above 25 months is due to the development of immunity to microorganisms following active or asymptomatic infection (Desalegn et al*.,* 2011; Motarjemi et al*.,* 1993).

This study also found more boys than girls presenting with diarrhoea. This is in contrast with the higher number of girls reported by Kolahi et al. (2008) in Iran, Shah et al. (2003) in Pakistan, and Gascon (2000) in Tanzania. The reason for this may be due to the fact that boys are more likely to explore while playing leading to a higher tendency of picking things possibly containing diarrhoeagenic agents.

Also, a large proportion of these cases of diarrhoea seen in this age group were among those not exclusively breastfed (Table 4), and thus conferring higher odds of having diarrhoea. Exclusive breastfeeding in infancy is known to protect against diarrhoea with maternally acquired antibodies helping to fight infective agents responsible for the disease. There is a general decline in

these antibodies in those not exclusively breastfed, and hence the high risk of developing diarrhoea. Besides, complementary foods are usually introduced at this stage increase risk of contamination, especially in the developing world like ours where safe water and basic sanitation is lacking (Dewey and Adu-Afarwuah, 2008).

The evaluation of the antibiotic susceptibility of the different bacteria showed all the strains had varying percentage resistance to commonly used antibiotics such as Nalidixic acid and Ciprofloxacin. This means that these antibiotics are fairly effective in the treatment of diarrhoea. It is noteworthy that the arbitrary empirical use of antibiotics might be responsible for the emerging resistance pattern as shown by this study (Ifeanyi et al*.,* 2010). The resistance of enteropathogenic bacteria to commonly prescribed antibiotics is increasing in developing and developed countries.

In assessing the antibiotic susceptibility profiles of the isolates, *E. coli* was susceptible to 3 (42.9%) and resistant to 4 (57.1%) of the seven antibiotics used, *Salmonella* was susceptible to 5 (71.4%) and resistant to 2 (28.6%), *Klebsiella pnemoniae* was susceptible to only 1 (14.3%) and resistant to 6 (85.7%), *S. aureus* was susceptible to 3 (42.9%) and resistant to 4 (57.1%), *Enterobacter* sp was susceptible to only 1 (14.3%) and resistant to 6 (85.7%), *Pseudomonas* sp was susceptible to 4 (57.1%) and resistant to 3 (42.9%), *Shigella* sp was susceptible to 6(85.7%) and resistant to only 1(14.3%) while *Proteus* sp. was susceptible to 5(71.4%) and resistant to 2(28.6%), as shown in Table 7. From this study some of the isolates were resistant to more than one drug, hence they were multi-drug resistant (Wasfy et al*.,* 2000; Akpomie and Akpan, 2013). Resistance of *E. coli* to antibiotics in this study is similar to reports by Adetosoye, 1980, who reported multidrug resistant *E. coli* from humans with one strain containing resistant factors to chloramphenicol.

The high prevalence of *E. coli* and incidence of other enteropathogens are a reflection of the poor environmental sanitation and very poor personal hygiene and unclean habits among some people, compounded by public ignorance and illiteracy. Preventing diarrhoea in children has been shown to reduce level of pupil absence in schools, while preventing long-term consequences such as malnutrition and stunting.

Conclusion

It was observed from this study that *E. coli* is the predominant enteropathogen amongst other enteropathogens causing diarrhoea in children less than 60 months, in UBTH. It is advised therefore, that appropriate measures should be taken by parents and care-givers to prevent the contamination of food and water by these enteropathogens to avoid occurrence of diarrhoea.

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