Bayero Journal of Pure and Applied Sciences, 3(1): 10 - 15
Received: June, 2009
Accepted: March, 2010

ISOLATION OF BACTERIA ASSOCIATED WITH DIARRHOEA AMONG CHILDREN ATTENDING SOME HOSPITALS IN KANO METROPOLIS, KANO STATE, NIGERIA

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ABSTRACT
Bacteriological investigations of Diarrhoeal diseases were carried out among 300 children who were between the ages of 0 – 24 months using stool samples from five different hospitals in the metropolitan Kano. The organisms were isolated and identified using cultural, serological biochemical test. Sensitivity testing of the isolates to some antimicrobial agents was carried out. Out of those number only a total of 122(40.7%) of children were found to have Diarrhoea associated with bacteria in which 34(27.9%) and 88(72.1%) were from breast-fed and bottle-fed children respectively. The Bacteria isolated were Escherichia coli which were the most predominant, followed by Salmonella species and then Shigella species. As at the time of the study, there was significance difference between breast-fed and bottle-fed in the incidence at P≥ 0.05. Among the antimicrobials used cotrimoxazole was found to be more effective than the others. Also, most of the children tested were found to have a common illness of diarrhoea followed by dysentery, abdominal pain and fever/ vomiting.

Keyword: Diarrhoeal diseases, Breast and Bottle milk, Water, Children, Antibiotics.

INTRODUCTION

Diarrhoeal diseases can be caused by many species of microbes that some time lead to dysentery; a type of diarrhoea in which the stools contain blood and mucus. For many years, Salmonellae and Shigellae were thought to be the only bacteria that caused Diarrhoeal diseases, but it is now clear that some strains of *Escherichia coli* cause the same symptoms. Campylobacter, Vibro, Aeromonas and Plesiomonas were also reported to cause Diarrhoeal diseases (Tesh and O' Brien, 1991). Diarrhoeal diseases and dysentery are also caused by an amoeba, *Entamoeba histolytica* (amoebic dysentery).

The influence of breastfeeding on the severity of illness in Diarrhoeal diseases was reported by Wadgaonkar *et al.* (2005) in which they suggested that; breastfeeding substantially shifts the spectrum of severity in Diarrhoeal diseases from severe to non severe illness. The high degrees of protection against severe Diarrhoeal diseases were also seen in malnourished children and in children reporting a recent history of measles. The protective effect of breastfeeding persisted even when the analysis was corrected for confounding effects of age, nutritional status, and early medication. Because Diarrhoeal diseases accounts for substantial morbidity and mortality in developing countries, prolonged breastfeeding is recommended (Wadgaonker *et al.*, 2005; Jebson *et al.* 1980).

Breastfeeding dramatically reduces gastrointestinal infections in infants by reducing external contaminants introduced through the bottle feeding. It also provides various Humoral and cellular constituents which protect against external pathogens example; *Escherichia coli*, Salmonellae, Shigellae, *Vibrio* and *Retrovirus* (Tauxe *et al.*, 2005). The antimicrobial factors identified in the breast milk are secretory IgA, transferin, lysozyme, milk lipid and lactobacillus promoting factors (Tauxe *et al.*, 2005).

In clinical bacteriology, the Shigellae are treated beside the Salmonellae, for both are intestinal pathogens and are isolated from feaces as non-lactose fermenting colonies on bile-salt-media. Biologically, however, they are more closely related to the Escherichiae, with which they share antigens and some strains of which cause invasive, dysentery-like infection of the intestinal mucosa. The strains *Escherichia coli* formerly called Alkalescensdispar (A-D) organisms resemble the *Shigella* in being non-motile, none lactose-fermenting or late-lactose-fermenting and non-gas-producing (Kauffmann, 2003).

Diarrhoeal disease is one of the principal cause of death all over the world and more so in developing countries. In the Paediatric age group, it accounts for fatalities than any other disease. Roughly, 25% of the deaths of infancy are due to Diarrhoeal diseases. Diarrhoeal disease and dysentery are usually acquired by drinking water contaminated with human feaces or by eating food washed with contaminated water or hand. Food can also be contaminated by flies, especially in areas where there are outdoor latrines.

Dysentery is a common disease in many developing countries where sanitary facilities may be minimal and

the water supply is not clean. Although dysentery is normally self-limiting in adults, it can be fatal to infants and young children. Dysentery is a major killer

The highest rates of complications occur in malnourished infants, but even well-nourished infants and young children can have more serious, sometimes fatal, form of the disease. (Cheesbrough, 2002; Tesh and O'Brien, 1999).

The main Aims and objectives of the study are, to determine the prevalence of Diarrhoeal diseases among breast and bottled fed children in kano metropolis, to isolate the bacteria associated with diarrhea among breast-fed children and to determine the antimicrobial resistance of the isolates.

of infants and children, but the attack rate in this age group is much higher than in adults.

MATERIALS AND METHODS

a). Study population

A questionnaire were distributed and faecal samples were collected from patients (children of less than 24 months) presenting with clinical symptoms of gastroenteritis such as diarrhoea, fever, dysentery (with mucus or blood) and abdominal pains attending five selected study hospitals.

b). Sample size: using formula:-

$$n = \frac{Pq}{(E/Z)^2}$$

Where; P = prevalence of previous studies = 18.4% (Kingston, (2003)
Q =100-p =100-18.4=81.6
E = allowable error = 5%
Z= standard normal distribution at 95% CI,= 1.96
n= number of sample to be collected. Therefore, when the values are substituted

$$n = \frac{18.4 \times 81.6}{(5/1.96)^2} = \frac{1501.44}{(2.55)^2}$$

$$= \frac{1501.44}{6.5} = 230.99 \text{ samples}$$

Approximately = 231 samples

However, for more accurate results in this research work, a total of 300 stool samples were collected from children of less than 24 months attending five hospitals in Kano metropolis.

c. Sample collection

A total of 300 faecal specimens were collected from children (children less than 24 months) by their mothers in clean, wide- mouthed containers, without disinfectant or detergent residue and tight-fitting leak-proof lids. When delay was unavoidable (more than 2 hours), faecal samples were placed in stuart's transport medium and refrigerated immediately (Perilla, 2003).

d. Culture of the faecal specimens:

The faecal specimens were cultured into liquid and onto solid media for isolation and identification of the Bacterial pathogens. Pre-enrichment broth (Selenite F) was used to allow the multiplication of bacteria; this was then subsequently sub-cultured onto a MacConkey agar (MCA) and Deoxycholate citrate agar (DCA) and then incubated aerobically at 37^0C for 24 hours (Cheesbrough, 2002).

e. Biochemical screening tests

Identification of bacterial isolates involves the use of biochemical screening media are usually used. One hundred and twenty two bacterial isolates (122) were subjected to various biochemical tests; Triple sugar iron (TSI), Motility, Indole, Urease and Citrate utilization tests (Cheesbrough, 2002; Perilla, 2003).

Triple sugar iron agar (TSI) test

At least one of each colony type of the well-isolated colonies was selected on plate using a sterile straight wire loop. The center of the colony was lightly touched and prepared TSI medium were inoculated by stabbing the butt and streaking the slants. These were then incubated at 37^oc for 24 hours (Cowan and Steel, 2002).

A yellow butt (acid) and red or pink (alkaline) slope indicates the fermenting of glucose only. Cracks and bubbles in the medium indicate gas production from glucose fermentation. A yellow (acid) butt indicates the fermentation of lactose. A red or pink (alkaline) slope and butt indicates no fermentation of glucose or lactose. (Cheesbrough, 2002).

Indole test:

Few colonies of the culture were inoculated into peptone water and incubated at 37^oC for 24 hours. Few drops of indicator (Kovac's reagent) were added and gently shake to mix well. Colour change was then observed.

If the layer of indicator reagent turns red within 1 minute (positive result). If the layer of indicator reagent remains yellow within 1 minute (negative result) (Cowan and Steel, 2002).

Urease test (Christensen's (modified) urea broth):-

Urea agars were inoculated heavily over the entire surfaces of the slants in bijou bottles. The cap were loosened and then incubated at 37^oC for 3-12 hours.

A urease-positive culture produces an alkaline reaction in the medium, evidenced by pinkish-red colour of the Medium. Urease-negative organisms do not change the colour of the medium, which is pale yellow-pink (Cowan and Steel, 2002).

Citrate utilization test using Simmon's citrate agar:-

Simmon's citrate slopes were prepared in bijou bottles as recommended by the manufacturer (stored at 2-8^{o}C). And the slopes were then stabbed and incubated at 37^{o}C aerobically for 48 hours (Cheesbrough, 2000). Blue colour indicates a positive reaction and if Simmon's citrate agar slopes remained as green in colour indicate negative reaction (Bello, 2002).

Motility Test (using motility agars):-

Motility agar were prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility was examined after 35-37^{o}C for 24 hour

Motility was indicated by the presence of diffuse growth (appearing as colouring of the medium) away from the line of inoculation (Cheesbrough, 2002; Perilla, 2003).

g. Susceptibility Testing of *Salmonella* species Isolates

The susceptibility testing was carried out using Mueller Hinton agar and were tested in vitro for susceptibility to five (5) different antimicrobial agents suggested by WHO (i.e Ampicillin, Trimethoprim-sulfamethoxole (cotrimoxazole), chloramphericol, Nalidixic acid and Ciprofloxacin) the following procedures were followed (Perilla, 2003).

Method:-

Using a sterile wire loop, 3-5 well isolated colonies were picked and emulsified in nutrient broth. The prepared turbidity was matched with a turbidity standard (0.5 McFarland) to have an equivalent suspension. Sterile swab was used to inoculate the suspension by streaking on the prepared and dried Mueller Hinton agar plate evenly. It was then allowed to stay for 3-5 minutes. Sterile forceps was used to place the antimicrobial discs on the inoculated plates. Within 30 minutes after applying the disc, the plate was incubated at 35^{O}C for 16-18 hours. By using Meter rule on the underside of plate, the diameter of each zone of inhibition was measured in millimeter. Zone diameter for ATCC 25922 was compared with NCCLS Published Limits; Interpretative chart was then used to interpret the zone sizes of Inhibition. Result was recorded as susceptible, intermediate susceptible, or resistant based on the Zones sizes of each antimicrobial disc used (NCCLS 2003; WHO, 2004; Andrews *et al.,* 2005).

RESULTS

From Table 1, out of 300 samples collected and processed, 101 samples were from the children with diarrhoea (33.7%), followed by dysentery (83), abdominal pain (67) and fever/ vomiting (49) with 27.7%, 22.3% and 16.3% respectively.

Table 1: **The distribution of samples in relation to age groups and common Illness children suffers in Kano metropolis**

Age group	Common illness				Total
(months)	Diarrhoea	Dysentery	Fever/ Vomiting	Abdominal pain	
0-4	10(3.3)	8(2.7)	10(3.3)	8(2.7)	36(12.0)
5-9	14(4.7)	11(3.7)	10(3.3)	8(2.7)	43(14.3)
10-14	20(6.7)	16(5.3)	12(4.0)	20(6.7)	68(22.7)
15-19	26(8.7)	20(6.7)	10(3.3)	18(6.0)	74(24.7)
20-24	31(10.3)	28(9.3)	7(4.0)	13(3.4)	79(26.3)
Total	**101(33.7)**	**83(27.7)**	**49(16.3)**	**67(22.3)**	**300(100)**

From Table 2, the frequency of diarrhoeal diseases were higher in 20-24 months age groups with 54 representing 18.00% followed by 15-19 (37), 10-14 (22), 5-9 (8) and 0-4 (1) age groups, representing 12.33%, 7.33%, 2.67% and 0.33% respectively. In the sexes male children were more infected than the female children in the same age group.

Table 2: Age and sex of children with diarrhoeal diseases among in Kano metropolis

Age group (months)	Sex		Total
	Males	Females	
0-4	1(0.33)	0(0.00)	1(0.33)
5-9	5(1.67)	3(1.00)	8(2.67)
10-14	12(4.00)	10(3.33)	22(7.33)
15-19	20(6.67)	17(5.67)	37(12.33)
20-24	29(9.67)	25(8.33)	54(18.00)
Total	**67(22.33)**	**55(18.33)**	**122(40.67)**

From Table 3, out of 122 bacterial isolates obtained from 300 stool samples collected and processed. 34(11.33%) were from culture of faecal samples from breast-fed children and 88(29.33%) were from culture of faecal samples from bottle-fed children. In addition, 0ut of 122 bacterial isolates obtained 45 were *Escherichia coli* with 15.00%, followed by *Salmonella species* (41) and *Shigella species* (36) representing 13.67% and 12.00% respectively. However, there was a significant difference between the numbers of breast fed and bottle fed children at $P \geq 0.05$.

Table 3: Bacterial isolates in relation to method of feeding of children in Kano metropolis

Bacterial isolates	Bottle-fed	Breast-fed	Total
Escherichia coli	10(3.33)	35(11.67)	45(15.00)
Salmonella species	11(3.67)	30(10.00)	41(13.67)
Shigella species	13(4.33)	23(7.67)	36(12.00)
Total	**34(11.33)**	**88(29.33)**	**122(40.67)**

From table 4: there was no significant difference between the number of children on treatment and those not on treatment among the different age groups at P$\geq$ 0.05 because, the calculated chi-square (t^2) value 21.02 obtained from the six hospitals were greater than the tabulated value X^2 = 9.488 at P$\geq$ 0.05 and 4 degree of freedom. However, in this study out of 122 clinical Bacteria isolated and tested, cotrimoxazole was the most effective antimicrobial agent with 89 representing (31.2%) susceptibility followed by ciprofloxacin (80), chloramphenicol (47), Nalidixic acid (40), , and ampicillin (29) with 28.1%, 16.5%, 14.1% and 10.2%, susceptibilities respectively (Table 5).

Table 4: The distribution of samples of children by treatment status in Kano metropolis

Age group (months)	Treatment status		Total
	On treatment	Not on treatment	
0-4	10(3.3)	26(8.7)	36(12.0)
5-9	25(8.3)	18(6.0)	43(14.3)
10-14	28(9.3)	40(13.3)	68(22.6)
15-19	48(16.0)	26(8.7)	74(24.7)
20-24	50(16.7)	29(9.7)	79(26.4)
Total	**161(53.7)**	**139(46.3)**	**300(100)**

Table 4: Results of the antimicrobial susceptibility patterns of Bacterial isolates among children in Kano metropolis

ANTIMICROBIALS	SXT	CH	CPX	NA	AMP
POTENCY (µg)	30	30	5	30	10
BACTERIAL ISOLATES	SUSCEPTIBILITY OF THE ISOLATES TO ANTIMICROBIAL AGENTS				
Escherichia coli =45	37(13.0%)	10(3.5%)	30(10.5%)	5(1.8%)	3(1.1%)
Salmonella species= 41	25(8.8%)	18(6.3%)	31(10.9%)	10(3.5%)	7(2.5%)
Shigella species= 36	27(9.5%)	19(6.7%)	19(6.7%)	25(8.8%)	19(6.7%)
Total= 122	89(31.2%)	47(16.5%)	80(28.1%)	40(14.1%)	29(10.2%)

Key: % = Percent, SXT= Cotrimoxazole, CH= Choloramphenicol, CPX= Ciprofloxacin, NA= Nalidixic acid, AMP= Ampicillin

DISCUSSION

In this research work, Breast-fed children were more infected with Diarrhoeal diseases than bottled-fed children. However, the increased prevalence observed (in 20-24 month's age group non in 0-4 months age group). Diarrhoea was the most common illness recorded by children with Diarrhoeal diseases, this could probably be because Parents take their children to the hospitals immediately they found out that their children were passing watery stool. These were in line with the findings of Stephen (2004); Gallies (2004); Arora (2001) and Kotloff *et al*. (2005). At the hospitals studied could be due to inadequate treatment of Diarrhoeal diseases leading to severe illness or due to the immune state (immunity). In addition, many hormones, growth factors and bioactive substances present in the maternal organs are now known to pass into the colostrums often exceeding concentration occurring in maternal plasma. As baby is growing, the concentration of breast milk reduces, also the concentration of most essential factors reduce (Zubairu, 2002). Change in diet from solely breast to a combination of foods may affect the immune status of the children. Most mothers nowadays are advised to feed their babies with breast milk only and should not give their babies even water until after the age of 6 months. This could be a reason why children in the ages 0-4 months were not recorded to have Diarrhoeal diseases in this work (Gallies, 2004).

In this work, it was observed that, there were higher risks of diarrhoeal diseases among those children that were bottle-fed than the children that depended solely on breast milk. This agrees with work of Wadgaonkar *et al.,* (2005) and Jolly, (2005). This may be due to the fact that, the bottle milk may not be properly pasteurized and bottle milk can easily be contaminated during preparation either from water, utensils or hands of persons preparing the milk (Jolly, 2005). The low or rare diarrhoeal diseases among breast fed children is probably because breast feeding especially during the first 6-9 months of life without supplements is the most effective preventive measure against infections generally among infants (Jolly, 2005). The breast milk has a high lactose content which when broken down by anaerobic *Lactobacilli* forms lactic acid that creates a low P^H environment which is detrimental to the proliferation of Shigellae, Salmonellae*, Escherichia coli* (Tauxe *et al.,* 2005). Also, human breast milk has other anti bacterial substance e.g. lactoferin, transferin, lysozymes, milk lipid and *Lactobacillus* promoting factors. However, breast milk is rich in humeral and cellular constituents that protects the body against external pathogen e.g. Shigellae, Salmonellae*,* Vibro, *Escherichia coli* etc. Also, breast feeding substantially shifts the spectrum of severity in Diarrhoeal diseases from severe to non severe illness (Jolly, 2005).

Results of the antimicrobial sensitivity demonstrated that, cotrimoxazole was the most effective antimicrobials while ampicillin was the least susceptible antimicrobial agent in the found in the present study. Bauer *et al*., (2004); Perilla, (2003) reported that, antimicrobial susceptibility of diarrhoeal pathogen usually affected because acquired antimicrobial resistance is a growing worldwide problem due to the increasing use of antimicrobials in humans, animals, and agriculture. In developing countries the situation is particularly serious for the following reasons: In many countries, antimicrobials can be obtained outside of recognized treatment centers, and taken without medical authorization or supervision. This leads to inappropriate use of antimicrobials and their being taken at sub-optimal dosages and for an insufficient length of time. Often the high cost of an antibiotic, results in an incomplete course being purchased, sufficient only to alleviate symptoms (Perilla, 2003).

There were high risks of Diarrhoeal diseases caused by *Shigella species*, *Salmonella species* and *Escherichia coli* among children. This could be due to so many factors such as developmental difference between children and adults with respect to anatomical nature of their organs infected (e.g. large intestine, terminal ileum, colon) that are weaker than that of older aged. In addition, these children are frequently exposed to the hospital environment where the organisms have high rate of colonization and are transferred mostly to them by the health workers that deliver health services to them or some time when interacting with infected children at home, or at day care centre etc. (WHO, 2004).The socio-cultural behavior of the children often playing with whatever comes their way, contributes to this and of course, the immune state (immunity), which at this stage, is usually not fully developed. The control of Diarrhoeal diseases is not only on early and appropriate treatment, but increasing fluids intake, UNICEF glucose-based, cereal-based oral hydration should be encouraged (Stephen, 2004).

Conclusion and recommendation

In conclusion, Bacteriological investigations of Diarrhoeal diseases were carried out among 300 children who were between the ages of 0 – 24 months in Kano metropolis. Out of those number only a total of 122(40.7%) of children were found to have Diarrhoeal diseases in which bottle-fed were infected than breast-fed children. The Bacterial species isolated comprised of *Escherichia coli* was found to be the most common among children, and then followed by *Shigella species.* Results of the antimicrobial sensitivity demonstrated that, cotrimoxazole was the most effective antimicrobials while ampicillin was the least susceptible antimicrobial agent in the found in the present study. However, in Nigeria, diarrhoea diseases are still the major cause of dysentery in children aged 0-9 months and many older children are hospitalized almost immediately after onset of disease (Iwalokun *et al.,* 2001).

Although, it appear to be quite marked differences in the relative importance of Diarrhoeal diseases in different parts of the country, and it is less of a scourge than in some other parts of the world, it is major attendance at health facilities, the second or third most common cause of admission to many of the hospitals in the country, and a significant and often preventable cause of death. Diarrhoeal diseases among children are believed to be very common in children, and can minimize or uncommon in a situation where personal hygiene quality of drinking water, quick isolation and treatment of infected cases as well as encouragement of breast feeding are maintained. This research shows a high incidence of Diarrhoeal diseases among children being bottle fed mainly because of contamination. Parents are strongly advice not to regard bottle-feeding of children with milk formula as main sources of feeding for their children. Rather they should stick to breast-feeding. This is because breast milk is not liable to easy contamination; it is convenient, less expensive, and available at correct temperature and concentration. The government should however endeavor to provide potable water to the community. Improving the sanitary awareness through basic health education and careful surveillance and monitoring incidence and spread of Diarrhoeal diseases, will go a long way to reduce the disease burden in children. The approach of oral dehydration therapy given to children by mother must be taught to reduce the debilitating effect of Diarrhoeal diseases (Kingston, 2003).

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