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# ISOLATION OF DIARRHOEAGENIC BACTERIA IN CHILDREN ATTENDING SOME SELECTED HOSPITALS WITHIN KADUNA METROPOLIS, KADUNA STATE, NIGERIA.

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ABSTRACT
Bacteriological investigations of diarrhoeal diseases were carried out among 100 children between the ages of 0 – 60 months using stool samples from three different hospitals in Kaduna metropolis. The organisms were isolated and identified using cultural and biochemical tests. Out of the 100 children only 44 (44%) were found to have diarrhoea associated with bacteria. The bacteria isolated were *Shigella* , 20 (45.5%), *Escherichia coli 15(34.1%)*, *Salmonella species 08 (18.1%)* and *Staphylococcus aureus 01 (2.3%)*. Most pathogens were isolated in females 26 (26%) as compared to males 18 (18%) but the difference was not statistically significant ($p>0.05$). However, statistical association was observed between age and the presence of the bacterial isolates with age group 25- 36 months having the highest occurrence of the bacterial isolates 15 (15%). This is followed by age group of 37 – 48 months 13 (13%), while the least bacterial isolates occurred in age group 0 – 12 months 3 (3%).

KEYWORDS: Diarrhoeal diseases, children, Isolation, Bacteria, Kaduna.

## INTRODUCTION

Acute diarrhea is a common cause of death in developing countries and the second most common cause of infant deaths world wide (Victora *et al.,* 2008). In Nigeria it is encountered both in urban and rural areas (Adegunloye, 2005). It is estimated that 1.3 billion episodes and 4 million deaths occur each year in children under five. About 80% of deaths due to diarrhea occur in the first two years of life (Rukunga *et al.,* 2002).

Diarrhea is the passage of unusually loose or watery stools, usually at least three times within 24 hour period. However, it is the consistency of the stools rather than the number that is most important. Frequent passing of formed stools is not diarrhea. Babies fed with only breast milk often pass loose pasty stools; this also is not diarrhea.

Prolonged diarrhea may lead to excessive loss of fluid, salt and nutrient in the feaces. The main cause of death from acute diarrhea is dehydration, which result from loss of fluid and electrolyte in stool. Another important cause of death is dysentery and under nutrition. Diarrhea is an important cause of under nutrition because patients eat less during diarrhea and their ability to absorb nutrients is reduced. Moreover, nutrient requirement is increased as a result of infection (Sinclair *et al.*, 2003).

Risk factors that predispose children to diarrhea include poor sanitation, poor social and economic status and malnutrition (Andu *et al.,* 2002).

The clinical syndromes of diarrhea include acute watery diarrhea, which refers to diarrhea that begins acutely and last less than 14 days (usually less than 7 days), and involve the passage of frequent loose or watery stool without visible blood. Vomiting may occur and fever may be present. Acute watery diarrhea causes dehydration which may result in death. The most important cause of acute watery diarrhea in young children in Nigeria include *rotavirus*, enterotoxigenic *Escherichia coli Shigella, Campylobacter jejuni,*and*Cryptosporidia, Vibrio cholerae, Salmonella* and enteropathogenic *Escherichia coli(*.Bahal *et al.*,2001 )

Another clinical syndrome of diarrhea is dysentery, which refers to diarrhea with visible blood in faeces, the effect of which include anorexia, rapid weight loss and damage to the intestinal mucosa by invasive bacteria. The organisms implicated in this type of diarrhea include *Shigella, Campylobacter jejuni, Salmonella* and very rarely *Entamoeba histolytica.*

Persistent diarrhea begins either as watery diarrhea or as dysentery. Marked weight loss is frequent and diarrhea stool volume may also be great, with a risk of dehydration. *(*.Bahal *et al*., 2001)

Transmission of agents that cause diarrhea are usually by the faecal oral route, which include the ingestion of faecal contaminated water or food, person to person contact and direct contact with infected faeces. Host factors that increase susceptibility to diarrhea include under nutrition, current or recent measles and immune deficiency or immunosupression (Andu *et al.,* 2002).In view of the above present work is aimed at determination of the bacteria associated with different diarrhea syndrome in children.

## MATERIALS AND METHODS

### Sample collection and handling

A total of 100 stool samples from children suffering from diarrhea were collected from Barau Dikko Children hospital, Yusuf Dantsoho Memorial hospital Tudun Wada and Biba Hospital Tudun Wada all within Kaduna metropolis. Sterile wide mouth screw capped bottles with collection spoons were given to the parents of the children and instructed on the proper method of collection. The importance of timing was also stressed as all samples were examined and cultured within 2h of collection.

### Isolation and identification of diarrhoeagenic bacteria

#### Microscopy

The stool samples were examined for consistency, colour, presence of blood, mucus or pus using direct technique as described in district laboratory practice in tropical countries .( Cheesbrough,2005 ) .

#### Culture

A sterile wire loop was used to pick each stool sample and inoculate into selenite F broth, then from the broth into MacConkey agar, Deoxycholate citrate agar and Salmonella-shigella agar using streak plate method. The inoculated plates were incubated at $37^{0C}$ for 18- 24 hours.

#### Identification of Bacteria.

All the plates were examined for growth and pure isolates were Gram stained and subjected to series of biochemical test.

#### Gram staining

A colony of the pure culture was emulsified in distilled water on a clean grease free slides and spread to make a smear. This was allowed to air dry and heat fixed by passing it gently over a Bunsen flame briefly under the slide. The smear was then flooded with crystal violet and allowed to stand for 1 min. This was then rinsed with water and Lugols iodine was added for 1 minute. The complex formed was washed with water and acetone was applied and allowed to stay for 5 seconds. The slide was rinsed with water and counter stained with neutral red for 2 minutes. The slide was rinsed with water and allowed to dry and examined using oil immersion objectives (x 100). (Cheesbrough 2005).

## BIOCHEMICAL CHRCTERISATION OF BACTERIAL ISOLATES

### Catalase test

A sterile wire loop was used to pick some colonies of bacterial isolates and mixed with 2-3drops of hydrogen peroxide on clean grease free slide. (Cheesbrough, 2005).

### Coagulase test

A part of the the pure isolate was emulsified in two drops of physiological saline. A loopful of citrated human plasma was added and examined after 2 minutes. (Cheesbrough, 2005).

Triple sugar iron agar (TSI) test
A colony 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 $37^0$c for 24 hours.

Indole test
The bacteria isolated were sub-cultured in nutrient broth and incubated for 24 hours. 3 drops of Kovac's indole reagent was added and mixed gently. (Cheesbrough, 2005).

Urease test
Urea agar was inoculated heavily over the entire surface of the slants in bijou bottles, incubated at $37^0_C$ for 24 hours..

Citrate utilization test
Simmons citrate slopes were prepared in bijou bottles. The slopes were then stabbed and incubated at $37o^C$ for 48 hours.

Motility test
A sterile straight wire loop was used to inoculate motility indole urea media with bacterial isolate and incubated overnight at $37^oC$.motility was shown by diffused turbidity in the medium (Cheesbrough,2005}

Triple sugar iron ager (TSI) test
At least one of each colony type of well isolated colonies was selected on plate using 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 slant these were inoculated at $37^o$c for 24 hours (Cowan and Steel, 2002)

RESULTS
A total of one hundred samples (100) were analyzed for the presence of bacterial agents as the cause of diarrhea. The overall percentage occurrence of bacteria was 44% positive.

Table 1 shows that 44 samples were positive for bacterial growth and the highest incidence occurred in the age group of 25 – 36 months (15.0%). The lowest occurrence was in age group of 0 – 12 months with 3 positive samples representing (3.0%). There is statistical association between age and bacterial diarrhea ($x^2 = 27.830$ $p<0.05$).

Table 1: Distribution of diarrhoeagenic bacteria according to age groups of children.

| Age group ( months) | No of sample examined | Positive | Percentage (%) |
|---|---|---|---|
| 0 -12 | 11 | 3 | 3.0 |
| 13- 24 | 23 | 8 | 8.0 |
| 25- 36 | 19 | 15 | 15.0 |
| 37-48 | 17 | 13 | 13.0 |
| 49-60 | 30 | 5 | 5.0 |
| Total | 100 | 44 | 44 |

Table 2 shows the incidence among the sexes with female having the highest with 26 (26.0) compared to males 18 (18.0%). But the difference was not statistically significant.

Table 2: Distribution of diarhoeagenic bacteria according to sex

| Sex | No. of sample examined | Positive | Percentage (%) |
|---|---|---|---|
| Male | 45 | 18 | 18.0 |
| Female | 55 | 26 | 26.0 |
| Total | 100 | 44 | 44 |

($x^2 = 0.531$, $p>0.05$)

Table 3 shows the distribution of the samples based on appearance of the stool samples collected. Loose sample with blood and mucus had the highest with 42 (42%) while watery with blood mucus and pus has the lowest with 08 (08%).

Table 3: Macroscopic characterization of the stool samples

| Appearance | No. of sample examined | Percentage (%) |
|---|---|---|
| Watery diarrhea | 22 | 22 |
| Bloody diarrhea | 28 | 28 |
| Loose sample with blood and mucus | 42 | 42 |
| Watery sample with blood, mucus, pus | 08 | 08 |
| Total | 100 | 100 |

Table 4 shows the occurrence of diarrhoeagenic bacteria in study subjects with gram negative bacteria { *Shigella spp, Salmonella spp, Escherichia coli* )being the main cause of bacterial diarrhea and *Shigella spp* having highest number of 20 (45.5%), followed by *Escherichia coli* with 15 (34.1%), *Salmonella spp* 08 (18.1%) and *Staphylococcus aureus* 01 (2.3%).

Table 4 occurrence of bacterial isolates in diarrhea stool sample

| Isolates | No. of sample | Percentage (%) |
|---|---|---|
| *Shigella spp* | 20 | 45.5 |
| *Salmonella spp* | 08 | 18.1 |
| Staphylococcus aureus | 01 | 2.3 |
| Escherichia *coli* | 15 | 34.1 |
| Total | 44 | 100 |

DISCUSSION

Generally, the aetiology of diarrhea in young children could be attributed to wide range of factors, but one of the main aetiology of the diarrhea is related to bacteria (such as *Salmonella spp, Shigella spp,Vvibrio, Escherichia coli, Aeromonas* and *Pseudomonas* (Abdullahi *et al.,* 2010).

In this study, the prevalence of bacteria aetiology of diarrhea is 44% which follows the same trend with the research conducted in Kano State which was found to be 40.67% (Abdullahi *et. al*., 2010). In Gabon prevalence of diarrhea with bacterial aetiology is 38% (Patwar, *et al.,* 1993). In Tanzania ,it was 36% (Molbak *et al.,* 1997). The study showed that *Shigella spp* appears to be the predominant bacteria causing diarrhea followed by *E. coli*, and *Salmonella* in that order. Fifty six percent (56%) of the hundred diarrhea cases investigated had no bacterial pathogen suggesting viral, protozoan or non pathogenic factors. Bacterial pathogens were isolated more in age group 25 – 36 months, (15.0%) followed by age group 37 – 48 (13.0%) with the least isolated from age group 0 – 12 months (3.0%) which suggest an association between age and bacterial diarrhea ($x^2 = 27.830$ $P<0.05$). The reason for high incidence of bacteria isolates in age group 25 – 36 months and 37 – 46 months could be due to the fact that children within this age group on their own cannot differentiate between what to eat and what not to eat; they have not learnt the rudiment of adherence to aseptic or hygienic practice; they can barely express themselves. Those between he age of 0 – 12 months are essentially under their mothers care, feeding mainly on breast milk thereby reducing their susceptibility to these pathogens. The predisposing factors that enhance spread and increase the risk of diarrhea in young children include failure to breast feeding exclusively for the first 4 – 6 months of life. The risk of developing diarrhea is greater in non-breast fed infants than those exclusively breast fed. Breast feeding until at least one year of age or prolonged breast feeding reduces incidence and severity of diarrhea disease (Abdullahi *et al.,* 2010). The uses of infant feeding bottle which may be contaminated with bacteria; under nutrition; immunodeficiency or immune suppression; current or recurrent measles attack are among the risk factor.

Most diarrhea episodes occur during the first 2 years of life due to combined effects of declining levels of maternally acquired antibodies, the lack of active immunity in the infant, the introduction of food that may be contaminated with faecal bacteria and direct contact with human or animals faeces when the infant start to grow. Most enteric pathogens stimulate at least partial immunity against repeated infection or illness, which helps to explain the declining incidence of disease in older children and adults (Patwari, *et al.,* 1993).

The study also shows that more bacterial pathogens were isolated in female (26.0%) than in males (18.0%) which is in contrast to the work of Abdullahi *et. al.,* 2010 where they reported that male children were more infected (22.33%) than female children (18.33%), although the difference was not statistically significant ($x^2 = 0.531$, $p>0.05$).

The physical appearance of the sample is very important when categorizing diarrhea. Watery sample, loose sample with blood and mucus; watery sample with mucus blood and pus; and bloody diarrhea were identified. This categorization is necessary as different sample appearance is associated with different causative agent. However, the appearance must be differentiated from normal liquid sample from exclusively breast fed infants who may pass several soft, semi liquid stools each day. For them, it is practical to define diarrhea as an increase in stool frequency or liquidity that is considered abnormal by the mother. Bacterial causes of watery diarrhea may be *Escherichia coli, Shigella, Campylobacter jejuni, Salmonella, Vibrio cholera*. For diarrhea with visible blood and mucus *Shigella* is the most important cause. In this study, loose stool sample with blood and mucus was predominant and it was observed that *Shigella spp* had the highest incidence of all the aetiologic bacteria isolated which is similar to the findings of Adegunloye (2005) which correlate the nature or appearance of stool sample and the aetiologic bacteria. Watery stool is mainly associated with causative agents like *Salmonella, Escherichia coli* and *Campylobacter jejuni*. The isolation of *Staphylococcus aureus* in one of the hundred samples analysed indicates the possibility of Staphylococcal food poisoning.

## CONCLUSION

This research finding show that, though there are a number of causative agents of diarrheal diseases, bacteria still remain one of the major causes with *Shigella,Salmonella* and *Escherichia coli* being most important bacterial pathogens among pediatric patient in the selected study hospital, in Kaduna.

RECOMMENDATION

Diarrhoeal diseases among children are believed to be very common and can be minimized by observing strict personal hygiene, quality of drinking water, quick isolation and treatment of infected cases as well as encouragement of breast feeding are maintained. Parents are strongly advise not to regard bottle feeding of children with milk formula as main source of feeding for the children. rather they should stick to breast feeding .Government should however endeavour to provide potable water to the community. Improving the sanitary awareness through basic health education, careful surveillance, monitoring incidence and spread of diarrhoeal diseases, may help to reduce the disease burden in children. The approach of oral rehydration therapy given to children by mothers must be taught to reduce the debilitating effect of diarrhoeal disease (Abdullahi *et al.,* 2010).

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