Bacterial Organisms Isolated From Children with Diarrhoea In Abeokuta, Nigeria

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Abstract: One hundred and fifty diarrhoea faecal samples collected from children presenting with diarrhoea were inoculated aerobically on sterile differential and selective media and incubated aerobically. Isolated pure cultures were subjected to various morphological and biochemical tests, followed by antibiotic sensitivity testing using Kirby-Bauer diffusion method. The bacteria isolated were *Escherichia coli, Klebsiella sp*, *Enterobacter sp*, *Proteus sp, Pseudomonas aeruginosa, Staphylococcus aureus, Shigella sp, Morganella morganii and Salmonella sp.* The susceptibility studies showed that *Escherichia coli* were resistance to cotrimoxazole, erythromycin, ampicillin and tetracycline. *Pseudomonas aeruginosa* isolates were resistance to ampicillin, erythromycin and cotrimoxazole, *Shigella sp*ecies were resistance to erythromycin and cotrimoxazole while *Samonella sp* were resistance to ampicillin, erythromycin and cotrimoxazole. Diarrhoeal diseases among children can be minimized by observing strict personal hygiene, drinking portable water, quick isolation and treatment of infected cases. Government should endeavour to provide portable water to the community. Also sanitary awareness through basic health education, careful surveillance, monitoring incidence and spread of diarrhoeal diseases is therefore recommended.
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1. INTRODUCTION

Diarrheal diseases are the major cause of death in children under 5 years of age in resource-poor countries, resulting in approximately 2.5 million deaths each year worldwide (Bryce *et al*., 2004). 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* (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 histolytical (*Bahal *et al.,* 2001).

Diarrhoea is one of the commonest problems found in pediatric clinics in any part of world. It is one of the leading causes of illness in young children in developing countries (Parashar *et al*., 2003). The public health significance of diarrhoeal disease cannot be over – emphasized and epidemiological studies of diarrhoea have been reported from several African countries including South Africa (Household *et al.*, 1988), Gabon (Presterl *et al.*, 2003), Egypt (Rao et al., 2003) and Kenya (Sang *et al.*, 1996).

Antimicrobial treatment tends to quicken the clinical resolution of diarrhea, prevent the progression of disease and reduce the severity of associated symptoms, such as fever, abdominal pain and vomiting. Furthermore, antimicrobial therapy decreases secondary cases, by halting person-to-person spread of most pathogens, which warrants special consideration for the use of antibiotics in the treatment of child-care workers, health professionals and workers in the catering industry or services. Prompt adoption of empirical antimicrobial therapy is

also useful in the setting of febrile acute bloody diarrhea in young children and is currently recommended by the World Health Organization (WHO, 1992). On the other hand, there are several arguments against the empirical use of antibiotics for acute infectious diarrhea. The most compelling of them is the fact that acute infectious diarrhea is typically a self-limiting disease, regardless of its etiology, with most cases resolving in less than three days (Goodman and Segretti, 1999). Risk factors that predispose children to diarrhea include poor sanitation, poor social and economic status and malnutrition (Andu *et al.,* 2002). The aim of this study is to determine the bacterial organisms isolated from children with diarrhea in Abeokuta, Nigeria.

2. MATERIALS AND METHODS

2.1. Sample Collection

A total of one hundred and fifty diarrhoea faecal samples were collected from patients aged 0 to 5 years old attending Sacred Heart Hospital, Lantoro Abeokuta, Ogun State, Nigeria. The diarrhoeal stool samples were collected into sterile, transparent, wide mouthed bottles. The name, age and sex of the patients were properly labeled on the universal bottles. This study was approved by the Ethical committee of the hospital.

2.2. Processing of Specimens

The specimens were processed according to the guidelines provided by Cheesbrough (2004) for the laboratory diagnosis of enteric pathogens.

2.3. Culture

The faecal samples were inoculated aerobically on sterile MacConkey agar, Eosin Methylene Blue Agar plates and Salmonella –shigella agar and incubated aerobically at 37^{o}C for 24 hours.

2.4. Characterization and identification of isolates

Isolated pure cultures of bacteria were subjected to various morphological and biochemical tests. After which they were identified using Bergey's Manual of Systematic bacteriology. The following tests were carried out: Gram stain, Motility, Oxidase test, Urease test, Indole test, Methyl red test, Vogue Proskauer test, Catalase tesr, Cogulase test and Citrate test, Fermentation of glucose, lactose and sucrose.

2.5. Antimicrobial sensitivity testing

Commercially available antimicrobial discs (Abtek Biological Ltd UK) were used to determine the drug sensitivity and resistance pattern of the isolates. A number of 6 different antibiotics with different disc concentration such as Gentamycin (Gen), Erythromycin (Ery), Ampicillin (Amp), Cotrimoxazole (Cot), Tetracycline (Tet) and Ceftriaxone (Cef) were used in this study. The antimicrobial sensitivity test of each isolate was carried out as described by the Kirby – Bauer disc diffusion method (Bauer *et al*; 1966) as recommended by the National Committee for Clinical Laboratory Standards. The turbidity of the bacterial suspensions was compared with 0.5Macfarland's barium sulfate standard solution. The standardized bacterial suspension was then swabbed and inoculated on to Muller Hinton Agar (Lab M Limited, UK) using sterile cotton swabs and left to dry for 10minutes, before placing the antimicrobial sensitivity discs. Antibiotic impregnated discs of 8mm diameter were used for the test. After incubation, the diameter of the zone of inhibition were measured and compared with zone diameter interpretative chart (CLSI 2007) to determine the sensitivity of the isolates to antibiotics.

3. RESULT

Out of the 150 faecal samples cultured. *Escherichia coli* accounted for 81(51.0%) of the bacteria isolated while *Klebsiella sp*, *Enterobacter sp, Proteus sp, Pseudomonas aeruginosa, Staphylococcus aureus*, *Shigella sp, Morganella morganii* and *Salmonella sp* constituted 22(14.0%), 19(12.0%), 14(9.0%), 10(6.3%), 7(4.4%), 3(2.0%), 2(1.3%) and 1(0.6%) respectively. Table 1 showed the percentage occurrence of the bacteria isolates in faecal samples.

Table 1: Percentage occurrence of the bacteria isolates in faecal samples

Isolates	**No. (%)**
Staphylococcus aureus	7(4.4)
Shigella species	3(2.0)
Escherichia coli	81(51.0)
Pseudomonas aeruginosa	10(6.3)
Klebsiella species	22(14.0)
Proteus species	14(9.0)
Morganella morganii	2(1.3)
Salmonella species	1(0.6)
Enterobacterspecies	19(12.0)
Total	**159(100.0)**

The susceptibility studies showed that most of *Escherichia coli* were susceptible to gentamycin (67.9%), ceftriaxone (66.7%), but were resistance to cotrimoxazole, erythromycin, ampicillin and tetracycline. The *Pseudomonas aeruginosa* isolates were 100.0% resistance to ampicillin, erythromycin and cotrimoxazole while *Shigella sp*ecies were 66.7% resistance to erythromycin and cotrimoxazole

respectively. *Samonella sp* were resistance to Ampicillin, Erythromycin and Cotrimoxazole (100.0%) and were sensitive to Ceftriaxone, gentamycin and tetracycline. The detail of the in - vitro susceptibility patterns of the bacterial isolates from diarrheoa faecal samples is shown in Table 2.

Table 2: *In - vitro* susceptibility patterns of the bacterial isolates from diarrheoa faecal samples

Isolates	Ampicillin	Ceftriaxone	Gentamycin	Erythromycin	Tetracycline	Cotrimoxazole
E. coli S	36(45.4)	54(66.7)	55(67.9)	33(40.7)	37(45.7)	31(38.3)
n = **81** R	45(55.6)	27(33.3)	26(32.1)	48(59.3)	44(54.3)	50(61.7)
Klebsiella sp S	12(54.5)	18(81.8)	18(81.8)	12(54.5)	7(31.8)	9(40.9)
n = **22** R	10(45.5)	4(18.2)	4(18.2)	10(45.5)	15(68.2)	13(59.1)
Enterobacter sp S	3(15.8)	12(63.2)	10(52.6)	10(52.6)	10(52.6)	7(36.8)
n = **19** R	16(84.2)	7(26.8)	9(47.4)	9(47.4)	9(47.4)	12(62.2)
Proteus sp S	8(57.1)	10(71.4)	10(71.4)	8(57.1)	7(50)	5(35.7)
n = **14** R	6(42.9)	4(28.6)	4(28.6)	6(42.9)	7(50)	9(64.3)
P. aeruginosa S	0(0.0)	5 (50.0)	7 (70.0)	0(0.0)	5 (50.0)	0(0.0)
n = **10** R	10(100)	5(50.0)	3(30.0)	10(100.0)	5(50.0)	10(100.0)
S. aureus S	5(71.4)	6(85.7)	4(57.1)	3(42.9)	5(71.4)	2(28.6)
n = **7** R	2(28.6)	1(14.3)	3(42.9)	4(57.1)	2(28.6)	5(71.4)
Shigella sp S	2(66.7)	3(100)	2 (66.7)	1(33.3)	2(66.7)	1(33.3)
n = **3** R	1(33.3)	0(0)	1(33.3)	2(66.7)	1(33.3)	2(66.7)
M. morganii S	1(50)	1 (50)	2(100)	1 (50)	1 (50)	0(0)
n = **2** R	1(50)	1(50)	0(0)	1(50)	1(50)	2(100)
Salmonella sp S	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)
n = **1** R	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)

Keys: S – Sensitive, R – Resistant

4. DISCUSSION

Diarrhoea due to bacterial infections is an important cause of morbidity and mortality in infants and young children in most developing countries including Nigeria (Adegunloye, 2005). In this study, a total of nine bacterial species namely *Escherichia coli, Klebsiella sp*, *Enterobacter sp*, *Proteus sp, Pseudomonas aeruginosa, Staphylococcus aureus, Shigella sp, Morganella morganii* and *Salmonella sp* were isolated from diarrhoeal faecal samples while Abdullahi *et al* (2010) identified six bacterial species (*Salmonella sp, Shigella sp, Vibrio sp, Escherichia coli, Aeromonas* and *Pseudomonas* sp) among children with diarrhoea attending some hospitals in Kano Metropolis, Kano State, Nigeria. This indicates that there are geographical differences in the spectrum of bacteria incriminated in childhood diarrhoea.

Out of the 150 diarrhoea faecal specimens analyzed in this study, 54% of the samples were positive for *Escherichia coli*. This is lower than 83.1% prevalent rate reported from similar study in Abakaliki, South eastern Nigeria (Ogbu *et al*., 2008) and higher than 34% prevalent rate recorded by Sule *et al* (2011) in Kaduna. The present prevalent rate is lower than 61.76% recorded by Babu *et al* (2009) in Rajah Hospital, Annamalainagar, Tamil Nadu, in India. *Escherichia coli* recorded highest prevalence among the nine bacterial species identified in this study followed by *Klebsiella sp* while *Samonella sp* was the least. This is in agreement with the report of Huilan *et al* (1991) and Clarke (2001) that stated that the bacterial pathogen most commonly associated with endemic forms of childhood diarrhoea is *Escherichia coli.*

The resistance of enteric pathogens to currently used antimicrobial agents has increased world over as a result of the widespread use of antimicrobials. All the bacterial isolates from this study displayed resistance to ampicillin, erythromycin and cotrimoxazole. There was high resistance of *E. coli* to most of the commonly used antibiotics (such as cotrimoxazole, erythromycin, ampicillin and tetracycline) in the treatment of diarrhoea in Abeokuta. The same trend was also recorded in Kenya among strains of pathogenic *E. coli* (Bii *et al,* 2005: Brooks *et al*, 2006).

The relative high rates of tetracycline resistance to enteric *E. coli*, in this study may probably be related to the indiscriminate use of the antibiotic. *Shigella* isolates were resistant to erythromycin and cotrimoxazole in this study similar antimicrobial resistance among *Shigella* isolates was observed in studies in Kenya (Maakooti *et al,* 1997: Shapiro *et al,* 1999). The only *Salmonella* isolate obtained in this study had lower levels of resistance compared to those of *E.*

coli and *Shigella* and was sensitive to gentamycin, ceftriaxone and tetracycline. It was noted that there is an emerging resistance of *Salmonella* isolates to gentamycin, in Nyanza and Mbagathi both in Kenya.

The *Shigella*, *Salmonella* and *Enterobacter* isolates in this study were resistant to ampicillin, cotrimoxazole and erythromycin, similar resistance of *Shigella* and *Salmonella* to ampicillin was also observed in Kenya (Brooks *et al*, 2006). This resistance may be due to widespread and indiscriminate use of these first-line inexpensive antibiotics in this environment. The resistance to ampicillin may be due to production of beta-lactamases enzymes, the most common mechanism for resistance to cotrimoxazole is acquisition of plasmid-encoded, variant diamino-pyrimidine folate reductase enzymes (World Health Organization, 2001).

Evidence from studies in other countries demonstrates a high prevalence of multiple antimicrobial resistances in normal bowel flora, which suggests that they may act as a reservoir for resistance available to enteric pathogens. A study of commensal gut flora of children in Sudan found that 39.0% of children had strains resistant to six antimicrobials and over 70.0% of the children had strains resistant to at least 4 out of 6 antimicrobials commonly used in the country (Shears *et al*, 1998). Diarrhoeal diseases among children can be minimized by observing strict personal hygiene, drinking portable water, quick isolation and treatment of infected cases. Government should endeavour to provide portable water to the community. Also sanitary awareness through basic health education, careful surveillance, monitoring incidence and spread of diarrhoeal diseases is therefore recommended.

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