Multi-Drug Resistant (MDR) *Escherichia coli* among Children suffering from Diarrhea infections in Abeokuta, Nigeria

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Abstract: A total of one hundred and fifty diarrhoea faecal samples were collected from children under 5 years old presenting with diarrhoea. Biochemically identified *Escherichia coli* isolates were subjected to the antibiotic sensitivity testing using Kirby-Bauer diffusion method. Of the 150 faecal samples collected, 81(54%) yielded growth of *Escherichia coli*. The distribution between feeding patterns and the number of *Escherichia coli* isolated were statistically significant (P<0.05) also, the distribution of mothers' level of education to number of *Escherichia coli* isolated from their children were statistically significant. The antibiotic susceptibility showed that 81 *Escherichia coli* isolates tested, were not all susceptible to all the drugs used. The *Escherichia coli* isolates were resistant to cloxacillin 54(66.7%), cotrimoxazole 50(61.7%), erythromycin 48(59.3%), ampicillin 45(55.6 %) and tetracycline 44(54.3%). Twenty one (25.9%) of the *Escherichia coli* were resistant to more than two classes of antibiotics. In conclusion, monitoring and regulating the distribution and use of antimicrobial by government may be necessary to reduce the resistance to drugs.

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1. Introduction

Escherichia coli is a bacterial organism that belongs to the family Enterobacteriaeceae. It is one of the main causes of both nosocomial and community acquired infections in humans. The organism is therefore of clinical importance. *E. coli* strains have been associated with a number of disease syndromes; among these, often severe and fatal infections are pyelonephritis, septicemia, meningitis, endocarditis, and epidemic diarrhea in adults and children. Diarrhoea is an important cause of morbidity and mortality in the pediatric population of developing countries (Guerrant *et al*., 1990). It ranks second only to respiratory diseases and is a major cause of morbidity among notifiable diseases in some part of the world (Coker *et al.*, 1998).

Diarrhoea is the condition of having three or more loose or liquid bowel movements per day (Coker *et al.*, 1998). The major biotypes of *E. coli* in diarrhoea are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and enteroadherent *E. coli* or diffusely adhering *E. coli* (DAEC) (Nataro and Kaper, 1998). This classification is based on agglutination using monovalent and polyvalent antisera (Gakuya, 2001).

In India, diarrhoeal infections are most commonly caused by *Escherichia coli (E. coli)*, with occasional outbreaks (Clarke, 2001). In Iran, it has been estimated that diarrhoea is responsible for 18 million cases of illness (MHME and UNICEF, 2000), 12 million medical visits, one million hospital admissions (Kolahi *et al.*, 2008), and 516 deaths in children younger than five years of age (Kolahi *et al.*, 2008).

In Nigeria, the incidence of acute watery diarrhoea is approximately 4.9 episodes per year and there are approximately 200,000 diarrhoea related deaths of children aged below five years with an average of 300 deaths per day (Federal Ministry of Health, 1992; Federal Office of Statistics, 1997).

Antibiotics resistance on enteric pathogen is of great public health concerned in the developing world where the rate of diarrhoea is highest. Currently we are faced with (multi) resistant bacteria that are difficult and sometimes impossible to treat (Levy, 2002). The tremendous therapeutic advantage afforded by antibiotics is being threatened by the emergence of increasingly resistant strains of microbes (Livermore, 2005). The problem has recently been worsened by the steady increase in multi-resistant strains and by the restriction of antibiotic discovery and development programs (Levy, 2002).

Multidrug resistant organisms (MDROs) are resistant to more than two classes of antimicrobial agents, the knowledge of susceptibility pattern is helpful in selecting the empirical therapy and improving the likelihood of a satisfactory outcome for patient (Sameera *et al*., 2010). It has been observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Hassan, 1985). This therefore demands the need for periodic screening of common bacterial pathogens for their antibiotic susceptibility profiles in different communities. According to Aibinu *et al*. (2004), *E. coli* is highly resistant to ampicillin, amoxicillin, tetracycline and trimethoprim - sulfamethoxazole. The widespread occurrence of drug resistant pathogens in our environment has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational prescription programs, making policy decisions and assessing the effectiveness.

This study aimed at determined the Multi-drug resistant (mdr) *Escherichia coli* among children suffering from diarrhoea infections in Abeokuta, Ogun State, Nigeria.

2. MATERIALS AND METHODS

2.1. Study Population

The study population was diarrhea patients aged ≤ 5 years old attending the Out Patient Department (OPD) of the Sacred Heart Hospital, Lantoro Abeokuta, Ogun State, Nigeria. This study was approved by the Ethical committee of the hospital.

2.2 Sample Collection

A total of one hundred and fifty diarrhoea faecal samples were collected from the patients. 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.

2.3 Processing of Specimens

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

2.4 Culture

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

2.5 Antibiotic susceptibility testing

Commercially available antimicrobial discs (Abtek Biological Ltd UK) were used to determine the drug sensitivity and resistance pattern of the *Escherichia coli* isolates. A number of 12 different antibiotics with different disc concentration such as Gentamycin (Gen) 10µg/disc, Erythromycin (Ery) 15µg/disc, Ampicillin (Amp) 10µg/disc, Ceftriaxone (Cef) 30µg/disc, Cotrimoxazole (Cot) 25µg/disc, Cefixime (Cf) 30µg/disc, Tetracycline (Tet) 30µg/disc, Streptomycin (Str) 10µg/disc, Cloxacillin 5µg/disc (Cxc), Amoxicillin (Amx), 25µg/disc, Cefuroxime (Cxm) 30µg/disc and Ceftazidime (Caz)30µg/disc, 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). The turbidity of the bacterial suspensions was compared with 0.5 Macfarland's barium sulfate standard solution. The standardized bacterial suspension was inoculated on to Muller Hinton Agar (Lab M Laboratories, Mumbai, India) and left to dry for 10 minutes, 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. Standard strain of *Escherichia coli ATCC*25922 was used as control.

3. Results

Faecal samples from 76 female and 74 male participants were analyzed. Of the 150 faecal samples collected, 81(54%) yielded growth of *Escherichia coli*. Table 1 showed the prevalence of *Escherichia coli* in relation to age and sex of children tested.

Table 1: Prevalence of *Escherichia coli* in relation to age and sex of children tested.

Age group (Years)	No. Tested	No. (%) Positive	No. Females	No. (%) Positive	No. Males	No. (%) Positive
≤ 1	12	6(50)	6	3(50.0)	6	3(50)
≤ 2	22	11(50)	9	5(55.6)	13	6(46.2)
≤ 3	36	14(38.9)	17	9(52.9)	19	5(26.3)
≤ 4	46	29(63.0)	27	16(59.3)	19	13(68.4)
≤ 5	34	21(61.8)	17	11(64.7)	17	10(58.8)
Total	**150**	**81(54)**	**76**	**44(57.9)**	**74**	**37(50)**

Children who were fed on solid food had highest number 56(54.3%) positive for *Escherichia coli,* from their faecal samples while those who were mixed fed had 21(55.3%) positive for *Escherichia coli* from their faecal samples. Four *Escherichia coli* were recovered from faecal samples of exclusively breast fed children, and the differences were statistically significant (P<0.05). The distribution between feeding patterns and the number of *Escherichia coli* isolated is presented in table 2.

Table 2: Distribution of feeding pattern of children with number of *Escherichia coli* Isolated

Type of feeding	No. examined	No. (%) positive	p – Value
Exclusive breast feeding	9	4(44.4)	0.001
Mixed feeding	38	21(55.3)	0.001
Solid feeding	103	56(54.3)	0.002
Total	**150**	**81(54)**	**0.001**

The results showed that 42(53.2%) children with mothers who had only received primary school education tested positive for *Escherichia coli*. Twenty eight (66.7%) children with mothers who had obtained secondary school education and eleven (37.9%) children with mothers who had a post-secondary education tested positive for *Escherichia coli*. The results of this analysis were statistically significant. The distribution of mothers' level of education to number of *Escherichia coli* isolated from their children is presented in table 3.

Table 3: Distribution of mothers' level of education to number of *Escherichia coli* isolated from their children.

Level of education	No. examined	No. (%) positive	p – Value
Above secondary school level	29	11(37.9)	0.004
Up to secondary school	42	28(66.7)	0.003
Primary school and below	79	42(53.2)	0.002
Total	**150**	**81(40.8%)**	**0.001**

The antibiotic susceptibility showed that the 81 *Escherichia coli* isolates tested, were not all susceptible to all the drugs used. Fifty four (66.7%) *Escherichia coli* were resistant to cloxacillin, 50(61.7%) were resistant to cotrimoxazole, 48(59.3%) were resistant to erythromycin, 45(55.6%) were resistant to ampicillin while 44(54.3%) were resistant to tetracycline and 36 (44.45) were resistant to amoxicillin. Thirty three (40.7%) of the *Escherichia coli* were resistant to cefixime and streptomycin respectively, thirty one (38.3%) were resistant to cefuroxime and 27(33.3%) were resistant to ceftriaxone. Twenty six (32.1%) of the *Escherichia coli* were resistance to gentamycin while 23 (28.4%) *Escherichia coli* were resistant to ceftazidime. These were documented in table 4.

Table 4: Antibiotic resistance profile of *Escherichia coli* isolates detected from faecal samples

Antibiotic	Concentration	n = 81	No. (%) resistant
Cloxacillin	5µg		54(66.7)
Cotrimoxazole	25µg		50(61.7)
Erythromycin	15µg		48(59.3)
Ampicillin	10µg		45(55.6)
Tetracycline	30µg		44(54.3)
Amoxicillin	25µg		36(44.4)
Cefixime	5µg		33(40.7)
Streptomycin	10µg		33(40.7)
Cefuroxime	30µg		31(38.3)
Ceftriaxone	30µg		27(33.3)
Gentamycin	10µg		26(32.1)
Ceftazidime	30µg		23(28.4)

n = Number of *Escherichia coli* subjected to antibiotic susceptibility test

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). Out of the 150 diarrhoea faecal specimens analyzed in this study, 54% of the samples were positive for *Escherichia coli*. This is lower than 89.4% prevalence reported from similar study in Benin Teaching Hospital, Benin, Nigeria and 83.1% reported in Abakaliki, South eastern Nigeria (Ogbu *et al*., 2008) and 62.8% obtained in a study carried out in the Federal Capital Territory Abuja, Nigeria by Ifeanyi *et al* (2010) among children presented with diarrhoea, but higher than 34% recorded by Sule *et al* (2011) in Kaduna, another Northern State in Nigeria. Clarence *et al* (2007) had 41.3% prevalence of *Escherichia coli* in a study conducted among children with diarrhoea, attending Madonna University Teaching Hospital (MUTH) Elele, in Rivers State, in South south, Nigeria while Olanipekun, (1996) documented 26% prevalence among children with diarrhoea attending Jos University Teaching Hospital, Jos in Plateau State, in North east, Nigeria. The present prevalence is lower than 61.76% recorded by Babu *et al* (2009) in Rajah Hospital, Annamalainagar, Tamil Nadu, in India and also lower compared with other studies carried out in Iran (Alikhani *et al*, 2006; Jafari *et al*, 2009).

Escherichia coli recorded highest prevalence among the nine bacterial species identified in this study followed by *Klebsiella sp*. 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*. *It is* also in agreement with Podewils *et al* (2002) that stated that *Escherichia coli* is one of the leading causes of diarrhoea in developing countries in children under 5 years old, with significant morbidity and mortality. World Health Organization in 1991 stated that diarrhoea caused by *E. coli* has been recognized as an important health problem among children in the developing countries and is a research priority of the diarrhoeal disease control program of the WHO. Ogunsanya *et al* (1994) and Jindal *et al* (1995) in their separate works also agreed that *E. coli* play an important role in the etiology of diarrhoea. All these submission is in agreement with the finding from this current investigation of diarrhoea among children in Abeokuta.

Isolation rate of *Escherichia coli* was higher among children within ≤ 4years of age, followed by children within ≤ 5years of age in this study. This finding is similar to reports published by several authors, Requa *et al* (1990), Olanipekun (1996) and Kandakai-Olukemi *et al* (2007) where they observed that the highest incidence of gastroenteritis in children was found among children age 4 years old and above. The group with the highest *Escherichia coli* belong to pre-primary age, their eating habits which might not be supervised all the time might have contributed to this high level of the infection. The result showed that children with mothers who had received primary school education had highest number of diarrhoea infection while mothers who had received above secondary school education had least in this study. The high number of the infection among children that their mothers had received primary school education is due to their low educational level as well as their poor knowledge of sanitation and food hygiene-related-practices. This result is in agreement with the report of Elmahde, (1987) that stated that illiteracy of mothers is a predisposing factor that contributes to infants and young children acquiring the infection.

The multi - drug resistant *Escherichia coli* detected from the diarrhoea faecal samples in this study were 21(25.9%). All of the *E. coli* isolates displayed resistance to one or more antimicrobials. There was high resistance of *E. coli* to most of the commonly used antibiotics (such as cloxacillin, cotrimoxazole, erythromycin, ampicillin and tetracycline) in the treatment of diarrhoea in Abeokuta. The widespread misuse of antimicrobial agents in the treatment of infections in this community might be the reason of this serious problem of antimicrobial resistance. This is also the trend in a research conducted in Rajah Muthiah Medical College and Hospital, Annamalainagar, Tamil Nadu in India, in which high rate of drug resistance to commonly used antimicrobial agents in the treatment of diarrhoea was also observed (Babu *et al*, 2009), according to Karlowsky *et al* (2004) pathogenic isolates of *E. coli* have relatively high potentials for developing resistance while Temu *et al* (2007) stated that the emergence and spread of antimicrobial resistance in bacteria of medical importance imposes serious constraints on the options available for treatment of many infections, and also raised concern among general practitioners and pediatricians in developing countries.

Resistance to ampicillin was the fourth highest (55.6%) in this research as opposed to 100% resistance reported by Aibinu *et al* (2004). The result obtained is in contrast also, to a study conducted by Celebi *et al* (2007) who reported highest rate of resistant to ampicillin while 62.7% of the *Escherichia coli* isolates showed resistance rate to cotrimoxazole in this study. This is higher than 53% and 57.9% resistance recorded by Densenclos *et al* (1998) and Aiyegoro *et al* (2007) respectively while 54.3% *Escherichia coli* isolates had resistance to tetracycline which is lower than 67% resistant rate documented by Densenclos *et al* (1998).

From this report, except the antibiotic susceptibility test-result is known, ampicillin, tetracycline, cloxacillin and co-trimoxazole are not advised to be used for treatment of *Escherichia coli* infection in Abeokuta.

The prevalence of *Escherichia coli* resistance to cloxacillin, cotrimoxazole, erythromycin and ampicillin 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) while the relative high rates of tetracycline resistance in strains of enteric *E. coli*, in this study may probably be related to the indiscriminate use of the antibiotic (Usein *et al*., 2009).

Bacterial resistance to commonly used antibiotics is a threat to public health throughout the world. It is noteworthy that the arbitrary empirical use of antibiotics might be responsible for the emerging resistance pattern as shown by this study. Since there is resistance to first-line drugs that are cheaper, more expensive drugs will be required for effective treatment and this may pose a major challenge to the health care system. *Escherichia coli* isolates recorded a moderate sensitivity to amoxicillin (55.6%) in this study this is in contrast to high resistance rate observed in South Africa, Israel, (62% - 84%) and Hong Kong, Philippines (64 - 82%) (Stelling *et al*, 2005).

Relative high rate of sensitivity were observed against aminoglycosides (gentamycin and streptomycin), corroborating data in the literature which suggest a good activity of these antimicrobials against enteric Gram-negative band cells (Usein *et al*., 2009). Ceftazidime, a third generation Cephalosporin, was the most effective antibiotic with 71.6% sensitivity across all the *Escherichia coli* isolates obtained from diarrhoeal faecal samples of children within five years of age in Abeokuta South western Nigeria. When considering a drug of choice for empirical treatment of diarrhoeal infections, if there is need to start antibiotics treatment on children presenting with severe diarrhoeal in Abeokuta, Ceftazidime, a third generation cephalosporin can be considered based on the result obtained from this current work, since appropriate antibiotic therapy for diarrhoea reduces mortality and also shortens the duration of symptoms.

Escherichia coli cause the highest diarrhea in the children 0 – 5 years in this study with 54% isolation rate. The observation of *Escherichia coli* resistance to the tested antimicrobial drugs should be considered in empirical therapy, especially in clinical situations in which the characteristics of this disease could suggest the involvement of *E. coli*. Mothers should be encouraged to practice good personal hygiene and to exclusively breast-feed infants for at least 6 months that is recommended by World Health Organization because of the protective role that breast milk plays against bacterial gastroenteritis.

The finding of resistance to the antimicrobial drugs is significant and should inform a regional clinical alert. These *Escherichia coli* isolates represent the bacterial population circulating in the study community. There should be effective legislation by the government to control the indiscriminate purchase and use of antibiotics without prescription to prevent continuous abuse in Nigeria and to minimize or to reduce rate *Escherichia coli* and other bacterial developed resistant to commonly prescribed antibiotics. Also, there is a need to regulate the use of antimicrobial to reduce the resistance to drugs. Finally, the government and other health agencies should highlights the necessity of sanitary control system as well as monitor and regulate the distribution of antibiotics. These factors may help reducing childhood diarrhoeal infections of bacterial agents in Nigeria particularly in those caused by multi drug resistance *Escherichia coli* and other pathogenic bacteria.

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