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Multi-drug resistant pattern of *Escherichia coli* isolates from diarrhoeic children in Zaria, Nigeria

Susan M. David[1*], Joseph O. Ehinmidu[2], Busayo O. Olayinka[2], Adebola T. Olayinka[3] and Olorunfemi S. David[4]

[1]*Department of Pharmaceutical Microbiology and Biotechnology, University of Ilorin, Ilorin. Nigeria.*
[2]*Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria. Nigeria.*
[3]*Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Shika. Nigeria.*
[4]*Department of Internal Medicine, Bingham University Teaching Hospital, Jos. Nigeria.*

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Abstract
E. coli, which is one of the main causes of both nosocomial and community acquired infections, particularly diarrhoea in children and has been variously reported to develop or acquire resistance to a variety of antibiotics. Seventy (70) isolates of *Escherichia coli* from stool of diarrhoeic children below the age of five (5) attending two hospitals and a primary healthcare centre in Zaria were screened for their susceptibility to a panel of nine commonly used antibiotics. Sixteen isolates (22.9%) were found to be resistant to all the antibiotics. Majority of the isolates were resistant to the inhibitory effects of the penicillins (amoxicillin, ampicillin), tetracycline, sulphonamide/ diaminopyridines and some cephalosporins, but susceptible to the fluoroquinolones (ciprofloxacin) and gentamicin. A high proportion of the isolates were multi-drug resistant, with seventeen phenotypic patterns. This raises serious issues as regards effective management of infection that could be caused by these organisms. The multiple antibiotic resistance indices (MARI) for the MAR isolates was found to be high, between 0.3 to 1.0 suggesting that the isolates originated from an environment where antibiotics were often used and the continued usefulness of these antibiotics in the treatment of *E. coli* infections in children need to be reviewed.

Keywords: *E. coli;* Antibiotic resistance; Diarrhoea; Children; MARI

INTRODUCTION

Diarrhoeal diseases account for 1 in 9 child-deaths worldwide, making diarrhoea the second leading cause of death among children under the age of 5 and has a detrimental impact on childhood growth and cognitive development [1]. Diarrhoea disease majorly caused by *E. coli* through water, fresh fruits and faecal-oral contamination, was estimated to kill about 1.8 million people each year [2]. In 2010, there were 1,731 billion episodes of diarrhoea (36 million of which progressed to severe episodes) in children younger than 5 years and in 2011,700 000 episodes of diarrhoea led to death [3]. In developing countries, children under three years old experience an average of three episodes of diarrhoea every year. Each episode of diarrhoea deprives the child of the nutrition necessary for growth due to malnutrition, and malnourished children are predisposed to falling ill due to diarrhea [4].

Rotavirus and *Escherichia coli* (which is a normal commensal of the GIT) are

* Corresponding author. *E-mail*: susanmosebolatan@gmail.com *Tel*: +234 (0) 7039137090
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reported to be the two most common causes of diarrhoea in developing countries [5]. *E. coli* is one of the main causes of both nosocomial and community acquired infections in humans and is therefore an organism of clinical importance in the lives of children [6]. Antibiotic resistance is a global public health issue that is impacted by both human and non-human antimicrobial usage. The continuing emergence, development and spread of pathogenic organisms that are resistant to antibiotics are a cause of increasing concern to health care practice [4]. The use of antibiotics has been reported to be one of the factors contributing to the emergence of bacterial resistance [7,8]. *E. coli* may contribute to the dissemination of antibiotic resistant microorganisms between human and animal populations, and it may constitute the route by which resistance genes are introduced in environmental bacterial ecosystems [9,10].

A lot of work has been done on diarrhoea in the World, Africa and in Nigeria, particularly in the Southwestern part of Nigeria. There are few documented studies on *E. coli* in diarrhoeic stools of children from the Northern part of this country. This study investigated the prevalence and antibiotic susceptibility of *E. coli* from diarrhoeic stools of children below 5 years in Zaria, Nigeria.

EXPERIMENTAL

Sample collection and isolation of *E. coli.* Children (0-5 years) who presented with diarrhoea with or without gastroenteritis within July 2010 and March 2011 were recruited for the study. Stool samples were collected from diarrhoeic children below five years of age from two hospitals and a Primary Health care centre in Zaria after ethical consent to carry out the study was granted from the hospital authorities. The hospitals are: Hajia Gambo Sawaiba General hospital, Kofan-Gayan, Zaria city, Zaria; Kaduna State Primary Health Care Centre, Samaru; and Institute for Child Health, Ahmadu Bello University Teaching Hospital, Ban-Zazzau, Zaria.

Isolation, identification and biochemical test. Sample collection and isolation of *E. coli* were carried out using the method described by Cheesbrough [11]. The stool samples were collected into sterile specimen containers and transported to the laboratory at ambient temperature (15°C to 30°C). The stool samples were inoculated into sterile nutrient broth and incubated at 37°C overnight. Overnight nutrient broth stool cultures were then sub-cultured on MacConkey agar and Eosin Methylene Blue Agar plates. The plates were incubated at 37^{o}C for 18 hours for primary isolation. Lactose fermenting colonies from MacConkey and Eosin-Methylene Blue agar were subjected to microscopic examination to determine cell morphology and Gram stain reactions. Cultural characteristics, colonial morphology of the isolates and Gram staining reactions were determined as described Cheesbrough [11]. Gram-negative bacilli were biochemically identified and confirmed as *Escherichia coli* using non-citrate utilization, indole formation, methyl red production, Voges Proskauer test and fermentation of sugars.

Antibiotic sensitivity pattern. Antibiotic susceptibility of *Escherichia coli* isolates to nine (9) commonly prescribed antibiotics was determined according to Wolf [12], as modified by CLSI [13], Antibiotic sensitivity discs used in this study were: Amoxicillin (10µg), Ceftriazone (30µg), Ceftazidime (30µg), Gentamicin (30µg), Cefuroxime (30µg), Ciprofloxacin (10µg), Cotrimoxazole (25µg), Tetracycline (30µg) and Ampicillin (10µg). All the antibiotic discs were obtained from Oxoid Ltd, England. An overnight culture of each isolate was prepared in nutrient broth. Dry sterile plates of prepared Mueller Hinton's agar were flooded with standardized inoculums (10^6 cfu/ml) of 18

hours culture of *E. coli* isolate and left to dry for 15 minutes in properly disinfected incubator. Thereafter the various antibiotic sensitivity discs were aseptically placed on the dried Mueller Hinton agar plates and left for one hour before they were incubated at 37^0C for 18-24 hours. After incubation, the plates were examined for zones of inhibition, which were measured in millimeters. The result was interpreted using CLSI [14] interpretation chart.

RESULTS

One hundred and fourteen (114) samples of diarrhoeic stools were collected from patients in the three selected locations for the study. Ten samples were collected from the Institute of Child Health, Ban-zazzau, 43 samples were collected from Samaru Primary Health care centre and 61 samples were collected from the General Hospital, Kofan-Gaya. Seventy isolates were identified *as E.coli* with isolation rates of 70%, 74.4% and 50.8% respectively. Higher proportion of *E. coli* positive stools were observed in samples from the Primary Health Centre Samaru and Kofar Gaya General Hospital, compared with proportion from Institute of Child Health, Ban-zazzau. The total isolation rate of *E. coli* from all the stool samples in this study was 61.4% (70/114).

The result of the antibiotic susceptibility testing shows that Gentamicin and Ciprofloxacin gave the highest level of inhibition against the isolates with a percentage of 64.3% and 60.0% respectively. This was moderately followed by ceftriaxone, which gave about 41.4% susceptibility. On the other hand, cefuroxime, ceftazidime and sulphamethoxazole/trimethoprim showed very low inhibitory effects against the isolates with percentage susceptibility of 14.3%, 11.4% and 2.9%respectively. Amoxicillin, ampicillin and tetracycline had no inhibitory effect on the isolates.

The isolates were observed to display high level of resistance to the antibiotics used. All the isolates from the three study sites were resistant to amoxicillin, ampicillin and tetracycline. High level of resistance was also observed with cotrimoxazole, ceftazidime, ceftriaxone and cefuroxime while low level of resistance was seen for ciprofloxacin and gentamicin.

The findings in this study shows seventeen phenotypic patterns of multiple antibiotic resistance, and 69 (98.6%) isolates which were observed to be resistant to 3 or more antibiotics with multiple antibiotic resistance index of ≥ 0.3 which indicates that the isolates originate from an environment where antibiotics are frequently being used .

DISCUSSION

The percentage produce of *E.coli* in the stool samples in this study showed that there was a high prevalence of *E. coli* infection among children. This correlates with the reports of several other workers. For example, Akinyemi [15] reported an incidence of 45.6% of *E. coli* in children and adult patients with acute gastroenteritis in Lagos, Southwestern part of Nigeria while Ifeanyi [16] recorded an incidence rate of 62.5% of *E. coli* as the most frequently isolated bacteria (62.8%) in all the age groups studied in the Federal Capital Territory of Nigeria. They further reported that *E. coli* was significantly associated with diarrhoea at age of 13-24 months. Nwanze [17] in a study carried out at Federal Medical Centre, Owerri, Nigeria, reported that the incidence of diarrhoea was more common in males than females, and more prevalent in the age group of 0-5years.

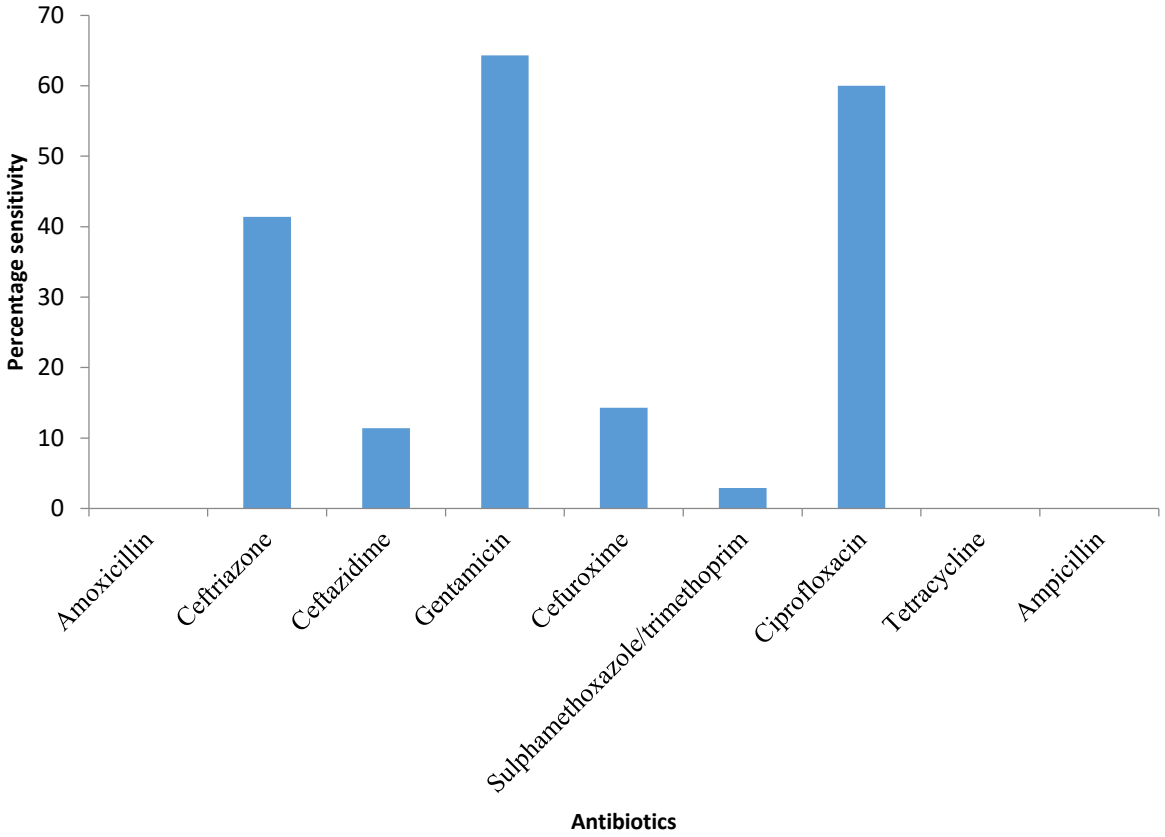

Figure 1. Antibiotic susceptibility profiles of *E. coli* isolates from diarrhoea patients

Table 1: Distribution and percentage of *E. coli* isolation from three study centres.

Hospital	No of samples collected	% with *E.coli* isolated
Kofan Gayan	61	50.8
Samaru	43	70.0
Ban-zazzau	10	74.4
Total	114	61.4

Table 2: Antibiotic resistance profile of *E. coli* isolates using zone of inhibition from the three hospitals

S/N	Antibiotics	Kofan Gaya n=31 (% resistance)	Ban-zazzau n=7 (% resistance)	Primary health n=32 (% resistance)
1	Amoxicillin	31 (100)	7 (100)	32 (100)
2	Ceftriazone	17 (54.84)	1 (14.29)	23 (71.88)
3	Ceftazidime	27 (87.10)	3 (42.86)	32 (100)
4	Gentamicin	11 (35.48)	2 (28.58)	12 (37.5)
5	Cefuroxime	29 (95.55)	4 (57.14)	27 (84.38)
6	Cotrimoxazole	31 (100)	5 (71.43)	32 (100)
7	Ciprofloxacin	16 (51.61)	1 (14.29)	11 (34.38)
8	Tetracycline	31 (100)	7 (100)	32 (100)
9	Ampicillin	31 (100)	7 (100)	32 (100)

Table 3: Profile of multiple antibiotic resistance of *E. coli* isolates.

Phenotypic resistance pattern	No of antibiotic resistance	No of isolates
Aml, Cro, Caz, Cn, Cxm, Sxt, Cip, Te, Amp	9	16
Aml, Cro, Caz, Cxm, Sxt, Cip, Te, Amp	8	6
Aml, Cro, Caz, Cn, Cxm, Sxt, Te, Amp	8	2
Aml, Caz, Cxm, Sxt, Cn, Te, Amp	7	1
Aml, Cro, Caz, Cxm, Sxt, Te, Amp	7	16
Aml, Caz, Cxm, Sxt, Cip, Te, Amp	7	2
Aml, Cn, Cxm, Sxt, Cro, Te, Amp	7	1
Aml, Cn, Sxt, Cip, Te, Amp	6	1
Aml, Caz, Cxm, Sxt, Te, Amp	6	14
Aml, Caz, Cn, Sxt, Te, Amp	6	3
Aml, Caz, Sxt, Cip, Te, Amp	6	1
Aml, Cxm, Sxt, Cip, Te, Amp	6	1
Aml, Cxm, Sxt, Te, Amp	5	1
Aml, Sxt, Cip, Te, Amp	5	1
Aml, Caz, Sxt, Te, Amp	5	1
Aml, Cn, Sxt, Te, Amp	5	1
Aml, Cxm, Te, Amp	4	1
Aml, Te, Amp	3	1

Aml=Amoxicillin; Cro = Ceftriaxone; Sxt = Sulfamethoxazole/Trimethoprim; Caz = Ceftazidine; Cxm = Cefuroxime; Te = Tetracycline; Cn = Gentamicin; Cip = Ciprofloxacin; mp = Ampicillin

Table 4: Multiple antibiotic resistance index of *E. coli* isolates.

MAR INDEX	NO OF ISOLATES	PERCENTAGE OF ISOLATES
0.3	1	1.4
0.5	1	1.4
0.7	34	48.6
0.8	18	25.7
1.0	16	22.9

The results of the antibiotic susceptibility in this study also correlates with the reports of previous studies that documented high resistance of *E. coli* isolates to ampicillin, amoxicillin, tetracycline and trimethoprim-sulfamethoxazole [17-20] Nwanze [17] found that *E.coli* isolates were highly resistant to tetracycline, cotrimoxazole, ampicillin and amoxicillin but sensitive to nitrofurantoin and ofloxacin. Oluremi [20] also reported high level of antibacterial activity for ciprofloxacin (71.4%), but moderate antibacterial activity for ofloxacin and low level of antibacterial activity for gentamicin, nalidixic acid, nitrofurantoin, co-trimoxazole, tetracycline, Augmentin® and amoxicillin. The prescription of broad-spectrum antibiotics, which are sometimes given in place of narrow spectrum antibiotics as a substitute for culture and sensitivity tests, with the consequent risk of selection of antibiotic-resistant mutants [21], could be a reason for the observation of low sensitivities of *E. coli* isolates in this study. This situation is usually made worse by patients not completing their course of medication, probably because of ignorance or poor financial level. In contrast to the above, Nkang [22] in a study in Calabar, Nigeria found that *E. coli* was susceptible to antibiotics like ciprofloxacin, gentamicin, tetracycline, sulphadoxine/ trimethoprim, amoxicillin and ampicillin among others with 90.9% susceptibilty percentage. Resistance of the *E. coli* isolates to multiple antibiotics was common in this study. The high level of beta-lactam resistant *E. coli* isolates may probably suggest frequent abuse of beta-lactam antibiotics in the study area. The observed moderate level of gentamicin activity in this

study may be due to the fact that it is a parenteral antibiotic while observed moderate level of ciprofloxacin activity could possibly be due to fact that quinolones are not used to treat children below 12 years. Nonetheless, decreasing antibacterial activity of fluoroquinolones has been reported in many countries and the emergence of resistance of bacterial isolates to fluoroquinolones is now a reported common phenomena. The observed level of resistance of fluoroquinolones in the study corroborates the earlier findings of Bolon [23] and Oteo [24], as reported earlier; the majority of the isolates were multidrug resistant. The high MARI of the isolates indicate the isolates originate from an environment where antibiotics are frequently being used. It is also possible that the resistance observed in the study is innate. If this current trend continues, the emergence of resistant *E. coli* strains to other useful antibiotics may probably follow and a grave situation may soon arise when there will be no antibiotics to treat life threatening bacterial infections caused by these resistant *E. coli* strains.

Conclusion. Findings from this study showed that *E.coli* is a major cause of diarrhoea in children under five years in the three study health institutions, and therefore an indicator of diarrhoea in the study environment. The multi-drug resistance of the majority of the isolates to the common antibiotics should be a matter of great concerns to the medical professionals and the general community.

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