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Dissemination of Multi-Drug Resistance in *Escherichia coli* Isolated from Apparently Healthy School Children in Ondo State, Nigeria

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Abstract

This study was conducted to identify and characterize the multi-drug resistant *Escherichia coli* isolated from urine and faecal samples collected from Ondo state, between December, 2012 and March, 2013. *Escherichia coli* isolates (n=206) were identified by conventional microbiology culture and were characterized using biochemical test. The *E. coli* isolates were subjected to eleven (11) most commonly used antimicrobial agents to determine their resistance. From the result, 100%, 100%, 98.1%, 97.1%, 94.7%, 94.7%, 83.5%, and 67.5%, 35.78%, 35.61% and 19.35% of the tested *E. coli* isolates from urine and faecal samples were found resistant respectively to ceftazidim, cefuroxime, augumentin, trimethoprime, tetracycline, cefixime, gentamicin, chloramphenicol, ciprofloxacin ofloxacin and nitrofurantoin. Multi-drug resistance (MDR) profile indicated that *E. coli* isolates were resistant to 7 out of 11 antibiotics tested, 6.79% of the *E. coli* isolates were resistant to all the eleven antibiotics tested while 0.97% were resistant to at least four antibiotics. However, multi-drug resistance index (MDRI) indicated that all isolates were resistant.

Introduction

Multidrug resistance observed in different microorganisms, is a condition enabling a disease-causing organism to resist distinct drugs or chemicals of a wide variety of structures and functions targeted at eradicating the organism. Multidrug resistant bacteria, thus, refers to those which are resistant to a vast range of antibiotics with structural independence (at least to three or more antibiotics) [1]. The patterns of resistance of microorganisms are different in various geographic areas [2]. Recognition of these patterns in different communities is vital to distinguish uncommon or new patterns. In order to study the molecular epidemiology of multidrug strains of *E. coli*, phenotypic and genotypic methods of characterization are best used to get accurate result. A current phenomenon of great concern among the medical communities in developing countries is the rising multidrug resistant organisms, and the challenges of curing the infections in children.

Escherichia coli, being an enteric Gram-negative bacillus, are very adaptive and easily recognized as non-invasive commensal. Most strains of *E. coli* live as commensals; many perhaps are opportunistic pathogens of human and even animals [3]. Eighty and twenty percent of girls and boys respectively stand the chance of infections including pylonephritis and cystitis at least once in their childhood [4]. A worrisome increase in antibiotic resistance among the *E. coli* isolates has been observed during the past few years [5]. Such escalated resistance is due to mechanisms of mutation and then resistance gene transfer by horizontal gene transfer (HGT) and spontaneous genetic variation [6-8]. Since a plasmid or transposon can carry several resistance indexes, resistance to several antimicrobial agents may be acquired simultaneously and results in multiple drug-resistant (MDR) organisms [9].

Among the enzymes involved in drug resistance exhibited by *E. coli* is the extended-spectrum β-lactamases (ESBLs). These are a group of enzymes which are able to hydrolyze theoxyimino-cephalosporins and monobactams, but not the cephamycins or carbapenems (imipenem, meropenem, doripenem and ertapenem) and cause resistance [10]. The ESBLs are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. The ESBLs are grouped into the class A Ambler which is SHV or TEM types that have evolved from parent enzymes (TEM-1,-2 and SHV-1) due to point mutations around the active site of the β-lactamases [10]. ESBLs are mostly located on large plasmids harbouring resistant genes to other antibiotics groups and therefore, exhibit multidrug-resistant (MDR) phenotypes associated with resistance to aminoglycosides and co-trimoxazole.

ESBL-producing bacteria are isolated with increasing frequency from patients in extended-care facilities [10,11]. Infections caused by ESBL-producing bacteria are often associated with increased morbidity, mortality and higer cost of healthcare [12,13]. Organisms that produce ESBLs are of clinically importance because of their antibiotics resistance abilities. A report from the Infectious Diseases Society of America (IDSA) in 2006 profiled Klebsiella spp. and *E. coli* producing ESBL as important organisms that are drug-resistant to which new therapies are urgently needed [14]. This study is therefore focused on multi-drug resistant pattern among *E. coli* isolated from primary pupils in Ondo state, Nigeria.

Materials and Methods

Sampling and bacterial identification

A total of 807 urine and faecal samples of apparently healthy children were collected from five major towns in Ondo state, Nigeria between December, 2012 and March, 2013. The towns where sampling was carried out include Akure, Owo, Ondo, Okitipupa and Ikare-

Akoko. Samples were collected inside well-covered and sterile universal bottles, kept under cold condition, and then transferred to the laboratory within 2 h of collection for culturing. Questionnaire containing information about age (2 years-15 years), gender, and health history was filled for each pupil.

Isolation and characterization of *E. coli*

Urine samples collected were streaked directly on Eosin methylene blue (EMB) agar plate using a sterile inoculating loop while faecal samples were first diluted with 5 ml of sterile distilled water, then a loop-full each was streaked on EMB agar plate. All inoculated plates were incubated at 37°C for 24 h. Colonies with greenish-black metallic sheen on EMB after incubation were suspected to be *E. coli* and were thereafter Gram-stained and viewed under an oil-immersion microscope. Also, all the suspected *E. coli* isolates were sub-cultured on MacConckey agar plates and incubated at 37°C for 24 h. The final pure colonies were characterized by biochemical tests such as citrate utilization test, urease test, H2S production test, indole test, methyl red test, Voges-Proskauer test and sugar fermentation test [15].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the isolates was performed using the kirby-bauer disc diffusion method and mueller-hinton agar according to clinical and laboratory standards institute (CLSI, 2012) instructions. The eleven antibiotics used include augmentin (30 μg/disc), nitrofurantoin (300 μg/disc), ciprofloxacin (5 μg/disc), ceftazidime (30 μg/disc), cefuroxime (30 μg/disc), gentamicin (10 μg/disc), cefixime (30 μg/disc), ofloxacin (5 μg/disc), tetracycline (30 μg/disc), chloramphenicol (30 μg/disc), and trimethoprim (5 μg/disg). The results were interpreted in accordance with CLSI criteria. Resistance to more than four antibiotics was designated as multidrug resistance (MDR). MDR index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was exposed [16]. Isolates with MDRI values of more than 0.2% or 20% were considered highly resistant.

Statistical analysis

Data obtained are presented as mean ± SE (standard error). The significance of difference between different treatment groups was tested using one-way analysis of variation (ANOVA) with the aid of statistical package for social sciences (SPSS, IL) version 17 at P ≥ 0.05.

Results

Isolation of antibiotic-resistant *E. coli*

Selective processing of 807 fecal and urine samples yielded 206 unique *E. coli* isolates, which constituted the study population (Table 1). Of the 206 isolates, 125 (60.7%) and 81 (39.3%) were from faecal and urine samples respectively. Of the *E. coli* isolates, 79 (38.35%), 9 (4.37%), 31 (15.05%) 53 (25.73%) and 34 (16.5%) were from Akure, Ondo, Owo, Okitipupa and Ikare-Akoko respectively.

The results of the antibiotic sensitivity test are presented in Figures 1-4 below. Overall, 204 isolates were classified as resistant on the basis of reduced susceptibility to at least five of the eleven antibiotics used. The remaining two isolates were susceptible to at least seven of these antibiotics. The resistant isolates were distributed as follows: Nitrofurantoin, 40 (19.42%); Ciprofloxacin, 83 (40.29%); Gentamicin, 172 (83.49%); Augumentin, 202 (98.06%); Ceftazidime, 0 (100%); Cefuroxime, 0 (100%); Cefixime 195 (94.66%); Ofloxacin 93 (45.15%); Tetracycline, 195 (94.66%); Chloramphenicol 139 (67.48%) and Trimethoprim 200 (97.09%).

Location	Sex	Sample Type	Isolated *E. coli*	Number of Sample	Prevalence (%)
Akure	Male	urine	5	60	8.3
		stool	17	37	46
	Female	urine	31	71	43.7
		stool	26	37	70.3
Ondo	Male	urine	3	30	9.7
		stool	1	5	20
	Female	urine	3	32	9.4
		stool	2	10	20
Okitipupa	Male	urine	2	82	2.4
		stool	15	28	53.6
	Female	urine	15	66	22.7
		stool	21	41	51.2
Owo	Male	urine	1	82	1.2
		stool	17	28	60.7

	Female	urine	5	79	6.3
		stool	8	19	42.1
Ikare-Akoko	Male	urine	1	38	2.6
		stool	5	9	55.6
	Female	urine	15	36	41.7
		stool	13	17	76.5

Table 1: *E. coli* isolates by location, sample and prevalence.
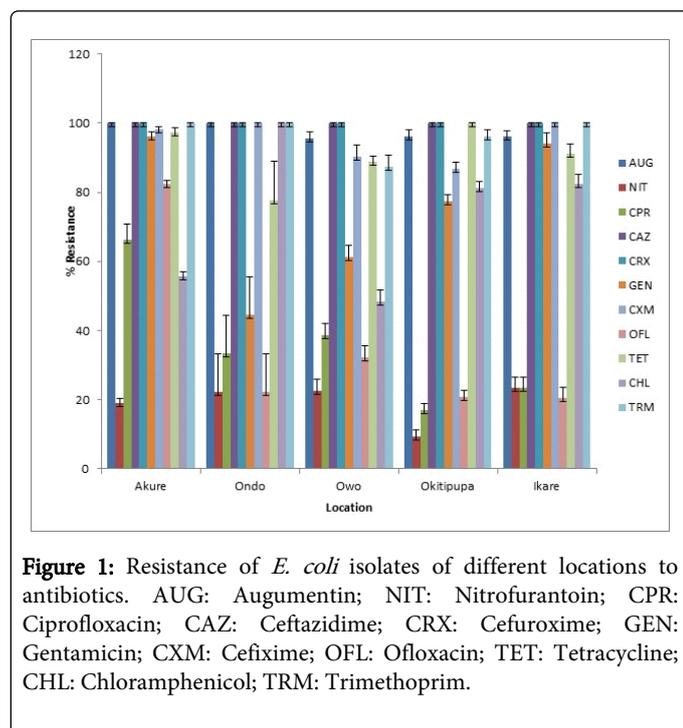

Figure 1: Resistance of *E. coli* isolates of different locations to antibiotics. AUG: Augumentin; NIT: Nitrofurantoin; CPR: Ciprofloxacin; CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CXM: Cefixime; OFL: Ofloxacin; TET: Tetracycline; CHL: Chloramphenicol; TRM: Trimethoprim.

Multidrug resistance (MDR) and *E. coli* isolates resistance profile

The result of multidrug resistant *E. coli* isolates is presented in Tables 2 and 3. Multidrug resistance is defined as resistance by a microorganism to three or more different classes of the antibiotics tested. From the result, fourteen (6.79%) of the isolates were resistant to all the eleven antibiotics tested, 39 (17.48%) to ten, 43 (20.87%) to nine, 56 (27.18%) to eight, 36 (14.56%) to seven, 12 (5.83%) to six, 4 (1.94%) to five and 2 (0.97%) to four antibiotics respectively. Diversities in MDR patterns were observed among the isolates. Most isolates demonstrated multi-drug resistance index (MDRI) above 0.2% or 20%, that is, they were resistant to at least four of the antibiotics used: AUG, NIT, CPR, CAZ, CRX, GEN, CXM, OFL, TET, CHL and TRM.

Discussion

This study focuses on multi-drug resistant pattern among strains of *E. coli* isolated from primary pupils in Ondo state, Nigeria. The variability of drug resistance as observed in this work from the various locations is as well documented in literatures. The rate of *E. coli* resistance to drugs had been shown to vary from one geographical location to the other. The data of surveillance from intensive care units (ICUs) of hospitals in North America and in some European countries revealed that, *E. coli* is either the most common or the second most common isolates from clinical specimens [17].
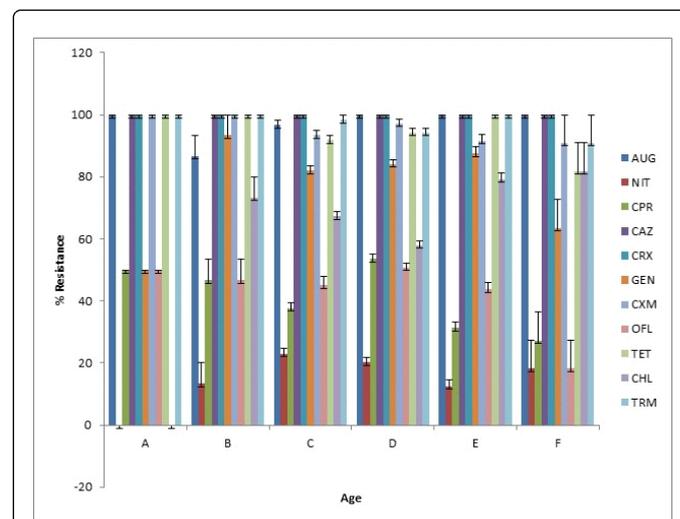

Figure 2: Resistance of *E. coli* isolates from different age groups to antibiotics. AUG: Augumentin; NIT: Nitrofurantoin; CPR: Ciprofloxacin; CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CXM: Cefixime; OFL: Ofloxacin; TET: Tetracycline; CHL: Chloramphenicol; TRM: Trimethoprim. A=2-3 yrs, B=4-5yrs, C=6-7, D=8-9, E=10-12, F=13-15.

The recent global report on surveillance of antimicrobial resistance showed that, the emerging resistance issues in *E. coli* are represented by resistance to fluoroquinolones (e.g. ciprofloxacin and ofloxacin) and to extended-spectrum cephalosporins (e.g. ceforoxime, ceftazidime and cefixime) [5], which are the major antimicrobial chemotherapy of *E. coli* infections, and this has been a growing concern among clinical practitioners. Although resistance to these agents can show remarkable geographic variability, resistance rates are quite higher in several European countries, and increasing trends have been observed in most continents.
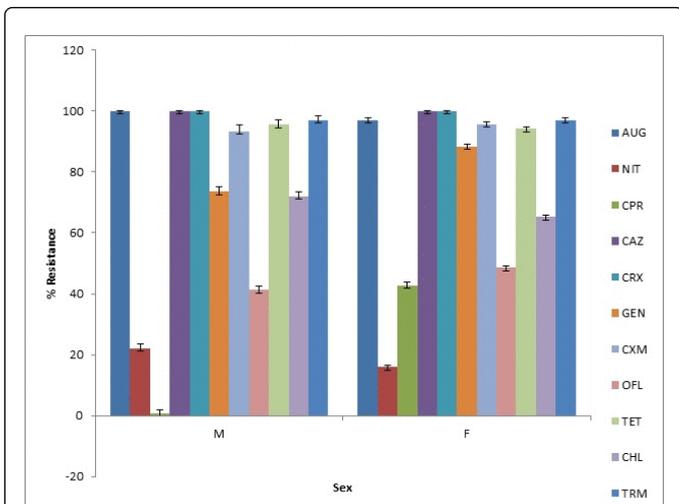
Figure 3: Resistance of *E. coli* isolates from male and female pupils to antibiotics. AUG: Augumentin; NIT: Nitrofurantoin; CPR: Ciprofloxacin; CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CXM: Cefixime; OFL: Ofloxacin; TET: Tetracycline; CHL: Chloramphenicol; TRM: Trimethoprim.
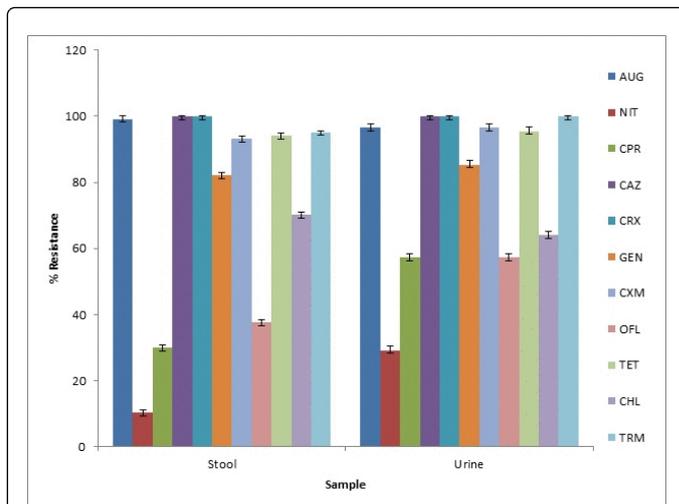

Figure 4: Resistance of *E. coli* isolates from stool and urine samples to antibiotics. AUG: Augumentin; NIT: Nitrofurantoin; CPR; Ciprofloxacin; CAZ; Ceftazidime; CRX; Cefuroxime; GEN; Gentamicin; CXM: Cefixime; OFL: Ofloxacin; TET: Tetracycline; CHL: Chloramphenicol; TRM: Trimethoprim.

Number of classes of Antibiotics	Occurrence (n=206)					Total MDR (%)
	Akure (n=79)	Ondo (n=9)	Owo (n=31)	Okitipupa (n=53)	Ikare (n=34)	
11	9 (11.39)*	0	2 (6.45)	2 (3.77)	1 (2.94)	14 (6.79)
10	27 (34.18)	1 (11.11)	4 (12.90)	4 (7.55)	3 (8.82)	39 (17.48)
9	24 (30.38)	0	4 (12.90)	5 (9.43)	10 (29.41)	43 (20.87)
8	12 (15.19)	5 (55.56)	4 (12.90)	22 (41.51)	13 (38.24)	56 (27.18)
7	7 (8.86)	1 (11.11)	11 (35.48)	11 (20.76)	6 (17.65)	36 (14.56)
6	2 (2.53)	1 (11.11)	2 (6.45)	6 (11.32)	1 (2.94)	12 (5.83)
5	0	0	2 (6.45)	2 (3.77)	0	4 (1.94)
4	0	0	2 (6.45)	0	0	2 (0.97)
MDR: Multidrug Resistance; (%)* percentage of MDR on location basis						

Table 2: Multidrug resistance (MDR) *E. coli* isolated from urine and faecal samples.

Number of Classes of Antibiotics	Sample Code	Resistance Profile	Multi-Drug Resistance Index (MDRI%)
11	43	AUG, NIT, CPR, CAZ, CRX, GEN, CXM, OFL, TET, CHL, TRM	100
10	63	AUG, CPR, CAZ, CRX, GEN, CXM, OFL, TET, CHL, TRM	90.9
9	56	AUG, CPR, CAZ, CRX, GEN, CXM, OFL, TET, TRM	81.82
8	73	AUG, CAZ, CRX, CXM, OFL, TET, CHL, TRM	72.73
7	07	AUG, CAZ, CRX, CXM, OFL, TET, TRM	63.64
6	553	AUG, CA, CRX, CXM, TET TRM	54.55

5	380	AUG, CA, CRX, CXM, TRM	45.46
Each sample code represents a group of isolate per resistance profile			

Table 3: *E. coli* Resistance profile and multi-drug resistance index (MDRI).

Antibiotic resistance can be associated strongly with spontaneously occurring genetic variation, the driving force of biological evolution. Already available data on spontaneous genetic variation and novel experimental results, in particular from microbial genetics, revealed a multitude of specific molecular mechanisms that contribute to the overall spontaneous genetic variation. These identified molecular mechanisms of genetic variation are categorized into three natural strategies:

- mutational changes of nucleotide sequences, such as a nucleotide substitution, the insertion or the deletion of one or a few adjacent nucleotides during DNA replication;
- mutational changes which occur as a result of duplication, deletion, inversion or translocation of a DNA segment carried in the genome;
- the acquisition new gene by horizontal gene transfer of a segment of foreign DNA where transformation, conjugation and viral infection contribution play a key role.

It is clear that the maintenance of any novel genetic variant is depending on Darwinian natural selection [6-8].

From the foregoing, the implication of location in the variability of drug resistance cannot be overemphasized. High level of resistance to antibiotics observed in this work was in contrast to research conducted by Shohreh et al. [18] where most of these antibiotics maintained lower resistance of an average of 20%. However, Hassan et al. [19] revealed that *E. coli* had a high level of resistance to these antibiotics too. This suggests that, there might have been a transfer or acquisition of antibiotic resistant gene among the *E. coli* isolates within the past few years.

Besides, since most patients, particularly in developing nations, cannot afford the medical visit and laboratory tests, they repeat empirical therapies which are not effective enough. Studies have also shown that, even in the USA and other developed countries, many antibiotics are prescribed and consumed unwisely, which results in the emerging resistance [20].

From the result, despite the differences in sex-specific resistance, the magnitude of these differences was generally less than 8% and thus may not represent clinically (10%) meaningful differences [21]. Most research works have documented that *E. coli* infection in males tends to resist antibiotics than females, which are actually in a relationship with the present study with the exception of augmetin, cefixime and ciprofloxacin, where higher resistance was observed in females. However, pupils from whom samples were collected in this study were of lower age than those in the literatures (18 years-64 years) [21,22]. Additionally, *E. coli* resistance to antibiotics in males has previously been associated with involvement in prostate occurrence in roughly 90% of cases [23].

It was clear that, *E. coli* isolated from the urine was more resistant to antibiotics than faecal samples, which is consistent with other works. The observed differences of *E. coli* resistance between urine and faecal samples may be associated with an analysis on the relationship between antibiotics use and resistance. Costelloe et al. [24] found weak, but detectable associations after 12 months of exposure between antibiotic use and resistance. The result was that the residual effect of intermittent antibiotic administration is likely to be an important cause of the high endemic levels of antimicrobial resistance in a community [24].

E. coli isolates were also analyzed for multidrug resistance (MDR) and multi-drug resistance index (MDRI). The most resistant *E. coli* isolate was from male urine and faeces; however 6.79% of the *E. coli* was resistant to all the eleven antibiotics tested. In addition, all *E. coli* isolates from urine and faeces displayed resistance to at least four (0.97%) antibiotics. Multidrug resistance was observed in all sources, in which large percentage of male and of female urine and faecal isolates showed this characteristic. Moreover, some isolates from male and female were resistant to 10 out of 11 antibiotics. This observation is similar to the work carried out by Oluduro et al. [25]. According to the work, high prevalence of MDR strains was obtained. Similarly, most *E. coli* isolates indicated multi-drug resistance index above 0.2% or 20%, which means that, they were actually very resistant to the various antibiotics. This occurrence might be associated with the fact that very large population of the *E. coli* isolates might have been exposed to several antibiotics or antibiotics with similar targets/modes of action.

Conclusion

In conclusion, there is a high level of antibiotic resistance observed in the *E. coli* isolates. This antimicrobial resistance, most significantly to the frontline antibiotics such as ciprofloxacin, ofloxacin, tetracycline, chloramphenicol and others, is of major concern.

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