Antibiotics Sensitivity Pattern of *Escherichia coli* isolated from Children of School Age in Ondo State, Nigeria

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Abstract: Two hundred and six (206) *E. coli* strains were isolated from school age children from five major towns in Ondo state and all isolates subjected to antimicrobial susceptibility testing. Most isolates were resistant to commonly used antibiotics such as Augmentin, Ceftazidime, Cefuroxime, Gentamicin, Cefixime, Tetracycline, Chloramphenicol, and Trimethoprim, but showed >50 percent susceptibility to Nitrofurantoin, Ofloxacin and Ciprofloxacin. 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 is shown here to vary from one location to the other within Ondo state in Nigeria.

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1. Introduction

Escherichia coli is an enteric Gram-negative bacillus, which is well known as non-invasive commensal. Even though most strains of *E. coli* live as commensals, some perhaps are opportunistic pathogens of human and animals (Belanger *et al*., 2011). Certain *E. coli* isolates have been found to produce a toxin, which was initially called verotoxin because of its distinct effect on vero cells. Enterohaemorrhagic *E. coli* (EHEC) is the main group of verotoxigenic strains which has emerged as the leading cause of haemorrhagic colitis and haemolytic uremic syndrome (HUS) in humans.

EHEC can be transmitted primarily through the ingestion of faecal contaminated foods, particularly undercooked beef. However, a large number of outbreaks of EHEC have also been associated with consumption of contaminated drinking water or contact with recreational water (Bonyadian *et al.,* 2010). Infection with Shiga toxin (Stx)-producing *Escherichia coli* (STEC) can result in a spectrum of cases, ranging from asymptomatic carriage to uncomplicated diarrhea, haemolytic uremic syndrome (HUS), bloody diarrhea, hemolytic anemia, thrombocytopenia, and acute renal failure. High mortality and morbidity rates have been reported for HUS, which can occur from infection with STEC strains. The pathogenesis of STEC has been reported to be related to several bacterial virulence factors. Some of these virulence factors include the intimin (eae) protein, two shiga toxins called stx1 and stx2, and the plasmid-encoded protein known as hemolysin (ehly) (Hassan *et al.*, 2013).

Microbial infections, if not adequately or properly managed, can lead to chronic ill-health, poor quality of life or even death. Thus there is a need for the use of suitable anti-microbial agents to control and contain the effects of infections. The use of appropriate antimicrobial agents can prevent the sequelae of infections. To be appropriate, the choice of an antimicrobial agent should be contingent or based on the type of pathogenic microorganism flora common in an area and known sensitivity pattern in that region. This is as a result of the phenomenon of resistant to antibiotics which have been found useful as an aid to the right choice of antibiotics (Omoigberale *et al*., 2005, Olatunji *et al*., 2009, Mordi *et al.*, 2010).

The present study was designed to determine the susceptibility pattern of characterized *E.coli* strains isolated from children in five major towns of Ondo state, to thirteen commonly used antibiotics.

2.0 Materials and Methods

2.1 Collection of Samples, Isolation and characterization of *E. coli*

Urine and faecal samples were collected from School pupils in five major towns of Ondo state. The towns include Akure, Owo, Ondo, Okitipupa and Ikare-Akoko. Urine samples were streaked directly on Eosin methylene blue (EMB) plate using a sterile inoculating loop, while faecal samples were diluted with 5ml of sterile distilled water, then a loop-full each was streaked on EMB agar plate. Inoculated plates were incubated at 37^{o}C for 24h. After incubation, *E. coli* grown on EMB agar plates appeared as greenish-black metallic sheen. The suspected *E. coli* colonies were sub-cultured on MacConkey agar plates and incubated at 37^{o}C for 24h. The final pure colonies were gram-stained and viewed

under the oil-immersion microscope and then characterized biochemically as described by Cheesbrough (2006).

2.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the isolates was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar according to Clinical and Laboratory Standards Institute, CLSI (2010). Poured plates were inoculated with standardized inoculum before antibiotics discs were incorporated onto the agar. Augmentin (30µg/disc), Nitrofurantoin (300µg/disc), Ciprofloxacin (5µg/disc), Ceftazidime (30µg/disc), Cefuroxime (30µg/disc), Gentamicin (10µg/disc), Cefixime (30µg/disc), Ofloxacin (5µg/disc), Tetracycline (30µg/disc), Chloramphenicol (30µg/disc), and Trimethoprim (5µg/disc) were tested. The plates were incubated at 37^{o}C for 24h, after which the diameter of inhibition zone was measured in mm.

2.3 Statistical analysis

The data obtained are presented as mean ± SE (standard error). The significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) using SPSS version 17 software at P≤0.05.

3.0 Results

3.1 Antimicrobial susceptibility testing on *E. coli*

The antibiotic sensitivity test of *E. coli* isolated from urine and stool samples of children across the various locations revealed that, the rate of drug-resistance varies from one location to the other. However, *E. coli* isolates collected from Akure, Ondo, Okitipupa, Owo and Ikare regions showed that only Nitrofurantoin remained active at >75% against the vast majority of isolates across locations, while Ofloxacin and Ciprofloxacin maintained > 65% and 60% respectively, except at Akure where less than 20% susceptibility was recorded. Meanwhile, *E. coli* susceptibility to the remaining antibiotics was observed to be very low (Tables 1-3).

Table 1: Percentage antibiotics resistance of *E. coli* isolates in different locations

Antibiotics	Akure	% Ondo	Antibiotics Owo	Resistance Okitipupa	Ikare
AUG	100.0±0^{a}	100.0±0^{a}	95.7±1.86^{a}	96.23±1.79^{a}	96.08±1.7^{a}
NIT	19.0±1.7^{e}	22.22±11.01^{e}	22.58±3.03^{e}	9.43±1.90^{e}	23.53±2.94^{e}
CPR	66.35±4.59^{d}	33.33±11.11^{d}	38.71±3.23^{d}	16.98±1.95^{d}	23.53±2.84^{d}
CAZ	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}
CRX	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}
GEN	96.2±1.30^{c}	44.44±11.09^{c}	61.29±3.13^{c}	77.36±2.09^{c}	94.12±3.01^{c}
CXM	98.31±0.70^{ab}	100.0±0^{ab}	90.32±2.88^{ab}	86.79±2.11^{ab}	100.0±0^{ab}
OFL	82.26±1.25^{d}	22.22±10.89^{d}	32.27±2.93^{d}	20.75±2.25^{d}	20.59±3.07^{d}
TET	97.46±1.05^{b}	77.78±11.90^{b}	88.76±1.62^{b}	100.0±0^{b}	91.18±3.12^{b}
CHL	55.68±1.08^{c}	100.0±0^{c}	48.39±3.19^{c}	81.13±1.69^{c}	82.35±2.68^{c}
TRM	100.0±0^{ab}	100.0±0^{ab}	87.28±3.5^{ab}	96.23±1.82^{ab}	100.0±0^{ab}

Each value is a mean of 3 replicates. Values in a column with the same superscript(s) are not significantly different at p≤ 0.05.
AUG= Augmentin; NIT= Nitrofurantoin; CPR= Ciprofloxacin; CAZ= Ceftazidime; CRX=Cefuroxime; GEN= Gentamicin; CXM= Cefixime; OFL= Ofloxacin; TET= Tetracycline; CHL= Chloramphenicol; TRM= Trimethoprim.

Table 2: Percentage antibiotics resistance of *E. coli* isolates in relation to age

Antibiotics	2-3yrs	4-5yrs	% 6-7yrs	Antibiotic 8-9yrs	Resistance 10-12yrs	13-15yrs
AUG	100.0±0^{a}	86.67±5.77^{a}	96.72±1.44^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}
NIT	00^{e}	13.33±6.07^{e}	22.95±1.51^{e}	20.29±1.40^{e}	12.5±2.08^{e}	18.18±8.09^{e}
CPR	50.0±0^{d}	46.67±6.23^{d}	37.7±1.54^{d}	53.62±1.50^{d}	31.25±1.89^{d}	27.27±1.79^{d}
CAZ	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}
CRX	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}
GEN	50.0±0^{b}	93.33±6.17^{b}	81.97±1.44^{b}	84.06±1.05^{b}	87.50±2.08^{b}	63.54±9.19^{b}
CXM	100.0±0^{a}	100.0±0^{a}	93.44±1.23^{a}	97.10±1.23^{a}	91.67±2.19^{a}	90.91±8.88^{a}
OFL	50.0±0^{d}	46.67±5.67^{d}	45.14±2.86^{d}	50.73±0.98^{d}	43.75±1.91^{d}	18.18±9.09^{d}
TET	100.0±0^{a}	100.0±0^{a}	91.80±1.67^{a}	94.2±1.08^{a}	100.0±0^{a}	81.82±7.97^{a}
CHL	00^{c}	73.33±5.97^{c}	67.21±1.77^{c}	57.97±1.65^{c}	79.17±2.09^{c}	81.82±9.01^{c}
TRM	100.0±0^{a}	100.0±0^{a}	98.36±1.04^{a}	94.2±1.45^{a}	100.0±0^{a}	90.9±9.10^{a}

Each value is a mean of 3 replicates. Values in a column with the same superscript(s) are not significantly different at p≤ 0.05.
AUG= Augmentin; NIT= Nitrofurantoin; CPR= Ciprofloxacin; CAZ= Ceftazidime; CRX= Cefuroxime; GEN= Gentamicin; CXM= Cefixime; OFL= Ofloxacin; TET= Tetracycline; CHL= Chloramphenicol; TRM= Trimethoprim.

Table 3: Percentage antibiotics resistance pattern of *E. coli* isolates in relation to sample and sex

Antibiotics	% Antibiotic Resistance			
	Stool	Urine	Male	Female
AUG	99.15±1.06^{ab}	96.63±1.02^{ab}	100.0±0^{ab}	97.1±1.73^{ab}
NIT	10.26±1.17^{h}	29.21±1.10^{h}	22.06±1.47^{h}	15.94±1.45^{h}
CPR	29.92±0.86^{g}	57.3±1.13^{g}	0.73±1.48^{g}	42.75±0.73^{g}
CAZ	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}
CRX	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}
GEN	82.06±0.85^{d}	85.39±0.93^{d}	73.53±1.39^{d}	88.41±1.66^{d}
CXM	93.16±0.95^{c}	96.63±1.11^{c}	93.41±1.97^{c}	95.65±1.03^{c}
OFL	37.61±1.09^{f}	57.30±1.18^{f}	41.18±1.07^{f}	48.55±0.53^{f}
TET	94.02±1.00^{c}	95.51±0.89^{c}	95.59±1.17^{c}	94.2±1.23^{c}
CHL	70.09±0.89^{e}	64.03±0.82^{e}	72.06±1.28^{e}	65.22±1.91^{e}
TRM	95.21±0.46^{b}	100.0±0^{b}	97.06±1.65^{bc}	97.1±1.33^{bc}

Each value is a mean of 3 replicates. Values in a column with the same superscript(s) are not significantly different at $p \leq 0.05$.
AUG= Augmentin; NIT= Nitrofurantoin; CPR=Ciprofloxacin; CAZ= Ceftazidime; CRX= Cefuroxime; GEN= Gentamicin; CXM= Cefixime; OFL= Ofloxacin; TET= Tetracycline; CHL= Chloramphenicol; TRM= Trimethoprim

4.0 Discussion

The variability of drug resistance as observed in this work from the various locations has been well documented in literatures. The rate of *E. coli* resistance to drugs has 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 (Jones *et al.*, 2004). Also, it was observed that, *E. coli* isolated from individuals suffering from dysentery were found to be resistant to Nitrofurantoin which happens to be considered as a frontline antibiotic (Hassan *et al*., 2013). From the foregoing, the implication of location in the variability of drug resistance cannot be overemphasized. High level of resistance to drugs observed in this work was in contrast to research conducted by Shohreh *et al*., (2011) where most of these antibiotics maintained lower resistance of an average of 20%. But, Hassan *et al.*, (2013) showed that, there was high level of *E. coli* resistance to these antibiotics too. This suggests that, there might have been sporadic development of antibiotic resistant gene characters within the past few years. Since most UTI 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 USA and other developed country many antibiotics are prescribed and consumed unwisely, which results in the emerging resistance.

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 (McGregor *et al*., 2013). Although most research works have documented that *E. coli* infection in males tend to resist antibiotics than females, which are actually in relationship with the work under consideration with exception of few drugs, where they either have same value or females took slight lead. However, pupils from whom samples were collected in this study were of lower age than those in the literatures (18-64years) (McGregor *et al*., 2013, Linhares *et al*., 2013). Additionally, *E. coli* resistance to antibiotics in males has previously been associated with involvement in prostate occurrence in roughly 90% of cases (Ulleryd, 2003).

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*., (2010) 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 (Costelloe *et al.*, 2010).

In conclusion, there is a high level of antibiotic resistance observed in the *E. coli* isolates. This antimicrobial resistance, most importantly to the frontline antibiotics such as Ciprofloxacin, Ofloxacin, Tetracycline, Chloramphenicol and others, is of major concern. There is therefore, need for concerted efforts between Clinicians and Public health workers in educating the people from this state on the menace of indiscriminate use of antibiotics, especially when not prescribed by Physicians. Government, through appropriate agencies should draw out drug sales and use policies/legislatures that will put to check the sales of antibiotics to people without a supporting document for prescription by qualified personnel(s).

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