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Antimicrobial Resistance Profile of *Escherichia coli* Isolates from Different Clinical Samples in Abeokuta, Ogun State

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Authors' contributions

This work was carried out in collaboration between both authors. Author SLO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IAA managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The alarming increase of antibiotic resistance of *Escherichia coli* has posed a great challenge in the public health sector. Thus, this microorganism is a leading cause of different human infections and it can be found in various environments. The aim of this study is to investigate the antimicrobial susceptibility patterns and the multiple antimicrobial resistance profile of *Escherichia coli* isolates obtained from some hospitals in Abeokuta, Ogun State, Nigeria. Isolates of *E. coli* were obtained from different clinical samples and were re-identified morphologically and biochemically. *E. coli* was isolated from 30% out of a total of 70 clinical samples analyzed for isolation and identification. The isolation rate of *E. coli* was highest in urine samples 10(47.6%) when compared to other clinical samples. There was significant increase in the resistance rate of *E. coli* to tetracycline (14.3%), ceftazidime (14.2%), and ampicillin (14.2%).Also, an increased sensitivity rate to augmentin (71.4%), ofloxacin (66.7%), cefuroxime (66.7%), ciprofloxacin (61.9%) and ceftazidime (61.9%) were observed. Furthermore, the overall multiple drug resistance rates

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obtained was 14(66.7%) and it was established that, multiple antimicrobial resistance of the *E. coli* isolates was plasmid mediated. *E. coli* isolates exhibited high resistance rate to multiple antimicrobial agents, however, its sensitivity to augmentin, ofloxacin, cefuroxime, ciprofloxacin and ceftazidime showed that these antimicrobials are still effective against *E. coli* infections in the study area.

Keywords: Antimicrobial resistance; Escherichia coli; kirby bauer disk diffusion.

1. INTRODUCTION

The prevalence of resistant microorganisms has rapidly increased particularly in the last four decades, with the greatest impact in sub-Saharan Africa [1]. Bacteria have developed mechanisms to resist, evade or remain resistant to the actions of all classes of antibiotics [2] The problem of antimicrobial resistance (AMR) has grown over the years to become a threat that endangers the existence of the human race [3]. Several studies revealed that different species of bacteria from diverse sources (human, animal, environmental) are able to exchange and shuffle genes, including those that exhibit antimicrobial resistance [4]. Bacterial infections caused by gram negative bacterial strains are on the increase in the last fifty years, especially those that are caused by Enterobacteriaceae. *Escherichia coli* (*E. coli)* causes about 80% of community-acquired infections while some strains show resistance due to frequent inappropriate use of antibiotics [5].

Escherichia coli is a common inhabitant of the human and animal gut, but can also be found in some other environments such as water, soil and vegetation. It is the leading pathogen causing urinary tract infections and it is among the most common pathogens causing blood stream infections, wounds, otitis media and other complications in humans. *E. coli* is also the most common cause of food and water-borne human diarrhoea worldwide and it is causing many deaths in children under the age of five years in many developing countries [6].

Increased antibiotic resistance in pathogens leads to an increased rate of mortality and morbidity, enhanced transmission and increased associated health care costs. The emergence of antimicrobial resistance is not only limited to the older and more frequently used classes of drugs but to the newer and more expensive drugs such as carbapenem as well [7].

Reported rates of multi-drug resistance (MDR) in microorganisms have been on the increase and the infections, caused by these microorganisms are no longer limited to hospital or clinical environments [8]. Consequently, the increased prevalence of MDR bacteria in the environment and the potential impact on human and animal health is of world-wide concern [9]. This study therefore examines antimicrobial resistance profile of *Escherichia coli* isolates, from different clinical samples.

2. MATERIALS AND METHODS

2.1 Study Site

The study was carried out at State Hospital, Abeokuta, Sacred Heart Hospital, and Federal Medical Centre, Abeokuta**,** in south west Nigeria. These are the three main hospitals in Abeokuta, the state capital of Ogun state.

2.2 Sample Collection

A total of 70 clinical isolates, isolated from urine, pus, sputum, eye, ear and wound swabs (Urine 15, sputum 10, wound discharge 20, ear discharge 15, eye discharge 10), Were collected at selected hospitals in Abeokuta (Sacred Heart, State Hospital; and Federal Medical Centre), from January to April,2018.

2.3 Isolation and Identification of *E. coli*

Urine samples were plated on cysteine lactose electrolyte deficient medium (CLED), MacConkey agar and Blood agar (Oxoid, Basingstoke, UK) using calibrated wire loops after which it was incubated at 37°C for 24 hours. Samples from discharging ears, eye swab and pus from wound were collected using sterile cotton swabs. Specimens were inoculated onto 5% Sheep's blood agar, chocolate agar, and MacConkey agar plates (Oxoid Ltd, Basingstoke, UK). The plates were incubated at 37°C and examined after 24 and 48 hours.

2.4 Characterization of *E. coli* Isolates

Isolates collected from various hospitals in Abeokuta were re- identified morphologically and biochemically using gram staining, spore

staining, capsule staining, motility, indole, citrate, urease, oxidase, catalase, coagulase, methyl-red, Voges – Proskauer and sugar fermentation test. The results were interpreted according to Bergy Manual of Determinative Bacteriology (1984).

2.5 Antibiotic Sensitivity Test by Disc Diffusion Method

The antibiotic sensitivity test was performed as described by Kirby Bauer to determine the antibiotic profile of the isolates. Mueller Hinton agar was purchased and agar plates were prepared according to manufacturer's instructions. The 0.5 McFarland standard broth suspension of *E. coli* were flooded on it, after which a multidisc containing different antibiotic at different concentration (in microgram) were carefully placed. The plates were incubated at 37°C for 24hrs, and the diameter zones of inhibition of the drugs against the organism were read and recorded [10].

The isolates were tested against tetracycline (30µg); cefuroxime (30µg); gentamycin (10µg); trimethoprim (5µg) + sulphamethaole (25µg); ofloxacin (10µg); ceftazidime (30µg); ampicillin (10µg); augmentin (20µg) and ciprofloxacin (10µg).

2.6 Plasmid Profiling

The extracted plasmid DNA molecular weight was executed using gel electrophoresis. The molecular weight of plasmids from *E. coli* isolates was determined by comparing with molecular weight DNA marker (Lambda DNA/Hind *III* digest and 1 kb DNA ladder) and images of gels were captured on DigiDoc-It Imaging System.

Data analysis was achieved using SPSS version 20.0, andthe statistical tool used wasChi square (χ^2) at p-value < 0.05.

3. RESULTS

A total of 70 clinical isolates comprising urine, pus, sputum, eye, ear and wound swabs of patients were analyzed for isolation, identification and antimicrobial susceptibility testing of *E. coli. E. coli* was isolated from 21samples and out of the positive cases, the isolation rate of *E. coli* was highest in urine samples 10(47.6%). This is closely followed by wound discharge 5(23.8%), Ear discharge 3(14.3%), Sputum 2(9.5%) and 1(4.8%) in Eye discharge.

Table 1. Distribution of *E. coli* from clinical sources

Sample	Number of sample tested	Number positive for *E. coli*	% of positive cases
Urine	15	10/21	47.6
Sputum	10	2/21	9.5
Wound discharge	20	5/21	23.8
Ear discharge	15	3/21	14.3
Eye discharge	10	1/21	4.8
Total	70		100

Table 2. Overall antimicrobial susceptibility patterns of *E. coli*

Antibiotics	Antibiotic susceptibility pattern (N=21)					
	Sensitive		Intermediate		Resistant	
	N	%	N	%	N	%
TET	10	47.6	8	38.1	3	14.3
CFX	14	66.7	6	28.6	1	4.8
AUG	15	71.4	4	19.0	2	9.5
CFZ	13	61.9	5	23.8	3	14.2
GEN	11	52.4	8	38.1	2	9.5
TRIS	12	57.1	8	38.1	1	4.8
OFX	14	66.7	5	23.8	2	9.5
AMP	11	52.4	7	33.3	3	14.2
CPX	13	61.9	7	33.3	1	4.8

Key: N= number of bacteria isolates; TET= tetracycline (30µg); CFX= cefuroxime (30µg); GEN= gentamycin (10µg); TRIS= trimethoprim (5µg) + sulphamethaole (25µg); OFX= ofloxacin (10µg); CFZ= ceftazidime (30µg); AMP= ampicillin (10µg); AUG= augmentin (20µg) and CPX= ciprofloxacin (10µg)

Table 3. Multiple antimicrobial resistance patterns of *E. coli*

Source/Sample	R0 n (%)	R1 n (%)	R2 n (%)	R3 n(%)	R4 n (%)	R5 n (%)	R6 n (%)	R7 n (%)	R8 n (%)	R9 n (%)
Urine(n=10)	2(20.0)	2(20.0)	2(20.0)	1(10.0)	1(10.0)	1(10.0)	0(0.0)	0(0.0)	1(10.0)	0(0.0)
Sputum(n=2)	1(50.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Wound discharge(n=5)	2(40.0)	1(20.0)	0(0.0)	1(20.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	0(0.0)
Ear discharge(n=3)	1(33.3)	1(33.3)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Eye discharge(n=1)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total(n=21)	7(33.3)	4(19.0)	3(14.3)	3(14.3)	1(4.8)	1(4.8)	0(0.0)	0(0.0)	2(9.5)	0(0.0)

Key: R0= Sensitive to all tested antimicrobials; R1, R2, R3, R4, R5, R6, R7, R8 and R9 -Resistant to one, two, three, four, five, six, seven, eight and nine antimicrobials, respectively

Overall antimicrobial susceptibility patterns of *E. coli* isolated from various clinical samples, showed high resistance rates to Tetracycline (14.3%), Ceftazidime(14.2%), and Ampicillin (14.2%) however, high sensitivity rates to augmentin (71.4%), ofloxacin (66.7%), cefuroxime (66.7%), ciprofloxacin (61.9%) and ceftazidime (61.9%) were recorded.

4. DISCUSSION

Antimicrobial resistance is characterized with high mortality rates and high medical costs, it has a significant effect on the efficacy of antimicrobial agents MDR initiates obstruction in disease containment by increasing the possibility of spreading of resistant pathogens, thus, decreasing the effectiveness of treatment and leading to treatment failure in patient [11].

The antimicrobial resistance in *E. coli* has globally increased and its susceptibility a pattern varies based on the differences in environment, population and exposure to antibiotics. For this study, a total of 70 clinical specimens comprising urine, pus, sputum, eye, ear and wound swabs of patients were analyzed for isolation, identification and antimicrobial susceptibility testing of *E. coli. E. coli* was isolated from 21samples and out of the positive cases, the isolation rate of *E. coli* was highest in urine samples 10(47.6%). This is closely followed by wound discharge 5(23.8%), Ear discharge 3(14.3%), Sputum 2(9.5%) and 1(4.8%) in Eye discharge (Table 1).

Table 2 shows the overall antimicrobial susceptibility patterns of *E. coli* isolated from various clinical samples. There was high resistance rates to tetracycline (14.3%), Ceftazidime(14.2%) and Ampicillin (14.2%) .However, high sensitivity rates to augmentin (71.4%), ofloxacin (66.7%), cefuroxime (66.7%), ciprofloxacin (61.9%) and ceftazidime (61.9%) were recorded. This result, corroborates the findings of [12].

The overall multiple drug resistance rate was 14(66.7%) however, 7(33.3%) of the isolates were sensitive to the nine antimicrobials tested (Table 3). This resistance of *E. coli* to the antibiotics used in this study might be due to, the prolonged usage and regular abuse by the populace, since antibiotics are still sold over the counter, in some pharmaceutical and patent medicine stores in Nigeria [13].

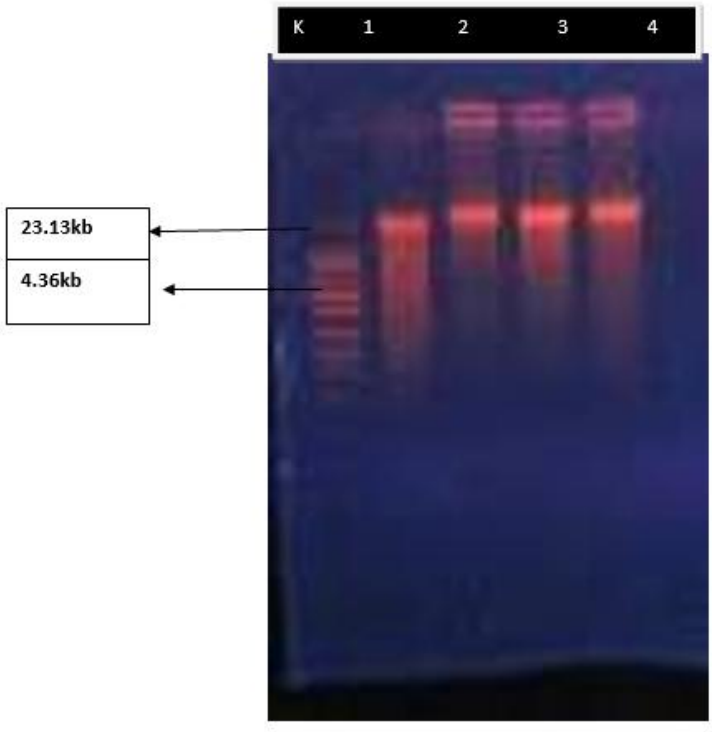

Fig. 1. Plasmid profile of test isolates of *E. coli*
(Lane K shows the DNA ladder, lanes 1-4 show Plasmid DNA of *E. coli* isolates)

Furthermore, Fig. 1 shows the bands and locations of the DNA after fragment separation. The observed lanes (1-4) possess the same molecular weight (23.13 kb) with the DNA ladder. This result however, proves that the multiple drugs resistant of *E. coli*, to various antibiotics, was harbored by the plasmid (plasmid mediated).

The resistance of *E. coli* to some of the antibiotics used in this study could be traced to the misuse and abuse of the screened antibiotics in the population. However, the rate of resistance observed in this study conforms to the findings of [14].

5. CONCLUSION

E. coli isolates has exhibited high resistance rate, to multiple antimicrobial agents, however, its sensitivity to augmentin, ofloxacin, cefuroxime, ciprofloxacin and ceftazidime, showed that these antimicrobials ,are still effective against *E. coli* infections in the study area. Furthermore, it was observed that, the resistance genes of *E. coli* in this study, were harbored by plasmid.

Antimicrobial resistance remains a global public health challenge. And this calls for a periodic need to dismay the spread of this threat, furthermore, awareness on the causes of antibiotic resistance should be given a higher priority.

ETHICAL APPROVAL

The Ethics committee of state hospital Ijaye approved the collection of the isolates from hospital laboratory, prior to the commencement of the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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