Clinical Microbiology: Open Access
ISSN: 2327-5073

Igwe et al., Clin Microbiol 2016, 5:6
DOI: 10.4172/2327-5073.1000268

Research Article Open Access

Virulent Characteristics of Multidrug Resistant *E. coli* from Zaria, Nigeria

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Received date: October 22, 2016; **Accepted date:** December 12, 2016; **Published date:** December 26, 2016

Abstract

Most multidrug resistant (MDR) *Escherichia coli* isolates (resistant to more than 3 classes of antibiotics) exhibit co-virulent characteristics that contribute to mortality and morbidity as a result of resistance to commonly prescribed antibiotics in the clinics. This study evaluated phenotypically some virulent characteristics in *E. coli* that contribute to the expressed MDR properties of *E. coli* using standard microbiological methods. Eighty seven *E. coli* isolates were confirmed as *E. coli* from urinary tract infection and diarrhoea patients in selected hospitals in Zaria Nigeria using Microgene identification kit, out of which 58.6% (51) were observed to be MDR. Significant number of the MDR isolates (70.6% (36)) were extended spectrum beta-lactamase producers, 45.1% (23) were resistant to cefoxitin and produce *ampC*. While further analysis on the isolates showed that 23.5% (12) were biofilm producers, 47.1% (24) were heteroresistant to cefoxitin while 5.9% (3) produced carbapenemase. This study showed that most MDR *E. coli* from UTI and diarrhoea could exhibit more than one virulent characteristics. Hence, isolates with MDR should be subjected to various tests in other to validate the mechanisms of resistance. This will encourage better treatment options and good periodic surveillance in prescription and dispensing of antibiotics in clinical settings.

Keywords: *E. coli;* Virulent characteristics; Multidrug resistance; UTI; Diarrhoea

Introduction

Virulence is the quantitative ability of an organism to cause disease (pathogenicity) that includes the characteristics of an organism (mechanisms used by these pathogens) to invade and circumvent host defense mechanisms and contributing to the chronicity of the diseases [1]. Virulent factors are properties (gene products) that enable a microorganism to establish itself on or within a host of a particular species and enhance its potential to cause disease. Virulence factors include bacterial toxins, cell surface proteins that mediate bacterial attachment, cell surface carbohydrates, and proteins that protect a bacterium and hydrolytic enzymes that may contribute to the pathogenicity of the bacterium [2].

Uropathogenic and diarrhoeagenic strains of *E. coli* are characterized by the expression of distinctive bacterial properties and products (virulent factors) which encourages their ability to cause more severe infections [3]. Such known factors in *E. coli* include extended spectrum betalactamases enzymes (betalactamases, cephalosporinases, carbapenemases), *ampC* genes, biofilms adhesins (*P fimbriae*), the aerobactin system, hemolysin, K capsule, and resistance to serum killing [4]. Extended-spectrum beta-lactamase–producing *E. coli* (ESBL *E. coli*) are highly resistant to many antibiotics other than betalactames, and infections by these strains are difficult to treat due to the acquisition of plasmid mediated multidrug resistance genes [5,6].

In most instances, only two oral antibiotics (ciprofloxacin and nitrofurantoin) and a very limited group of intravenous antibiotics (gentamicin and ceftriaxone) still remain effective against ESBL-producing *E. coli* [6]. Carbapenemases are also secreted by *E. coli* against the last drug (carbapenems) of hope for enteric infections [7]. *E. coli* that produce carbapenemases are tagged nightmare bacteria as they could cause 23% mortality in 7 days, 42% in 30 days, and 60% by the end of hospitalization in patient with bloodstream infections [8,9]. *AmpC* β-lactamases are clinically important cephalosporinases encoded on the chromosomes of many of the *Enterobacteriaceae* and a few other organisms, where they mediate resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and β-lactamase inhibitor-β-lactam combinations [10,11]. Bacterial adhesion onto mucosal or urothelial cells is encouraged by the presence of fimbriae in biofilm producing organism [12]. In UTI infection, relapse or re-infection are common with organisms that produce biofilms and they are known to exhibit increased tolerance toward antimicrobial agents, decreased susceptibility to stresses imposed by the host defense system and administered antibiotics [13].

Organisms that produce biofilms have been observed to exhibit multidrug resistance (MDR) to aminoglycosides, carbepenems, tetracyclines, and sulfonamides compared to those strains characterized as weak biofilm producers [14]. This results into a state of chronic pathogenicity to the host [15]. These forms of genes/virulent factors contribute significantly to antibiotics resistance, which influence increased mortality and morbidity, prolonged hospital stay and creates economic burden on the patients, hence, the need to evaluate these virulent factors among clinical isolates of *E. coli* from diarrhea and UTI patients in Zaria, Nigeria is imperative.

Methodology

Sample collection, identification and biochemical test

A total of 132 presumptive non-duplicated *Escherichia coli* (*E. coli*) isolates from urine and stool samples submitted for microbial analysis in 4 hospitals (Ahmadu Bello University Teaching Hospital Shika, St.

Luke Anglican Hospital Wusasa, Gambo Sawaba General Hospital Kofan-Gaya, and Ahmadu Bello University Clinic (Sickbay), Main Campus Samaru), were aseptically collected for the period of 6 months (April-September, 2014) and subjected to biochemical tests using Microgen GNA kit.

Antibiotic susceptibility testing

Antibiotic susceptibility pattern of the isolates from UTI and diarrhoea patients that were confirmed to be *E. coli* were determined using disc diffusion method according to Cheesbrough [16] and CLSI [17].

Phenotypic detection of ESBLs production

The double disc synergy test was adopted as described by Tsering et al. [18] to detect the production of ESBL by the *E. coli* isolates.

The identified and confirmed *E. coli* isolates with multidrug resistance characteristics were standardized in normal saline using MacFarland 0.5 turbidity standard. The standardized organisms were then streaked onto prepared Mueller Hinton agar and allowed to dry for 5 mins at room temperature. Using a sterile pair of forceps, cefpodoxime (10 µg) and ceftriaxone (30 µg) discs were gently placed on the agar at a distance of about 15 mm, center to center from a combination disc of amoxicillin-clavulanic acid (20:10 µg respectively). The plates were then incubated for 18-24 h at 37°C. *E. coli* ATCC 25922 which was susceptible to all the antibiotics tested was used as a negative control. Positive result of ESBLs was interpreted as any isolate that has the zone around the test antibiotics disc increased towards the center disc of amoxicillin-clavulanic acid and a ≥ 5 mm increase in zone diameter for either antimicrobial agent (cefpodoxime and ceftriaxone) compared to its zone when tested alone signifies positive result.

Heteroresistance to Cefoxitin

The isolates were checked for colonies growing within the zone of inhibition (squatter colonies) according to Denamur et al. [19]. The presence of squatter colonies reflects a high frequency of mutations conferring resistance to antibiotics. Confirmed isolates of *E. coli* were grown in nutrient agar for 18-24 h at 37°C and standardized in sterile normal saline using McFland 0.5 turbidity standard. The standardized isolates were streaked onto prepared sterile Mueller Hinton agar and allowed for 15 mins before cefoxitin antibiotic was place on the inoculated Mueller Hinton agar and allowed for 15 mins for pre-diffusion time. The plates were incubated at 37°C for 18-24 h and the result was obtained in diameter (mm) and interpreted as described by CLSI [20]. Within the clear zone of inhibition, some tiny colonies were observed and documented.

Detection of *AmpC* production

Resistance to cefoxitin (a presumptive test for *ampC*-betalactamase production) was tested using the disc diffusion method according to Cheesbrough [16] and interpreted using CLSI [20] criteria. Isolates that yielded a zone diameter less than 18 mm were screened positive and they were further subjected to confirmatory test [21,22]. This test was carried out as described by Jarlier et al. [23]. All presumptive positive isolates from the presumptive test were streaked on nutrient agar and incubated for 18-24 h at 37°C. Single colonies were picked and suspended in sterile normal saline for standardization using 0.5 MacFarland turbidity standards for comparism. Prepared Mueller Hinton agar was streaked with the confirmed and standardized isolates of *E. coli* from the presumptive test and allowed to dry for 5 mins. Cefoxitin disc (10 µg) was placed in the middle of the inoculated plate and sterile 6mm disc impregnated with sterile 20 µl normal saline were placed side by side as if they were to touch the cefoxitin discs. The plates were incubated for 18-24 h at 37°C and *E. coli* ATCC 25922 was used as a negative control. A positive test appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disc. A negative test had an undistorted zone.

Biofilm evaluation

The presence of curli fimbriae and cellulose in ESBL producing *E. coli* was determined by growing an overnight culture of the isolates on Congo red (CR) medium and incubated at 28°C for 48 h according to Castonguay et al. [24]. Colony morphology was evaluated according to Bokranz et al. [25]. Basic morphotypes: rdar (violent colony, expressed curli fimbriae and cellulose), pdar (pink colony expressed cellulose), bdar (brown colony expressed curli fimbriae) and saw (no expression of curli fimbriae or cellulose) were used for identification of biofilm producers.

Hospitals	Incidence			
	Diarrhoeic Samples Submitted	E. coli (%)	UTI Samples Submitted	E. coli (%)
ABUTH	348	54 (15.5)	552	326 (59.1)
ABUSB	72	28 (38.9)	116	78 (67.2)
SLAH	28	6 (21.4)	47	19 (40.4)
HGSGH	59	20 (33.9)	80	35 (43.8)
Total	507	108 (21.3)	795	458 (57.6)

Keys: ABUTH: Ahmadu Bello University Teaching Hospital, Shika; ABUSB: Ahmadu Bello University Sick Bay; SLAH: St. Luke Anglican Hospital, Wusasa; HGSGH: Hajiya Gambo Sawaba General Hospital, Kofan-Gayan.

Table 1: Occurrence of *E. coli* among UTI and diarrheic patients in Zaria, Nigeria.

Carbapenemases detection (Modified Hodges Test)

The MDR-ESBL producing *E. coli* isolates were screened for the presence of carbapenemases according to CLSI [17]. Meropenem and Imipenem discs were placed on the surface of inoculated Mueller Hinton Agar plates using a sterile forceps. The discs were placed about 30 mm apart and the plates were incubated for 24 hours at 37°C after which zones of inhibitions were read. Isolates that showed a zone of inhibition ≤ 21 mm in diameter for Meropenem or ≤ 23 mm in diameter for Imipenem were considered as suspected carbapenemase producers and were subjected to confirmatory test by the Modified Hodges Test (MHT). A Mcfarland turbidity standard of *E. coli* ATCC 25922 was evenly inoculated with a sterile cotton swab on surface of MHA plates. After which 10 µg meropenem disc was placed at the center of the MHA plate. Using a sterile swab sticks, 4 straight lines of the MDR-ESBL producing *E. coli* isolates were streaked from the edge of the Meropenem disc to the end of the plate. The plates were incubated at 37°C for 24 h and later examined for a cloverleaf type indentation or flattening at the intersection of the test organism and *E.*

coli ATCC 25922 within the zone of inhibition of the carbapenem susceptibility disc as described by Anderson [26].

Lab codes	Antibiotics resistance pattern	NAR	Class of antibiotics resistance	GRT	Resistance category
THU1	OFX, ATM, CN,CIP, CPD, CRO, CPO, CTX, SXT, C, AML, TE	12	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR
THU2	OFX, CIP, CPD, CPO, CTX, SXT, C, AML	8	FLU, CEPH, MISC, PEN	4	MDR
THU5	CPO, CTX, SXT, C, AML, TE	6	MON, CEPH, MISC, PEN, TE	5	MDR
THU6	ATM, CRO, CPO, CTX, C, AML, TE	7	MON, CEPH, MISC, PEN, TE	5	MDR
THU7	OFX, CIP, CPD, CPO, CTX, SXT, C, AML, TE	9	FLU, CEPH, MISC, PEN, TE	5	MDR
THU8	OFX, ATM, CIP, CPD, CPO, CTX, AML, TE	8	FLU, MON, CEPH, PEN, TE	5	MDR
THU10	OFX, ATM, CN, CIP, CRO, CPD, CPO, CTX, SXT,F, AML, TE	12	FLU, AMIN, MON, CEPH, MISC, PEN, TE	7	MDR
THU13	OFX, ATM, CIP, CRO, CPD, CPO, CTX, C, AML, TE	10	FLU, MON, CEPH, MISC, PEN, TE	6	MDR
THU14	ATM, CPO, CTX, C, AML, F, TE	7	MON, CEPH, MISC, PEN, TE	5	MDR
THU19	OFX, ATM, CIP, CRO, CPD, CPO, CTX, SXT, AML, F, TE	11	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
THU20	CPO, CTX, SXT, C, AML, TE	8	FLU, AMIN, CEPH, PEN, TE	5	MDR
THU21	ATM, CN, CPD, CPO, CTX, AML, TE	7	MON, AMIN, CEPH, PEN, TE	5	MDR
THU25	OFX, ATM, CN, CIP, CRO, CPD, CPO, CTX, SXT, C, AML, F, TE	13	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR
THU27	OFX, CN, CIP, CRO, CPD, CPO, CTX, SXT, AML, F, TE	11	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
THS1	OFX, CN, CIP, CPD, CPO, CTX, SXT, C, AML, TE	10	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
THS2	OFX, ATM, CIP, CRO, CPD, CPO, CTX, C, AML	9	FLU, MON, CEPH, MISC, PEN	5	MDR
THS4	OFX, ATM, CIP, CRO, CPD, CPO, CTX, SXT, AML	9	FLU, MON, CEPH, MISC, PEN	5	MDR
THS5	OFX, CIP, CPO, CTX, SXT, C, AML	7	FLU, CEPH, MISC, PEN	4	MDR
THS6	OFX, ATM,CIP, CRO, CPD, CPO, CTX, SXT, C, AML	10	FLU, MON, CEPH, MISC, PEN	5	MDR
THS7	CN, ATM, CIP, CRO, CPD, CPO, CTX	7	FLU, MON,AMIN, CEPH	4	MDR
THS8	CN, OFX, ATM, CIP, CRO, CPD, CPO, CTX, SXT, C, AML, TE	12	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR
THS12	CN, OFX, ATM, CIP, CRO, CPD, CPO, CTX, SXT, C, AML, TE	12	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR
THS15	CN, OFX, CIP, CPD, CPO, CTX, SXT, AML, TE	9	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SBS1	CN, OFX, CIP, CPD, CPO, CTX, SXT, C, AML, TE	10	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SBS4	CPD, CPO, CTX, SXT, C, AML	6	CEPH, MISC, PEN	3	MDR
SBS8	OFX, CIP, CPD, CPO, SXT, C, AML	7	FLU, CEPH, MISC, PEN	4	MDR
SBS11	CN, OFX, CIP, CPO, CTX, SXT, AML, TE	8	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SBU2	CN, OFX, CIP, CPD, CPO, CTX, SXT, AML, TE	9	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SBU3	ATM, CPO, CTX, SXT, AML	5	MON, CEPH, MISC, PEN	5	MDR
SBU8	CN, OFX, CIP, CPO, CTX, SXT, AML, TE	9	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SBU12	CN, ATM, OFX, CIP, CRO, CPD, CPO, CTX, SXT, C, AML, F, TE	13	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR
SBU13	CN, ATM, OFX, CIP, CRO, CPD, CPO, CTX, SXT, C, AML, TE	12	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR
SBU15	CN, OFX, CIP, CPD, CPO, CTX, SXT, C, AML, TE	10	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SBU16	CN, ATM, OFX, CIP, CPD, CPO, CTX, SXT, C, AML, TE	11	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR

SBU17	CN, OFX, CIP, CPD, CPO, CTX, SXT, AML, TE	9	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SBU18	OFX, CIP, CPD, CPO, SXT, C, AML	8	FLU, CEPH, MISC, PEN	4	MDR
SBU20	CN, OFX, CIP, CPD, CPO, SXT, C, AML, TE	9	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SLS5	OFX, CIP, CN, CRO, CPO, CTX, C, TE	8	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SLS6	OFX, CIP, CN, CRO, CPO, CTX, AML, TE	8	FLU, AMIN, CEPH, PEN, TE	5	MDR
SLU3	OFX, CIP, AK, CN, F, SXT, CTX, AML, TE	9	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
SLU8	OFX, CIP, CPD, CPO, ATM, AML, TE	7	FLU, MON, CEPH, PEN, TE	5	MDR
SLU10	OFX, CIP, CPD, CPO, CTX, SXT, C, AML	8	FLU, CEPH, MISC, PEN	4	MDR
HGS2	CN, F, CPD, CPO, SXT, AML, TE	7	AMIN, CEPH, MISC, PEN, TE	5	MDR
HGS5	CN, ATM, OFX, CIP, F, CPD, CPO, CTX, SXT, AML, TE	11	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR
HGS6	CN, ATM, OFX, CIP, F, CPD, CTX, AML, TE	9	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR
HGU1	CN, OFX, CIP, CPD, CPO, CTX, SXT, AML, TE	9	FLU, AMIN, CEPH, MISC, PEN, TE	6	MDR
HGU7	ATM, F, CPD, CPO, CTX, SXT, AML, TE	8	MON, CEPH, MISC, PEN, TE	5	MDR
HGU9	F, CRO, CPD, CPO, CTX, AML, TE	7	MON, CEPH, MISC, PEN, TE	5	MDR
HGU14	ATM, F, CPD, CPO, CTX, AML, TE	7	MON, CEPH, MISC, PEN, TE	5	MDR
HGU15	CN, CPD, CPO, CTX, SXT, C, AML	7	AMIN, CEPH, MISC, PEN	4	MDR
HGU16	CN, ATM, OFX, CIP, CRO, CPD, CTX, C, AML, TE	10	FLU, MON, AMIN, CEPH, MISC, PEN, TE	7	MDR

FLU: Fluoroquinolone; MON: Monobactam; AMIN: Aminoglycoside; CEPH: Cephalosporin; MISC: Miscellaneous Antibiotics; CAB: Carbapenems; PEN: Penicillin; AK: Amikacin; OFX: Ofloxacin, F: Nitrofurantoin; ATM: Aztreonam; CN: Gentamicin; CIP: Ciprofloxacin; CPD: Cefpodoxime; CRO: Ceftriaxone; CPO: Cefpirome; CTX: Ceftaxime; SXT: Sulfamethoxazole-Trimethroprim, C: Chloramphenicol; IPM: Imipenem; AML: Amoxicillin; MDR: Multidrug-Resistant; XDR: Extensively Drug-Resistant; NIL: Neither MDR nor XDR, NAR: Number of Antibiotics Resistance; CART: Class of Antibiotics Each Isolate of *E. coli* is Resistant to; MDR: Non-Susceptible to ≥ 1 Agent in ≥ 3 Antimicrobial Categories; XDR: Non-Susceptible to ≥ 1 Agent in all but ≥ 2 Categories; PDR: Non-Susceptible to all Antimicrobial Agents Listed. PDR was not considered because not all the antibiotics contained in the proposal of Magiorakos et al. [54] are prescribed for infections associated with *E. coli* in A.B.U Teaching Hospital Shika, Zaria.

Table 2: Antibiotics susceptibility profile of *E. coli* isolates from UTI and diarrhoea.

Results

A total of 86 isolates were confirmed to be *E. coli* among the isolates evaluated after biochemical test. Ahmadu Bello University Sick bay had the highest presumptive *E. coli* confirmed as *E. coli* while the highest number of samples was collected from Ahmadu Bello University Teaching Hospital, Shika (Table 1).

59.3% (51) of the *E. coli* isolates were resistant to 4 and above antibiotics tested (MDR) (Table 2), of which high resistance were observed against amoxicillin (96.1%), cefpirome (94.1%), ceftaxime (90.2%), cefpodoxime (78.4%), ciprofloxacin and tetracycline (76.5%), ofloxacin (74.5%), cotrimozaxole (72.5%), while the isolates were susceptible to imipenem (0%) and amikacin (2%) (Figure 1).

The classification of resistant profile showed that all the isolates were multidrug resistant (Table 2).

High percentage (70.6% (36)) of the isolates that were MDR were observed to produce ESBL using both double disc diffusion method and Oxoid betalactamase's test strip, 45.1% (23) were resistant to cefoxitin and produce *ampC*. While further analysis on the isolates showed that 23.5% (12) were biofilm producers, 47.1% (24) were heteroresistant to cefoxitin while 5.9% (3) produced carbapenemase (Table 3 and Figure 2).

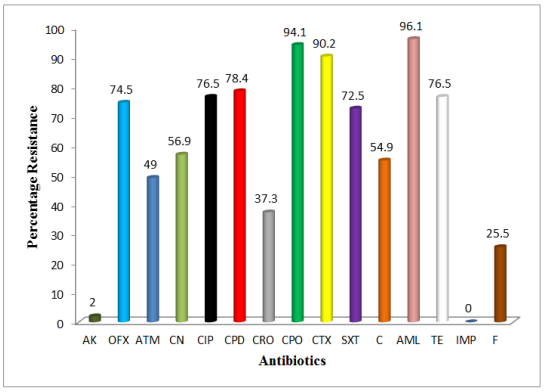

Figure 1: Percentage resistance to each antibiotic (AK: Amikacin; OFX: Ofloxacin; F: Nitrofurantoin; ATM: Aztreonam; CN: Gentamicin; CIP: Ciprofloxacin; CPD: Cefpodoxime; CRO: Ceftriaxone; CPO: Cefpirome; CTX: Ceftaxime; SXT: Sulfamethoxazole-Trimethroprim; C: Chloramphenicol; IMP: Imipenem; AML: Amoxicillin; TE: Tetracyclin).

S/N	Isolates	ESBL	CR	AGD	BP	H	CP
1	THU1	+	+	+	-	+	-
2	THU2	+	+	+	-	-	-
3	THU10	+	+	+	-	+	-
4	THU13	+	+	-	+	+	+
5	THU19	+	+	+	+	+	-
6	THU21	+	-	-	-	+	-
7	THU25	+	+	+	+	-	-
8	THU27	+	+	+	+	-	-
9	THS1	+	-	-	-	-	-
10	THS2	+	+	+	-	+	-
11	THS5	+	-	+	-	-	-
12	THS7	+	+	-	-	+	-
13	THS8	+	+	+	-	+	-
14	THS12	+	+	+	-	+	-
15	THS15	+	+	+	+	+	-
16	SBS1	+	+	+	-	+	-
17	SBS4	+	+	-	-	+	-
18	SBS8	+	-	+	-	-	-
19	SBU2	+	+	+	+	-	-
20	SBU3	+	-	-	-	-	-
21	SBU12	+	+	+	-	-	-
22	SBU13	+	+	+	-	+	-
23	SBU15	+	+	+	+	-	-
24	SBU16	+	+	+	-	+	-
25	SBU17	+	-	+	-	+	-
26	SBU18	+	-	-	-	+	-
27	SLS5	+	-	-	+	+	-
28	SLS6	+	-	-	-	+	-
29	SLU8	+	-	-	+	+	-
30	SLU10	+	+	+	+	+	-
31	HGS2	+	+	-	+	-	-
32	HGS5	+	-	+	-	-	+
33	HGS6	+	-	+	-	-	-
34	HGU1	+	-	-	+	-	+
35	HGU15	+	+	-	-	+	-
36	HGU16	+	+	+	-	+	-
37	ATCC35218	+	-	-	-	-	-

CR: Cefoxitine Resistant; AGD: *AmpC* Gene Detection; BP: Biofilm Production; H: Heteroresistance to Cefoxitin; CP: Carbapenemases Production

Table 3: Antibiotics virulence characteristics in MDR-ESBL Producing *E. coli.*

Pictorials representation of virulent characteristics in Multidrug Resistant *E. coli*

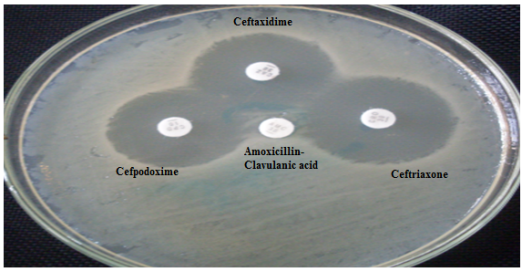

Figure 2: ESBL production among MDR *E. coli* isolates.

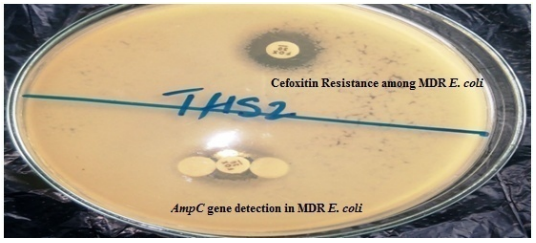

Figure 3: Cefoxitin resistance and *ampC* gene detection.

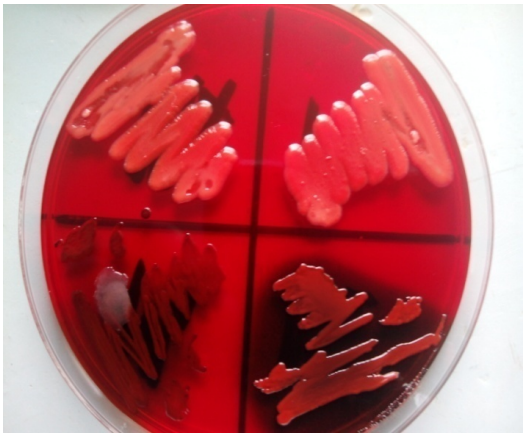

Figure 4: Biofilm production by MDR *E. coli* isolates.

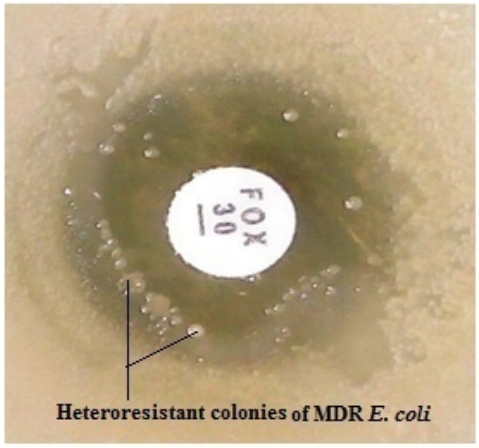

Figure 5: Heteroresistance to Cefoxitin (FOX).

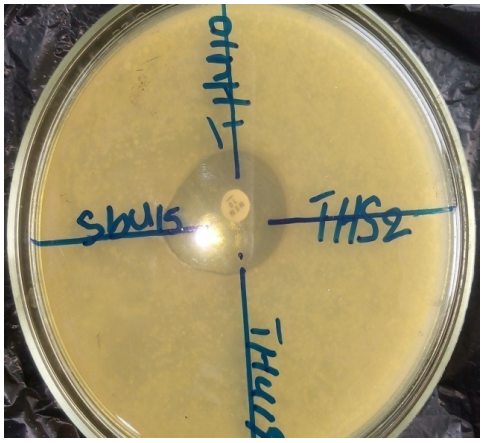

Figure 6: Carbapenemases production in MDR *E. coli* isolates.

Discussion

This study observed that significant number (59.3% (51)) of the *E. coli* isolates were resistant to 4 and above antibiotics tested (MDR). This might be an indication that a large proportion of the bacteria isolates have been pre-exposed to several antibiotics, and also, may be due to a combination of microbial characteristics such as selective pressure on antimicrobial usage, societal and technological changes that enhance the transmission of drug resistant organisms might be the cause of this high resistance [27]. High resistance was observed against amoxicillin (96.1%), cefpirome (94.1%), ceftaxime (90.2%), cefpodoxime (78.4%), ciprofloxacin and tetracycline (76.5%), ofloxacin (74.5%) and cotrimozaxole (72.5%), while the isolates were susceptible to imipenem (0%) and amikacin (2%). The high resistance to beta-lactam antibiotics (penicillins and cephalosporins) by *E. coli* might be due to the production of beta lactamase. Beta-lactamases are known to hydrolyze the amide bond of the β-lactam ring resulting in an inactive compound. Many of these β-lactamases are encoded by transposons, some of which may also carry resistance determinants to several other antibiotics: quartenary ammonium compounds, dyes (acriflavine and ethidium bromide) or heavy metals (lead, mercury and cadmium) [28,29]. The sensitivity of *E. coli* to imipenem, amikacin, ceftriazone, gentamicin and quinolones as observed in this study may be due to the fact that the imipenem and amikacin are expensive and not commonly sold over the counter, while nitrofurantoin, ciprofloxacin and ofloxacin are rarely prescribed for children but are often used in most adult's infections. The parenteral routes of ceftriazone and gentamicin reduce the abuse of these two antibiotics. Also concentration dependent bactericidal activity of gentamicin, its extended post-antibiotic effect, and the possibility of reduced nephrotoxicity and ototoxicity also affect the recommendation of gentamicin [30]. The mild susceptibility of *E. coli* to ofloxacin and ciprofloxacin observed in this study also concurs with the study of Kemebradikumo et al. [31] in Bayelsa, who reported that 61.5% of *E. coli* was sensitive to ofloxacin and 75% to ciprofloxacin. Factors such as low patient compliance, menace of substandard antibiotics which is common in developing countries, self-medication, and potentially sub-therapeutic prescription by health workers are some of the factors influencing multiple antibiotics resistance [32].

Infections with ESBL-producing organisms have been associated with poor outcomes [33]. The results of ESBL production suggest the presence of ESBL in 70.6% (36) of the 51 multidrug resistance *E. coli* isolates that were resistant to at least four (4) or more groups of antibiotics. The findings in this study concurs with the reports of Irith et al. [34] who reported 56.7%, Husam et al. [35] who reported 83% and Wani et al. [36] who reported 95.5% ESBL producing *E. coli* isolates from nosocomial and community-acquired infections.

The result of phenotypic evaluation for the presence of virulent characteristics in *E. coli* showed that 63.9% (23) MDR producing ESBLs were both cefoxitin resistant and AmpC gene producers (Figure 3). The high percentages of *AmpC* beta-lactamase gene producers among ESBL producing *E. coli* observed in this study is similar to the report of Silke et al. [37], who reported 69.2% *ampC* producers among *Enterobacteriaceae*. This result is contrary to the suggestion of Bradford [38] who claimed that cefoxitin would be the drug of choice besides carbapenems for the treatment of MDR isolates, as high percentages of this isolates are resistant to cefoxitin, an indication of chromosomally mediated *ampC* producer. On the other hand, Coudron et al. [39] suggested the combination of an antibiotic and an augmenting agent for the treatment of infections by resistant strains. This is also observed by Piroth et al. [40], who noted that β-lactam antibiotics and β-lactam inhibitors (oxyimino-cephalosporins (cefuroxime, cefotaxime, ceftazidime, ceftriaxone) and amoxicillin-clavulanic acid) could be used simultaneously against MDR isolates to induce high susceptibility of the resistant isolates. *ampC* beta-lactamases have been associated with high degradation of penicillins, expanded-spectrum cephalosporins (except cefepime and cefpirome), cephamycins, monobactams, and beta-lactam inhibitors [37]. In strains with loss of outer membrane porins, high resistance to carbapenems has been recorded [41]. Failure to detect these enzymes during routing laboratory practice has contributed to their uncontrolled spread and sometimes to therapeutic failures [42]. *ampC* beta-lactamases are inhibited by boronic acid and cloxacillin [43]. In *E. coli*, regulation of chromosomal *ampC* expression is by a weak promoter and strong attenuator genes encoded in a plasmid which result in a constitutive low-level *ampC* expression but mutations at the promoter site which could influence over expression that is different from the mode of expression in other *Enterobacteriaceae* has been reported [41,44]. Over expression of *ampC* beta-lactamases confers resistance to broad-spectrum cephalosporins including cefotaxime,

ceftazidime, and ceftriaxone and is a problem especially in infections due to *Enterobacter aerogenes* and *Enterobacter cloacae*, where an isolate initially susceptible to these agents may become resistant upon therapy [41].

Thirty three point three (33.3%) (12) of the isolates that were MDR produced biofilms in this study. This result is lower than the finding of Ghanwate [45] who reported 51.9% in India and Sevanan et al. [46] who reported 84.37% biofilm producing *E. coli* among UTI patients. This study support other findings that *E. coli* have high propensity to form biofilm that could render conventional antimicrobial therapy ineffective, as biofilm producing *E. coli* showed high resistance to tested antibiotics than non-producing isolates. Reports have also shown that biofilms encourage colonization and increased rate of persistent infections in *E. coli* [45,46]. Hence, biofilm assays may be helpful in selecting patients, who require a therapeutic approach to eradicate persistent biofilm-forming *E. coli* strains, to prevent subsequent relapses (Figure 4) [13].

Heteroresistance refers to phenotypic heterogeneity of microbial clonal populations under antibiotic stress, accruing due to mutation and adaptation whose mechanism is unknown. Resistance to antibiotics associated with heteroresistance is believed to have evolved as a result of differences in susceptibilities displayed by such subsets of bacterial cells to antibiotics. High percentages (66.7%) (24) of the MDR isolates were observed to produce heteroresistance to cefoxitin in this study. This is higher than that reported by Sun et al. [47] in China, who reported 25.0% heteroresistant formation to imipenem, 17.2% to ertapenem, and 3.9% to meropenem. Factors such as invasive intervention, antibiotic use and bacterial extended-spectrum β-lactamase (ESBL) production have been noted to contribute to invasive infections by *E. coli* heteroresistance [47]. Heteroresistance could give rise to the development of intrinsic and high-level resistance to virtually all antimicrobial agents available for clinical use especially in immunocompromised patients (Figure 5) [48].

Carbapenems, such as imipenem and meropenem are a class of β-lactam antibiotics with a broad spectrum of activity compared to other β-lactam classes. They are effective against MDR nosocomial *Acinetobacter baumannii* and *P. aeruginosa producing* β-lactamase enzymes [49]. Although carbapenem resistance is mediated by a variety of mechanisms, it has been rarely reported [50]. Carbapenemases producing strains have been reported to exhibit difficulty in treatment using β-lactamase inhibitors and resistance can spread widely into various Gram negative bacilli [51]. In this study, low percentage (8.3%) (3) of the MDR *E. coli* evaluated produced carbapenemases phenotypically, this further correlate with the antibiotic susceptibility study result that showed 3.4% (3) resistance to Imipenem (Figure 6). This report is in agreement with the low carbapenemases production (1.4% (2)) reported by Castanheira et al. [52] who found 2% imipenem resistance. This result though low but it's very significant as CDC had announced that care facilities should establish a protocol, in conjunction with CLSI guidelines, to detect resistance and carbapenemase production in Enterobacteriaceae, particularly *Klebsiella spp.* and *E. coli*, and immediately alert epidemiology and infection control staff members if identified. It was also recommended that all acute care facilities should review microbiology records for the preceding 6-12 months to ensure that previously unrecognized carbapenemase resistant *Enterobacteriaceae* cases have not occurred. If previously unrecognized cases are identified, facilities should conduct a point prevalence survey (a single round of active surveillance cultures) in units with patients at high risk (e.g., intensive care units, units where previous cases have been identified, and units where many patients are exposed to broad-spectrum antimicrobials) to identify any additional patients colonized with carbapenem-resistant or carbapenemase-producing *Klebsiella spp.* and *E. coli* [53].

Conclusion and Recommendations

E. coli, which are known to be the major bacteria pathogens in diarrhoea and UTI are largely resistant to betalactames, tetracyclines, sulfonamides and some cephalosporins, mildly susceptible to the fluoroquinolones and highly susceptible aminoglycosides, carbapenems, nitrofurantoin and some 2nd and 3rd generally cephalosporins in the studied environment. A high proportion of the *E. coli* isolates were also observed to be multidrug resistant and ESBL producers, encoded by plasmids. These isolates harbour several genes that mediate resistance to multiple antibiotics, which are structurally unrelated. Based on our finds, this study observed that potential risk of MDR spreading among hospitalized patients especially in those with invasive monitoring and treatment devices is possible, and any isolate with MDR properties from inpatient samples of any source should be reported immediately to appropriate authorities and investigated by a hospital's infection control personnel should be carried out immediatly. This will provide useful statistics and also enable hospital management to implement containment and preventive measures, including strict hand hygiene, contact precautions, healthcare personnel education, minimal use of devices, positive case isolation, antimicrobial stewardship, and screening for virulent factors carriage.

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