GSC Biological and Pharmaceutical Sciences, 2018, 05(01), 087–094

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Genetic basis of multiple drug resistant *Escherichia coli* from urine samples in Ikpoba Okha Local Government Area of Edo State, Nigeria

Eremwanarue Aibuedefe Osagie [1, 2] * and Ehiaghe Joy Imuetiyan [2]

[1] *Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.*
[2] *Lahor Research Laboratories and Diagnostics Centre, 121, Old Benin-Agbor Road, Benin City, Nigeria.*

Publication history: Received on 16 September 2018; revised on 03 October 2018; accepted on 08 October 2018

Article DOI: https://doi.org/10.30574/gscbps.2018.5.1.0102

Abstract

This study was aimed at characterizing bacteria at the molecular level and determining the genetic basis of the multidrug resistant bacteria isolates. Bacteria were isolated from eighty urine samples from urinary tract infection patients. Phenotypically identified isolates of *Escherichia coli* were selected. Multiple drug resistant (MDR) bacteria were generated from the isolates by carrying out antibiotic susceptibility tests using the Kirby-Bauer disc diffusion technique. MDR bacteria were selected and molecular characterization using polymerase chain reaction technique with species-specific primers was performed for confirming microbial identity. Plasmid DNA profiling was carried out to detect MDR *Escherichia coli* with plasmid and subsequent plasmid curing of isolates. The polymerase chain reaction result showed that all twenty isolates were *Escherichia coli*, fourteen out of the twenty isolates were multiple drug resistant *Escherichia coli*. The gel electrophoresis indicated that eleven out of the fourteen multiple drug resistant *Escherichia coli* contains plasmids with a molecular weight of 48.5kb. The *E. coli* isolates that habors plasmid were cured of the plasmids and the results of the antibiotics sensitivity tests showed that *E. coli* that showed resistance before curing became sensitive after curing. This study has shown that multidrug resistant *E. coli* are plasmid mediated in this locality.

Keywords: Antibiotics sensitivity testing; Multiple drug resistance; Bacteria; Plasmid DNA; Plasmid DNA curing

1. Introduction

Urinary tract infection is one of the significant illnesses that cause burden of national concern. Due to widespread and careless use of antibiotics at community level, we encountered more and more resistance pattern of micro-organisms to common antibiotics [1]. Urinary tract infections (UTIs) are among the most widespread infectious diseases of humans and a chief cause of morbidity and mortality [2]. It is estimated that 40–50 % of healthy adult women have experienced at least one UTI episode [3, 4].

UTI has become the most common hospital acquired infection, accounting for as many as 35.0% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients [5]. Previous reports have also suggested that UTI can occur in both males and females of any age [6, 7]. The leading causes of acute and uncomplicated UTI in patients have been reported to be due to *Escherichia coli*, *Staphylococcus aureus, Proteus* spp., *Klebsiella* spp. *and Pseudomonas aeruginosa* [8]. *Escherichia coli* are the most common organism associated with asymptomatic bacteriuria [9]. *Escherichia coli* are responsible for more than 80.0% of all UTIs and causes both asymptomatic and symptomatic UTI [9]. The main cause predisposing an individual to urinary tract infection has been attributed to poor personal hygiene and culture habit imposition [8].

* Corresponding author
E-mail address: eremwanarueaibuedefe@yahoo.com

Multiple drug resistance isolates causing UTI has serious implications regarding therapy against pathogenic isolates and for probable co-selection of antimicrobial resistance mediated by multiple drug resistant plasmids [10]. *E. coli* from clinical isolates are known to harbor plasmids of different molecular sizes. It has been widely reported that bacteria harbor antibiotic resistant genes which can be horizontally transferred to other bacteria [11]. The widespread occurrence of drug resistant *E. coli* and other pathogens in our environment has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational prescription programs, making policy decisions and assessing the effectiveness of both [12]. Therefore, this study was carried out to molecularly identify, determine antibiotic sensitivity pattern and plasmid profile of multidrug resistant *Escherichia coli* isolated from Urinary tract infection patients in Ikpoba Okha Local Governmen Area, Nigeria.

2. Materials and methods

2.1. Sample collection

A total of 80 clean-voided, mid-stream urine samples of about 20ml were collected from both inpatient and outpatient attending Lahor Medical Centre in Benin City with their respective bio-data. Urine samples were collected in sterile universal bottles.

2.2. Ethical clearance

Approval was obtained from the Medical Director of the hospital whose patients participated in this study and the patients gave their consent after being informed of the objectives of study.

2.3. Bacteriological procedures/identification of isolates

Specimens were aseptically inoculated onto MacConkey, Blood and Nutrient agar and incubated aerobically at 37°C for 24 hours and observed for colonial growth. Isolates were screened for *Escherichia coli*. The specimens were processed at Lahor research Laboratories, Benin City, Nigeria using standard microbiological methods. All isolates were identified using conventional techniques [13] and confirmed with polymerase chain reaction technique.

2.4. Antibiotic susceptibility testing

The susceptibility of isolates to commonly used antibiotic was determined by the Kirby-Bauer disk diffusion method for *in vitro* antibiotic susceptibility as described by NCCL (2002), against the following antibiotics for Gram negative bacteria include: Augmentin (AUG, 30µg), Ofloxacin (OFL 5µg), Cefixime (CXM 5µg), Gentamycin (GEN 30µg), Cefuroxime (CRX 30µg), Ceftazidime (CAZ 30µg), Ciprofloxacin (CPR 5µg), Nitrofurantion (NIT 300µg). The concentrations of antimicrobial sensitivity and interpretation of zones of inhibition were in accordance to Performance Standards for antimicrobial disk susceptibility tests of Clinical and Laboratory Standards Institute [14].

2.5. Bacteria genomic DNA extraction

Multiple drug resistant bacteria isolates were subcultured overnight in Luria-Bertani broth (Merck, Germany) and genomic DNA was extracted from typical colonies of *Escherichia coli* using Zymo research fungi/bacterial DNA MiniPrep DNA extraction kit (Irvine, CA, USA), according to manufacturer's instructions.

2.6. Identification of *Escherichia coli* using polymerase chain reaction technique

Polymerase Chain Reaction (PCR) was used for the amplification of *Escherichia coli* species-specific genes. Forward and reverse primers (URF-301-TGTTACGTCCTGTAGAAAGCCC; URR-432- AAAACTGCCTGGCACAGCAATT) were used in a Peltier thermal cycler PCR machine at Lahor Research Laboratories, Benin City, Nigeria. Quick load OneTaq one-step PCR master 2x (New England Biolab, USA) was purchased from lnqaba Biotech, Hartfield, South Africa incorporated and used according to the manufacturer's instruction. The PCR was performed in 25 µl reaction mixture containing 12.5 µl Quick load OneTaq one-step PCR master mix (2x), 1.25 µl of each species-specific forward primer (20 µM), 1.25 µl of each species-specific reverse primer (20 µM), 5.0 µl of nuclease free water and 5 µl of DNA template was added last. The PCR was started immediately as follows: Initial denaturation at 94 °C for 3 minutes, denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, extension at 72 °C for 1min, for 35 cycles, final extension at 72 °C for 15 minutes and final holding at 4 °C forever. Ten microliters (10 µl) of the amplified PCR products were fractionated on a 1.0% agarose gel containing ethidium bromide in Tris/Borate/EDTA (TBE) Buffer. Electrophoresis was performed at 90 volts for 60 minutes. Products were visualized by Wealth Dolphin Doc UV transilluminator and photographed. Molecular weights were calculated using molecular weight standard maker.

2.7. Plasmid DNA extraction and gel electrophoresis

Plasmid isolation was carried out using a commercial plasmid isolation kit (ZR Plasmid Miniprep Classic, USA) and the process was carried out according to the manufacturer instructions as described by D'Anteo [15]. Isolated plasmids were thereafter electrophoresed in a horizontal tank at a constant voltage of 90V for 60 minutes. After electrophoresis, plasmid DNA bands were viewed under UV transilluminator and photographed. Molecular weights were calculated using molecular weight standard maker which ranged in size from 0.5kb to 48.5kb, and results recorded.

2.8. Plasmid curing procedure

Multidrug resistant bacteria isolates were subjected to plasmid curing experiment using procedure as described by Ehiaghe et al. [16]. Freshly prepared nutrient broth (9 ml) was inoculated with 1 ml overnight culture that was grown in Luria broth (LB) containing antibiotics for 24 hours at 37 °C. The resultant mixture was incubated for 4 hours to allow for minimal growth of the microorganisms. Aliquot of 1 ml of the 10 % sodium dodecyl sulphate curing agent was added to 9 ml nutrient broth culture, and was incubated at 37 °C for 24 hours. The cured culture of 1 ml was inoculated unto 9 ml freshly prepared nutrient broth and incubated at 37 °C for 24 hours. The overnight broth culture was then used to carry out post susceptibility test on Muller-Hinton agar plate with the necessary antibiotic discs placed and incubated for 24 hours at 37 °C.

3. Results and discussion

The prevalence of antimicrobial resistance among microorganisms that cause UTI is increasing worldwide and is a major factor in selecting antibiotics for treatment. There are local variations in the antimicrobial susceptibility among urinary pathogens in different hospitals. Diagnosis of UTI is a good example of the need for close cooperation between the clinician and the microbiologist. The present study gives an insight about the common trend of increased resistance of uropathogens in this region which may be due to geographical variation or indiscriminate or sub lethal use of antibiotics. In this study, a total of 80 urine samples were collected. Of these, 20 (25%) isolates were found to be *E. coli.* All the 20 isolates revealed characteristic features of *E. coli*, which were Gram negative bacilli, produced pink lactose fermenting colonies on MacConkey agar and characteristic greenish metallic sheen on Eosin methylene blue agar. All the isolates showed typical IMViC pattern of *E. coli viz.,* Indole and Methyl Red tests positive, Voges Proskauer and Citrate utilization tests negative. Malonate utilization was negative for all the isolates. These findings were in hormony with that of Arya [17]. All isolates produce yellow colour colony on Triple Sugar Iron slant (TSI) as also reported by Lehman [18]. Glucose fermentation test positive for all the isolates and all isolates were motile which was in line with the report by Mittal *et al.*[19], they were positive for nitrate reduction test as also shown by Tiso and Schechter [20].

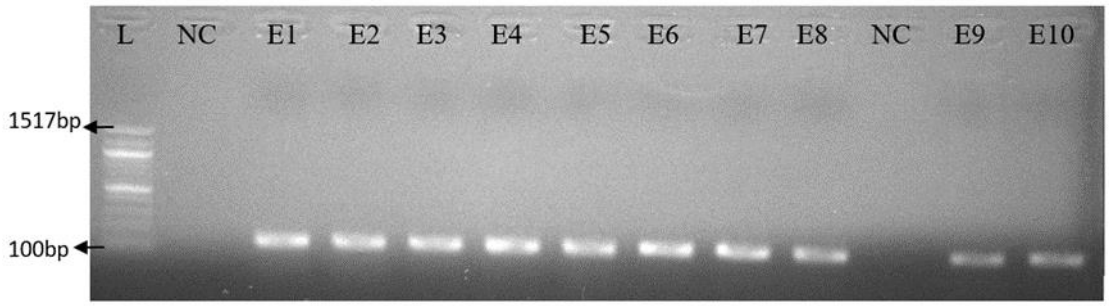

Figure 1 Polymerase chain reaction results for bacteria isolates analyzed with 1.0% agarose gel electrophoresis stained with ethidium bromide. L is 100 -1517 bp DNA ladder (molecular marker). Samples E1 –E10 are positive for *Escherichia coli* with band at 154 bp, NC: negative control

Polymerase chain rection (PCR) was performed for identification of bacterial isolates*. Escherichia coli* species-specific set of primers (URF-301 and URR-432) gave amplicon of 154 bp in all isolates screened (figure 1, 2). Thus, all the isolates were confirmed as *E. coli* by PCR. This corroborate with the result reported by Bej*et al.* [21] in the detection of low levels of microorganisms in environmental samples by using polymerase chain reaction.

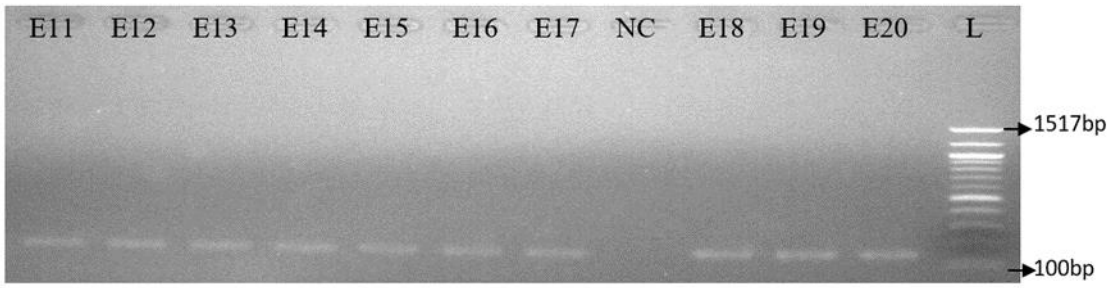

Figure 2 Polymerase chain reaction results for bacteria isolates analyzed with 1.0% agarose gel electrophoresis stained with ethidium bromide. L is 100-1517bp DNA ladder (molecular marker). Samples E11 – E20 are positive for *Escherichia coli* with band at 154 bp, NC: negative control

Table 1 Antibiotic sensitivity testing of *Escherichia coli*

Isolates	Antibiotic zone of inhibition (mm)							
	AUG	OFL	CXM	GEN	CRX	CAZ	CPR	NIT
E1	22.15 ±0.05	0.00±0.00	0.00±0.00	0.00±0.00	10.05±0.88	0.00±0.00	0.00±0.00	16.32±0.87
E2	0.00±0.00	20.10±0.34	0.00±0.00	0.00±0.00	0.00±0.00	15.08±0.40	0.00±0.00	0.00±0.00
E3	13.20±0.20	16.30±0.10	0.00±0.00	20.12±0.25	15.00±0.40	12.40±0.33	2.12±0.10	15.20±0.45
E4	19.19± 0.81	0.00±0.00	20.15 ±0.15	0.00±0.00	20.46 ±0.62	0.00±0.00	0.00±0.00	0.00±0.00
E5	2.00 ± 0.05	3.00±0.06	2.00 ± 0.03	0.00±0.00	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.02
E6	0.00±0.00	0.00 ± 0.00	0.00±0.00	19.25±0.33	0.00±0.00	25.12±0.66	0.00±0.00	0.00±0.00
E7	19.00±0.13	26.18±0.11	0.00±0.00	13.00±0.15	12.51±0.55	0.00±0.00	16.15±0.10	21.18± 0.32
E8	0.00 ± 0.00	14.12± 0.30	0.00 ± 0.00	0.00±0.00	0.00±0.00	0.00±0.00	20.11± 0.18	0.00±0.00
E9	12.15± 0.15	21.18±0.41	19.20±0.65	14.11± 0.22	24.10±0.21	1.12±0.40	0.00 ± 0.00	23.12±0.21
E10	0.00 ± 0.00	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.00±0.00	0.00 ± 0.00	22.12±0.44	26.00±0.26
E11	15.19± 0.75	0.00±0.00	18.10 ±0.14	0.00±0.00	0.00±0.00	0.00 ± 0.00	12.46 ±0.60	0.00±0.00
E12	0.00 ± 0.00	21.10±0.44	0.00 ± 0.00	0.00 ± 0.00	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00	23.00±0.20
E13	2.00 ± 0.10	3.00±0.20	4.00 ± 0.00	0.00±0.00	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00	2.10 ± 0.20
E14	0.00 ± 0.00	20.16±0.04	15.00±0.40	17.00±0.25	18.11±0.15	22.00±0.47	0.00 ± 0.00	24.00±0.21
E15	0.00 ± 0.00	21.10±0.44	0.00 ± 0.00	18.00±0.49	3.00±0.05	0.00 ± 0.00	2.00 ± 0.08	20.00±0.25
E16	13.00±0.49	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.00±0.00	0.00 ± 0.00	18.15±0.50	16.00±0.21
E17	17.15± 0.70	14.40 ±0.65	17.11 ±0.15	2.00±0.02	13.15±0.25	0.00 ± 0.00	0.00±0.00	20.18±0.30
E18	16.10±0.50	21.10±0.45	0.00 ± 0.00	17.15±0.24	20.13±0.14	25.00±0.44	0.00 ± 0.00	23.00±0.20
E19	15.19± 0.75	0.00±0.00	18.10 ±0.14	0.00±0.00	0.00±0.00	0.00 ± 0.00	12.46 ±0.60	0.00±0.00
E20	0.00±0.00	19.10 ±0.45	14.00 ±0.16	16.00 ±0.18	13.00 ±0.28	0.00 ± 0.00	15.16 ±0.55	18.06 ±0.51

AUG: Augmentin, OFL: Ofloxacin, CXM: Cefixime, GEN: Gentamycin, CRX: Cefuroxime, CAZ: Ceftazidime, CPR: Ciprofloxacin, NIT: Nitrofurantion, E: *Escherichia coli*

Antibiotic sensitivity testing of the isolates revealed that 14(70%) isolates out of the twenty *Escherichia coli* were found to be multidrug resistant isolates (Table 1). All isolates in this study were resistant to between 3 and 8 antimicrobial compounds, with different resistance profiles recognized. The resistance rate of *E. coli* in decreasing order are as follows: ceftazidime (75%), cefixime and ciprofloxacin (65%), gentamycin (60%), cefuroxime (55%) augmentin (50%), ofloxacin (45%), nitrofurantion (40%) as shown in table 2. Primarily, antimicrobial therapy is one of the primary control measures for reducing morbidity and mortality due to UTI. However, majority of *E. coli* isolates tested in this

study demonstrated resistance to ceftazidime (75%), cefixime and ciprofloxacin (65%), gentamycin (60%) and displayed multiple antimicrobial resistances as typified by resistance to as many as four different antimicrobial classes. These resistance levels are comparable to those previously reported for clinical *E. coli* isolates [22] and for both fecal and clinical *E. coli* isolates recovered from turkeys in West Virginia and Virginia [23]. Similar resistances have been reported in other countries as well for *E. coli* isolated from avian species [24, 25, 26], with the majority of isolates exhibiting resistance to tetracyclines, sulfa drugs, and aminoglycosides. On the contrary, Patel *et al.*[27] reported moderate percent of isolates (33.33%) were found to be resistant to ciprofloxacin which was also similar to the findings of Paul *et al.*[28] who observed less than 30 percent of *E. coli* isolates resistant to ciprofloxacin in their study while moderately high percentage (65%) of isolates were found to be resistant to antibiotic ciprofloxacin in our study. It has been reported that pathogenic isolates of *E. coli* have relatively high potential for developing resistance [29]. *Escherichia coli* isolates obtained from this research were more susceptible to nitrofurantion (60%) and ofloxacin (55%). It has been observed that antibiotic susceptibility of bacterial isolates is not constant, but dynamic and varies with time and environment [30].

Table 2 Antibiotic susceptibility pattern of *Escherichia coli*

Antibiotics	**Concentration (µg)**	***Escherichia coli***	
		Number (%) of resistant isolates	**Number (%) of Sensitive Isolates**
Augmentin	30	10(50)	10(50)
Ofloxacin	5	9(45)	11(55)
Cefixime	5	13(65)	7(35)
Gentamycin	30	12(60)	8(40)
Cefuroxime	30	11(55)	9(45)
Ceftazidime	30	15(75)	5(25)
Ciprofloxacin	5	13(65)	7(35)
Nitrofurantion	300	8(40)	12(60)

Bacteria resistance to specific antimicrobials is sometimes encoded by plasmids, which may distribute resistance in susceptible bacteria through horizontal gene transfer [31]. Figure 3 and 4 showed the Agarose gel electrophoretic analysis of the plasmids DNA from the multiple drug resistant *Escherichia coli* isolates. Lane M, is the standard molecular marker used (0.5kb - 48.5kb DNA ladder). Plasmid DNA analyses revealed that there were detectable plasmids in 10(71.4%) of the 14 multidrug resistant *E. coli* isolates tested. Four of the isolates possessed no plasmid. The ten isolates possessed single sized plasmids of the same molecular weight (48.5kb). *E. coli* isolates which harbors plasmid are: E1, E2, E4, E5, E6, E10, E11, E12, E13 and E15 while isolates E8, E16, E17 and E19 do not harbor plasmid. Resistance to antibiotics has been ascribed in most instances to the presence of plasmids [32]. In the study carried out in Benin City, Nigeria, 11.4% of the *Pseudomonas* isolates was plasmid mediated, and were highly transferable with a frequency range of $2x10^{-2}$ to $6x10^{-4}$[33]. The emergence of R-plasmids in this study could be ascribed to the indiscriminate and widespread use of antibiotics caused by over the counter availability of antibiotics as well as the higher exposure of people to enteric flora in places with poor sanitation [34, 35]. Smith *et al.* [36] reported that 47 of the *E. coli* isolated from animals in Lagos harbors detectable plasmids which ranged in sizes from 0.564kb to >23kb. Danbara *et al.* [37] reported plasmids of sizes between 3.9kb and 50kb in *E. coli* strains isolated from traveller's diarrhoea.

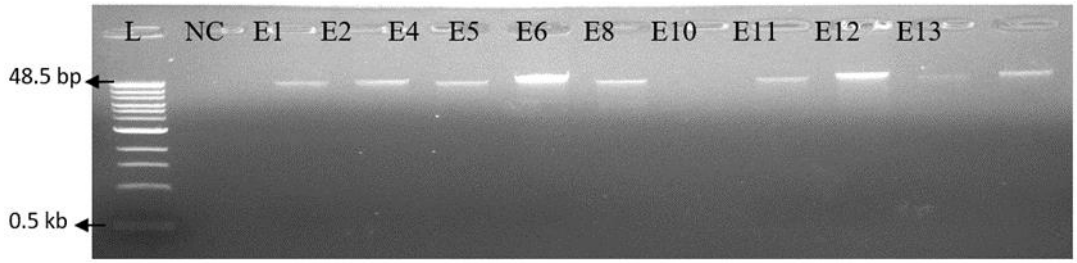

Figure 3 Agarose gel electrophoresis showing profiles of plasmid DNA of clinical isolates of *Escherichia coli*. Lane L is the molecular weight marker of size ranges from 0.5kb to 48.5kb. Isolates E1, E2, E4, E5, E6, E10, E11, E12 and E13 shows plasmid bands at 48.5kb while isolate E8 do not show plasmid band. NC: negative control.

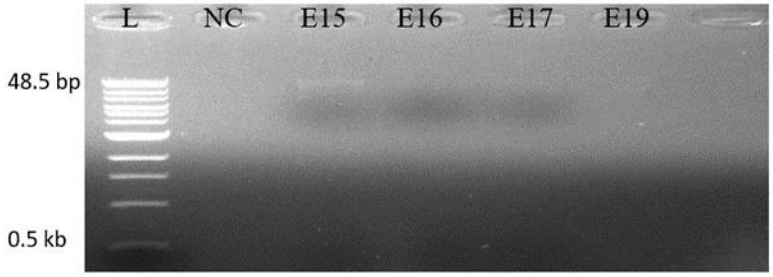

Figure 4 Agarose gel electrophoresis showing profiles of plasmid DNA of clinical isolates of *Escherichia coli*. Lane L is the molecular weight marker of sizes ranging from 0.5kb to 48.5kb. Isolate E15 shows plasmid band at 48.5kb while isolate E16, E17 and E19 do not show plasmid band. NC: negative control.

4. Conclusion

This study showed that *Escherichia coli* were most susceptible to nitrofurantion and ofloxacin. It was observed that all *Escherichia coli* used in this study were resistant to cefixime, gentamycin, cefuroxine, ceftazidime and ciprofloxacin. This study has also highlighted the emergence of multidrug resistant R-plasmids among *Escherichia coli* causing urinary tract infections in Ikpoba Okha Local Government Area, Edo State, South-south Nigeria. Concerted strategies at monitoring and prescribing habits of clinicians, the diagnostic efficiency of laboratory scientist, the dispensing habits of pharmacists, the inappropriate use of antibiotics, as well as encouraging good hygienic measures could help curtail possible transmission of MDR *E. coli* infections within and outside the hospital environment. Our findings also indicate that the *E. coli* recovered in this study expressed high levels of resistance to antimicrobials that are commonly used in clinical medicine. This could contribute to the spread and persistence of antimicrobial resistant bacteria and resistance determinants in humans and the environment.

Compliance with ethical standards

Acknowledgments

The authors wish to thank Prince Ikhuiwu, a technologist at Lahor Research Laboratories and Diagnostics Centre, Benin City, Nigeria for his assistance throughout the study.

Disclosure of conflict of interest

Author declares that there is no conflict of interests. Eremwanarue Aibuedefe O. conducted the set-up of the experiments, conducted the experiment and wrote the manuscript. Ehiaghe Joy I. also participated in conducting the experiment and in writing of the manuscript.

Statement of ethical approval

Ethical approval was obtained from the Medical Director of the hospital whose patients participated in this study.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Paryani JP, Memon SR, Rajpar ZH and Shah SA. (2012). Pattern and sensitivity of microorganisms causing urinary tract infection at Teaching Hospital. Journal of Liaquat University of Medical & Health Sciences, 11(02), 97 – 100.

[2] Roos V and Klemm P. (2006). Global gene expression profiling of the asymptomatic bacteriuria *Escherichia coli* strain 83972 in the human urinary tract. Infection and Immunity, 74, 3565 – 3575.

[3] Roos V, Schembri MA, Ulett GC and Klemm P. (2006a). Asymptomatic bacteriuria *Escherichia coli* strain 83972 carries mutations in the foc locus and is unable to express F1C fimbriae. Microbiology, 152, 1799 – 1806.

[4] Soto SM, Jimenez de Anta MT and Vila J. (2006). Quinolones induce partial or total loss of pathogenicity islands in uropathogenic *Escherichia coli* by SOS-dependentor-independent pathways, respectively. Antimicrobial Agents and Chemotherapy, 50(2), 649-653.

[5] Kolawole AS, Kolawole OM, Kandaki-Olukemi YT, Babatunde SK, Durowade KA and Kolawole CF. (2009). Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. International Journal of Medicine and Medical Sciences, 1(5), 163-167.

[6] Obiogbolu CH, Okonko IO, Anyamere CO, Adedeji AO, Akanbi AO, Ogun AA, Ejembi J and Faleye TOC. (2009). Incidence of urinary tract infections (UTIs) among pregnant women in Akwa metropolis, South-eastern Nigeria. Scientific Research and Essay, 4(8), 820-824.

[7] Okonko IO, Ijandipe LA, Ilusanya AO, Donbraye-Emmanuel OB, Ejembi J, Udeze AO, Egun OC, Fowotade A and Nkang AO. (2009). Incidence of urinary tract infection (UTI) among pregnant women in Ibadan, South-Western Nigeria. African Journal of Biotechnology, 8(23), 6649-6657.

[8] Ehinmidu, JO. (2003). Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. Tropical Journal of Pharmaceutical Research, 2(2), 223-228.

[9] Roos V, Ulett GC, Schembri MA and Klemm P. (2006b). The asymptomatic bacteria *Escherichia coli* strain 83972 out-competes UPEC strains in human urine. Infection and Immunity, 74, 615-624.

[10] Sherley M, Gardon DM and Collingnon PJ. (2004). Evolution of multi-resistance plasmids in Australia clinical isolates of *Escherichia coli*. Microbiology, 150, 1539-1546.

[11] Piddock LJV. (2006). Clinically relevant chromosomallyencoded multidrug resistance efflux pumps in bacteria. Clinical Microbiology Reviews, 19(2), 382-402.

[12] Omigie O, Enweani IB, Ohenhen RE, Umolu IP and BenEdo-Osagie O. (2006). Bacteriological survey of wound infections in Benin City, Nigeria. Nigerian Annals Natural Science, 6 (In press).

[13] Cheesbrough M. (2000). District Laboratory Practice Manual in Tropical Countries, part 2 Cambridge University Press, New York, 178-179.

[14] Clinical and Laboratory Standards Institute (2011). Performance standards for antimicrobial susceptibility testing 21st informational supplement, M100–S21. CLSI, Wayne, PA.

[15] D'Anteo S, Mannucci A, Meliani M, Verni F, Petroni G, Munz G and Vannini C. (2015). Nitrifying biomass characterization and monitoring during bioaugmentation in a membrane bioreactor. Environmental technology, 36(24), 3159-3166.

[16] Ehiaghe FA, Ehiaghe IJ, Agbonlahor DE, Oviasogie FE, Etikerentse SMO, Nwobu RAU, Akinshipe BO, Ikusemore AI, Aladenika ST and Enwa FO. (2013). Plasmid profiling and curing analysis of fluoroquinolone multidrugresistant *Pseudomonasaeruginosa.* Open Journal of Medical Microbiology, 3, 201-205.

[17] Arya G. (2006) Isolation and identification of *Escherichia coli* from diarrhoeic calf faeces by biochemical tests, antibiogram pattern and PCR based detection of toxigenic genes. M.V.Sc Thesis, submitted to Anand Agricultural University, Anand.

[18] Lehman D. (2013) Triple sugar iron protocols. Microbe Library.

[19] Mittal N, Budrene EO, Berner MP and Oudenaarden AV. (2003) Proceedings of the National Academy of Sciences, 100(23), 13259-13263.

[20] Tiso M and Schechter AN. (2015). Nitrate reduction to nitrite, nitric oxide and ammonia by gut bacteria under physiological conditions. PloS one, 10(3), e0119712.

[21] Bej AK, DiCesare JL, Haff L and Atlas RM. (1991). Detection of *Escherichia coli* and *Shigella spp*. in water by using polymerase chain reaction (PCR) and gene probes for uid. Applied Environmental Microbiology, 57, 1013-1017.

[22] Keyes K, Hudson C, Maurer JJ, Thayer S, White DG and Lee MD. (2000). Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. Antimicrobial Agents Chemotherapy, 44, 421-424.

[23] Altekruse SF, Elvinger F, Lee KY, Tollefson LK, Pierson EW, Eifert J and Sriranganathan N. (2002). Antimicrobial susceptibilities of *Escherichia coli* strains from a turkey operation. Journal of the American Veterinary Medical Association, 221, 411-416.

[24] Guerra B, Junker E, Schroeter A, Malorny B, Lehmann S and Helmuth R. (2003). Phenotypic and genotypic characterization of antimicrobial resistance in German Escherichia coli isolates from cattle, swine and poultry. Journal of Antimicrobial Chemotherapy 52, 489-492.

[25] Saenz Y, Zarazaga M, Brinas L, Ruiz-Larrea F and Torres C (2003). Mutations in gyrA and parC genes in nalidixic acidresistant *Escherichia coli* strains from food products, humans and animals. Journal of Antimicrobial Chemotherapy, 51, 1001-1005.

[26] Yang H, Chen S, White DG, Zhao S, McDermott P, Walker R and Meng J. (2004). Characterization of multiple antimicrobial resistant *Escherichia coli* isolates from diseased chickens and swine in China. Journal of Clinical Microbiology, 42, 3483-3489.

[27] Patel Hardik, Kalyani IH, Bariya A, Dodiya V, Sakhare P and Sharma K. (2017). Isolation, characterization, serotyping and antibiogram studies of *E.coli* collected from diarrhoeic neonatal kids. International Journal of Microbiology Research, 9(2), 854-856.

[28] Paul SK, Kan MSR, Rashid M A, Hassan J and Mahmud SMS. (2010). Bangladesh Journal of Veterinary Medicine, 8(1), 23-26.

[29] Karlowsky JA, Jones ME, Draghi DC, Thornsbery C, Sahm DF and Volturo GA. (2004). Prevalence of antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Annals of Clinical Microbiology and Antimicrobials, 3, 1-7.

[30] Hassan SH. (1995). Sensitivity of *Salmonella* and *Shigella* to antibiotics and chemotherapeutic agents in Sudan. Journal of Tropical Medicine and Hygiene, 88, 243-248.

[31] Hall BG and Barlow M. (2004). Evolution of the serine beta-lactamases: past, present and future. Drug Resistance Updates, 7, 111-123.

[32] Daini OA, Ogbolu DO and Ogunledun A. (2006). Plasmid determined resistance to quinolones in clinical isolates of Gram negative enteric bacilli. African journal of medicine and medical sciences, 35, 437-441.

[33] Yah SC, Eghafona NO, Enabulele IO, Aluyi HAS (2006). Ampicillin Usage and Ampicillin Resistant Plasmids Mediated *Escherichia coli* Isolated from Diarrhoeagenic Patients Attending Some Teaching Hospital in Nigeria. Shiraz E-Medical Journal, 7(4), 1-12.

[34] Aibinu IE, Adenipekun EA and Odugbemi TO. (2004). Emergence of quinolone resistance amongst *E. coli* strains isolated from clinical infections in some Lagos State Hospitals, in Nigeria. Nigerian Journal of Health and Biomedical Sciences, 3, 73-78.

[35] Ogbolu DO, Daini OA, Ogunledun A, Alli AO and Webber MA. (2011). High levels of multidrug resistance in clinical isolates of gram negative pathogens from Nigeria. International Journal of Antimicrobial Agents, 37, 62-66.

[36] Smith SI, Aboaba OO, Odeigha P, Shodipo K, Adeyeye JA, Ibrahim A, Adebiyi T, Onibokun H and Odunukwe NN.(2003). Plasmid profile of *Esherichia coli* 0157:H7 from apparently healthy animals. African Journal of Biotechnology, 2(9), 322-324.

[37] Danbara H, Komase K, Ivli Y, Shinohawa M, Arita H, Makino A and Yoshikawa M. (1987). Analysis of the plasmids of *Escherichia coli* 0148:H28 from travellers with diarrhoea. Microbial Pathogenesis, 3(4), 269-278.

How to cite this article

Eremwanarue AO and Ehiaghe JI. (2018). Genetic basis of multiple drug resistant *Escherichia coli* from urine samples in Ikpoba Okha Local Government Area of Edo State, Nigeria. GSC Biological and Pharmaceutical Sciences, 5(1), 87-94.