Multiple resistance in clinical isolates of *Escherichia coli* and plasmid-mediated Quinolone resistance genes

Abstract

Quinolone antibiotics have been commonly used to treat cases of multiple antibiotic resistance. Unfortunately, quinolone antibiotics have so much been resisted by infectious bacterial agents. This study aimed to evaluate the susceptibility of some clinical isolates of *E. coli* to some commonly used quinolone antibiotics and the determination of the plasmid-encoded quinolone resistance genes.

Our results showed the plasmid quinolone-resistance genes in the following prevalence: qnr genes: qnr S (71.4 %); qnr B (15.4 %); qnr S and B (12.1 %); aac (6) lb-cr (4 %); Efflux genes: oqxA (7.7 %); oqxB (25.3 %); qepA (12.1 %); oqxA and oqxB (5.5 %). We conclude that there is a high frequency of Plasmid-mediated quinolone resistance genes in *Escherichia coli* isolates from clinical samples in South-Eastern Nigeria. These could be responsible for the high incidence of quinolone resistance reported in Enugu. There is a need for whole-genome sequencing to map out all resistance genes.

Keywords: *Escherichia coli;* Quinolone antibiotics; plasmid; antibiotics; Quinolone genes

1. Introduction

Resistance of infectious microorganisms have been a great concern to all globally.[1] Antibiotic resistance occurs when any drug meant to hinder bacterial growth or kill the bacteria fail to exhibit any lethal action on the microorganism or hinder its growth, such that the bacteria grow and multiply in the presence of that drug.[2] The first serious consequence of antibiotic resistance in medical therapy was the gross dissemination of strains of *Staphylococcus aureus*, which were resistant to penicillin, described as penicillin-resistant *Staphylococcus aureus*.[3] Currently, Multi-Drug Resistance (MDR) has become major public health concern all over the world.[1, 4] Among the present-day superbugs are the multidrug-resistant *Escherichia coli (E. coli).*

Quinolones are a family of synthetic broad-spectrum antibiotics, which are drugs of choice commonly used to treat bacterial infections worldwide.[5, 6] Resistance to the first-generation

quinolones led to the manufacture and introduction of stronger members of the quinolones, otherwise known as the fluoroquinolones.[7, 8]

Many studies have attributed the frequency of quinolone resistance to carriage of plasmid, which has been found to contain genes for quinolone resistance.[9, 10] The Plasmid-mediated quinolone resistance includes the qnr genes, majorly *qnrA*, *qnr* B and *qnr* S; the aminoglycoside acetyltransferase gene (aac-6'-Ib-cr) and specific efflux pumps' genes, qepA, oqxA and OqxB.[11, 12]. Owing to the high resistance of *E. coli* to the quinolones, there is an urgent need to find out how *Escherichia coli* is resisting these drugs. This will enable further research for modifications on the drugs.

In this work, clinical isolates of *E. coli* were screened for quinolone resistance, after which the plasmid-mediated quinolone resistance was investigated. The resistance genes mediated by plasmids may be of epidemiologic importance in the rapid spread of antibiotic resistance even to bacteria of other species and consequently, elevate antibiotic resistance.

2. Materials and methods

2.1 Study Population and Sampling method

A total of 274 isolates of presumptively identified *E. coli*, were collected from 13 Medical Laboratories/Diagnostic Centers in Enugu State and from 10 different clinical samples. The samples include pus aspirates, catheter tip, ear swab, Endocervical swab (ECS), High Vaginal Swab (HVS), semen, stool from infants, throat Swab, urine and wound swab. A convenience method of non-probability sampling was employed.

2.2 Identification and Characterization of the *E. coli* Isolates

A loopful of the *E. coli* isolate from Brain Heart Infusion agar storage or directly from MacConkey or Blood agar medium was aseptically picked and streaked on Chromagar to enable the growth of pure colonies. This was incubated at 37 ^{o}C for 24 h. The colonies of *E. coli* would appear dark-blue while the non-*E. coli* (other coliforms) would appear colorless/ white. A pure colony identifiable as *E. coli* was streaked on Mueller- Hinton agar MHA (Oxoid) plate for luxuriant colonies. Further biochemical characterization, using Triple Sugar Iron agar (TSI); IMVC (I = indole; M= Methyl Red; V = Voges Proskauer; C = citrate) and Urease were carried out.

2.2.1 Triple Sugar Tests

Triple Sugar Iron medium is a differential medium that can distinguish between a number of Gram negative enteric bacteria based on their physiological ability to metabolize lactose and/or sucrose; produce acid and /or gas during fermentation and generate H_2S. The medium contained1.0% each of sucrose and lactose and 0.1% glucose. If only glucose was fermented, acid produced in the butt would turn it yellow, but insufficient acid products would form to affect the methyl red in the slant. If either sucrose or lactose were fermented, sufficient fermentation products would be formed to turn both the butt and the slant yellow. If gas was formed during the fermentation, it would show in the butt either as bubbles or as cracking of the agar. If no fermentation occurred (as for an obligate aerobe), the slant and butt would remain red. The medium also contained ferrous sulfate. If the bacterium formed H_2S, this chemical would react with the iron to form ferrous sulfide, which would be seen as a black precipitate in the butt (a black butt). The Triple sugar iron agar (oxoid) powder was prepared according to manufacturer's instruction It was then allowed to set in slants 1 inch deep. A single colony from the Muller Hilton agar (MHA) was streaked on the slant and stabbed on the butt. Incubation of the slant was done at 42°C for 18 hours for change of colour and possible result.

2.2.2 Indole Test

This tests the ability of an organism to degrade tryptophan based on the possession of tryptophanase enzyme, producing indole, a by- product. Indole is detected using Kovac's reagent which contains p-dimethylaminobenzaldehyde (DMAB). Positive test is indicated by red colour in the form of ring in the medium. The production of Indole was tested using SIM (Sugar, Indole and Motility) medium agar, (oxoid) product. The SIM medium was prepared according to company's specification. Once cool, the pure colony from MHA was inoculated once, by inserting a straight wire to about one third of the depth of the medium. The culture was incubated at 42°C for 18 hours. To test for indole production a drop of Kovac's reagent was put in the tube. A positive result was indicated by appearance of a red ring in the medium. No change in colour was observed for a negative result.

2.2.3 Methyl –Red (MR)- Voges Proskauer (VP) Test

Methyl red tests the ability of bacteria to metabolize glucose with production of mixed acid products (lactic, acetic, formic and succinic). This would result to a decrease in the pH of the

medium and a positive MR test. The pH must drop to 4.4 or less for the MR indicator to take on its acidic red color. Voges Proskauer tests the ability of bacteria to metabolize glucose with production of acetyl methyl carbine acetoin which is an intermediate product to butylene glycol. This intermediate neutral product is then assayed. In the presence of oxygen and 40 % potassium hydroxide, acetoin is converted to the diacetyl form, which results in a red color in the presence of alpha-napthol.

The methyl red (MR) and Voges-Proskauer (VP) broth, containing glucose and peptone was prepared and inoculated with a pure colony and incubated at 37^{O}C for 24 hours after which the MR-VP broth was split into two tubes. One tube was used for the MR test; and the other for the VP test. About 5 drops of the methyl red indicator was added to the first tube. Barrit's reagents, 3ml of solution A containing naphtholin and 1 ml of solution B containing 40 % potassium hydroxide solution were added to the second tube. A positive result was indicated by the appearance of red colour in the medium, while a negative result showed no change of colour.

2.2.4 Citrate Test

Principle: The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequen t increase in the pH of the medium is demonstrated by the colour change of a pH indicator. Citrate agar powder was prepared according to manufacturer's instruction and used in slants. Pure colony from MHA was inoculated on slant and stabbed to the butt. The slant was then incubated at 37 ^{O}C for 24hours. Positive test was indicated by a change in colour of the medium from green to blue; whereas no change in colour indicated negative result.

2.2.5 Urease tests

This tests the ability of bacteria to split urea into ammonia (NH_3), CO_2 and water (H_20), based on the possession of urease. Ammonia reacts in solution to form an alkaline compound, ammonium carbonate, which results in an increased pH of the medium and a color change in the indicator to pink red.

Urea Agar Base (Oxoid) was prepared according to manufacturer's instruction. One ampule of 40% sterile urea solution was added aseptically and mixed well. 10 ml was distributed into sterile tubes and allowed to set in the slope position. Pure colony from MHA was stab inoculated in the Urease agar, covered with cotton wool and incubated for 24 hours. Positive test was indicated by change in colour from yellow to pink colour.

2.3 Antimicrobial Sensitivity Tests

Confirmed *E. coli* were confirmed using *E coli* Chromagar and the biochemical tests and then tested for quinolone resistance using the disk diffusion method for carrying out the cultivation and sensitivity test of the *Enterobacteriaceae* as shown in Fig. 1. The standard guidelines employed were those of the Clinical and Laboratory Standards Institute (2019), and that of the British Society for Antimicrobial Chemotherapy (BSAC), as highlighted by Howe and Andrews (2012).[13] The bacterial colony was inoculated in 5ml sterile normal saline and adjusted to a 0.5 McFarland Standard. A sterile cotton wool swab was dipped in the prepared bacterial suspension and used to streak the surface of Mueller Hinton Agar plates, ensuring the entire smooth uniform coverage of the plates. Filter paper disks impregnated with known concentrations of the antibiotics: *Ciprofloxacin (10 ug), Ofloxacin (30 ug), Pefloxacin (10 ug), Sparfloxacin (10 ug), Moxifloxacin (5 ug) and Levofloxacin (20ug)* obtained commercially (Maxi-care) were used and deposited on the agar plate surface. The plates were incubated at 37 ^{o}C for 24 h. The result was interpreted using BSAC standard for Quinolone antibiotic breakpoint for Enterobacteriaceae using zone of inhibition.[13]

Fig. 1: Sensitivity pattern of the *E. coli* isolates

2.4 Extraction of Plasmid DNA

Extraction of Plasmid DNA from the isolates was done using Zymo Research Plasmid Miniprep *Classic* kit (ZR, USA). The plasmid wash buffer was constituted by adding ethanol (100%) to the supplied buffer. 0.5 ml of bacterial culture (bacterial colony emulsified in Tris EDTA buffer pH 8.0) stored in a clear 1.5 ml tube was centrifuged at full speed for 20 seconds in a micro-centrifuge. 200 µl of P1Buffer was added to the tube and the pellet was re-suspended completely (by vortexing). Then 200 µl of P2 Buffer was added and the contents mixed by inverting the tube 4 times, without vortexing, and then incubated for 2 minutes to ensure complete lysis of the cells. 400 µl of P3 Buffer was added and contents mixed gently but thoroughly, without vortexing. The sample turned yellow, indicating complete neutralization. The lysate was then allowed to incubate at room temperature for 2 mins and then centrifuged for 2 min at 15,000 rpm.

3. Results

3.1 Escherichia coli Isolates

Table 1 shows the sample types and the corresponding numbers of confirmed *E. coli* isolates. A total of 274 suspected *E. coli* isolates were collected from various medical laboratories. The analysis of these isolates indicated that 138 (50.4 %) were actually *E. coli* isolates, while 136 (49.6 %) belonged to other Enterobacteriaceae groups. Urine specimens gave the highest number of *E. coli* 102 (37.2 %).

Table 1: Clinical Sources of Samples for Isolation of *Escherichia coli*

Sample type	Presumed *E. coli*	Confirmed *E. coli*	Percentage
Pus Aspirate	1	1	0.4
Catheter tip	2	1	0.4
Ear swab	1	0	0
ECS	4	0	0
HVS	11	5	1.8
Semen	5	1	0.4
Stool	24	15	5.5
Throat Swab	1	0	
Urine	176	102	37.2
Wound Swab	49	13	4.7
TOTAL	**274**	**138**	50.4

ECS is an Endocervical swab, HVS is a high vaginal swab. About 50.4 % of pre-diagnosed E. coli from participating laboratories were confirmed E. coli using the E. coli Chromagar.

3.2 Susceptibility Test Result of the *E. coli* Isolates to Quinolone Antibiotics

Great majority of the *E. coli* isolates showed resistance to ciprofloxacin 88 (96.7 %); Ofloxacin 91 (100 %); Pefloxacin 84 (92.3 %); spafloxacin 85 (93.4 %) and moxifloxacin 90 (98.9 %) (Table 2).

Table 2: Susceptibility of *E. coli i*solates to Quinolone Antibiotics

Antibiotic	Sensitive	Intermediate	Resistant	Percentage of the Resistant Isolates
Ciprofloxacin	2 (≥20mm)	1(17-19mm)	88 (≤ 16mm)	96.7
Ofloxacin	0 (≥29mm)	0(26-28mm)	91 (≤ 25mm)	100.0
Pefloxacin	4 (≥20mm)	3(17-19mm)	84 (≤16 mm)	92.3
Pefloxacin	6 (≥20mm)	0 (17-19mm)	85 (≤16 mm)	93.4
Moxifloxacin	0 (≥20mm)	1 (17-19mm)	90 (≤ 16mm)	98.9
Levofloxacin	33 (≥17mm)	16(14-16mm)	41 (≤ 13mm)	45.1

3.3 Plasmid Mediated Resistance Gene

3.3.1 qnr Genes

qnr S genes were found the most prevalent quinolone (*qnr*) gene in the clinically isolated *E. coli*, with a prevalence rate of 71.4 %. *qnr* B was present in fifteen of the isolates at the rate of 15.4 %. *qnr* A was not detected in any of the isolates. Eleven (12.1 %) of the isolates showed both qnrS and qnr B. Table 3 shows the frequency of plasmid-mediated quinolone (*qnrA*, *qnrB*, *qnrS*) genes and Fig. 2a shows the *qnr* genes on agar gel. *oqxB* of the Efflux pump gene recorded the highest prevalence (25.3 %), followed by qepA (12.1 %), and then, *oqxA* (7.7 %) (Table 3).

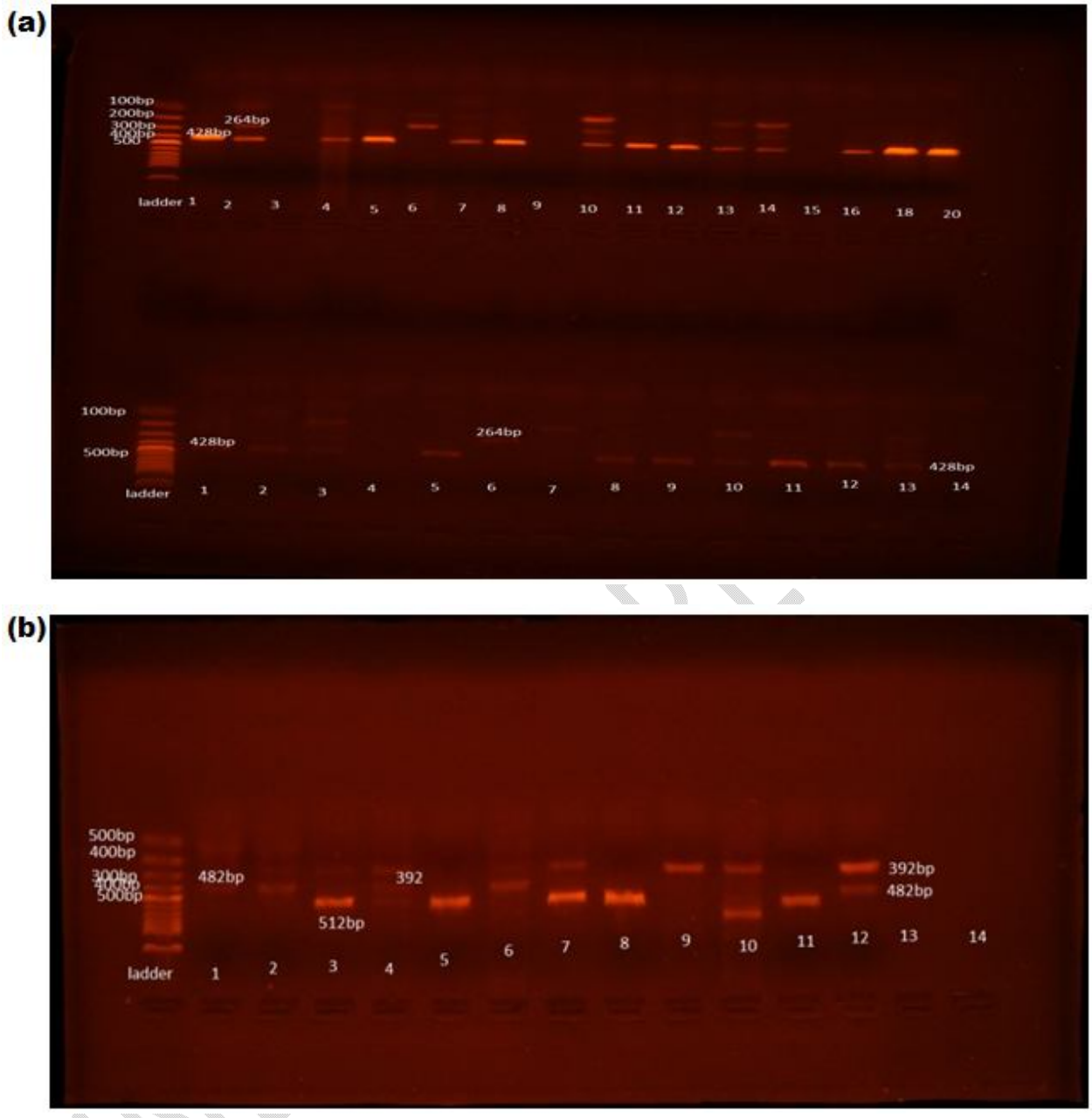

Fig. 2. (a) qnr genes (b) Aminoglycosyl acetyltransferase and Efflux genes

Table 3: Frequency of Plasmid-Mediated Quinolone Resistance Genes in *E. coli*

Type of gene	Specific gene	Positive Cases	Percentage
qnr	qnrS	65	71.4
	qnrB	14	15.4
	qnrA	0	
	qnrS & B	11	12.1

amg	aac (6) lb-cr	4	4.4
Efflux	oqxA	7	7.7
	oqxB	23	25.3
	qeqA	11	12.1
	oqxA & oqxB	5	5.5
No Gene		18	

3.3.2 The Aminoglycoside Acetyltransferase Gene

The Aminoglycoside acetyltransferase gene was seen only in four isolates (4%) (Table 3). Fig. 2b shows the aac (6) lb-cr, and the three efflux genes, *oqxA, oqxB and qepA*. The aac-(6) *lb-cr* is 482 bp and lies close to the 500 bp of the ladder; *oqxA* is 392 bp and is located close to the fourth bar (400 bp) of the ladder; *oqxB* is 512 bp and is found above the 500 bp of the ladder, while *qepA* is 199 bp and lies close to 200 bp of the ladder.

3.3.3 Clinical *E. coli* Isolates and PMQR Gene Carriage

Given the total of 91 *E coli* isolates subjected to Plasmid quinolone-resistant genes, 68 (74.7 %) were positive for *qnr* genes while 23 (25.3 %) were negative for qnr genes; 36(39.6 %) were positive for the *Efflux* pump genes, while 54 (59.3 %) were negative; only 4 (4.4 %) were positive for the *aac (6') lb-cr* while the great majority 87 (95.6 %) were negative as presented in Table 4.

Table 4*:* No of the *E coli* isolates Positive and Negative for the PMQRGenes ***(N=91)***

PMQR Gene	No positive	% age	No Negative	% age
Qrn	68	74.4	23	25.3
Efflux	36	39.6	54	59.3
Aac(6') ib-cr	4	4.4	87	95.6
Total Positive for PMQR Gene	73	80.2		93.4
Total negative for PMQR Gene			18	19.8

3.3.4 Plasmid Gene and the Antibiotic Profiles of the *E. coli* Isolates

Tables 5 and 6 showed the plasmid gene profile of the *E. coli* Isolates and their frequencies. The profile with the highest frequency is *Qnr* alone 36 (39.6 %), followed by *QNR /Efflux*, 28 (30.8 %), and then efflux alone 5(5.5 %). A total of 18(19.8 %) of the population were found to possess none of the genes. Going down the analysis into the *PMQR* gene profile in Table 6, Qnr S occurring alone has the highest-profile(30.8 %), followed by *QnrS/OqxB (QNR /Efflux*),12.1 % and then followed by *Qnr S/ QepA* (also *QNR /Efflux*), 8.8 % as detected in section 2.5.

Table 5: Frequency Table of the Plasmid Gene Profile of the *E. coli* Isolates

Gene profile	Frequency	Percentage frequency
QNR/ EFFLUX/ AMG	1	1.1
QNR / EFFLUX	28	30.8
QNR/ AMG/	1	1.1
EFFLUX/ AMG	1	1.1
EFFLUX/	5	5.5
QNR /	36	39.6
AMG/	1	1.1
None Present	18	19.8

Table 6: Quinolone Resistance Gene Profile of the *E. coli* Isolates

Gene profile	Frequency	Percentage frequency
QNR S/QNRB/OqXB	2	**2.2**
QNR S/QNRB/OqXA	0	**0.0**
QNR S/QNRB/qepA	2	**2.2**
QNR S/QNRB/AA	1	**1.1**
QNR B/OqxB	1	**1.1**
QNR B/OqxA	0	**0.0**
QNR B /qEpA	0	**0.0**
QNR S/OqxB	11	**12.1**
QNR S/ qEpA	8	**8.8**
QNR S/ qEpA/AA	1	**1.1**
QNR S/OqxA	1	**1.1**
QNR S/OqxA/AA	1	**1.1**
QNRS/OqxA/OqxB	3	**3.3**

QNR B/OqxA/OqxB	0	**0.0**
QNRS/OqxA/OqxB/AA	1	**1.1**
QNR S/QNRB	6	**6.6**
OqxA/OqxB	1	**1.1**
QNR S/	28	**30.8**
QNR B/	2	**2.2**
OqxA/	0	**0.0**
OqxB/	4	**4.4**
qEpA/	0	**0.0**
AA/	0	**0.0**
None /	18	**19.8**
Total	**91**	**100**

3.3.5 Quinolone Drugs and *E. coli* Resistance: Plasmid Gene Predominance and Frequency

Qnr S is shown to be the leading gene in all the Resisted quinolones. There is also a low frequency of Qnr S coexisting with Qnr B in all the drugs. The efflux pump gene, OqxB was the most frequent of the efflux pump genes and was found to occur alone or in combination with the other efflux or Qnr genes. In almost all the drugs (except in Levofloxacin that was least resisted), all the PMQR genes, Qnr, aac (6') lb-cr, and efflux pumps were all fully represented, alone or in combination with others as shown in Table 7.

Table 7: Frequency of the Plasmid Quinolone Genes in the Quinolone Drugs

Quinolone	**Aac (6') lb-cr + Efflux Genes**											
	Qnr S	**Qnr B**	**QNR B+ S**	**AA**	**Efflux Gene Absent**	**OqxA**	**OqxB**	**QepA**	**AA +OqxA**	**OqxA + OqxB**	**AA+ OqxA +OqxB**	**AA+ QepA**
cip (n=88)	64	14	11	4	52	6	22	11	1	3	1	1
oflo (91)	65	14	11	4	51	7	23	11	1	4	1	1
Pef (n=84)	61	12	10	4	47	7	23	11	1	4	1	1
spa(n=85)	61	12	10	4	49	6	22	11	1	3	1	1

Moxi(n=90)	53	13	10	4	52	6	22	11	1	4	1	1
levo(n=41)	30	6	5	1	22	3	14	4	0	1	1	0

Cip = ciprofloxacin; Oflo= Ofloxacin; pef= pefloxacin; Spa= Spafloxacin; Moxi = moxifloxacin; Levo= levofloxacin; AA=aac (6') lb-cr (aminoglycosyl acetyltransferase gene)

Table 8(a) and (b) show the antibiotic profile of the clinical *E. coli* isolates and the summary, respectively. The six and the five-antibiotic resistance profiles had the highest frequency of 27 (29.7 %) and 42 (46.2 %), respectively, with all the different genes elaborated.

Table 8(a): Antibiotic Profile of *E. coli* Isolates and their Plasmid Genes.

Antibiotic Profile	**Total No of Isolate**	**Qnr gene(s) Present**	**Efflux gene(s) Present**	**AMG gene Present**
Cip/Oflo /pef / Spa Moxi/ Levo	27	19	8	1
Cip/ Oflo/ pef /Spa/ Moxi	41	31	22	2
S/N	1	1	0	0
Cip/ pef /Spa/ Moxi	1	1	1	0
Cip/ Oflo/ Spa/ Moxi	2	2	0	1
Cip/ Oflo/ pef// Spa	8	7	3	0
Cip Oflo/ pef	1	1	0	0
Cip/ pef/ Moxi	1	0	0	0
Cip/ Spa/ Moxi	1	0	0	0
Oflo/ Moxi/ levo	1	1	1	0
Pef/ Spa/ moxi	1	1	1	0
Spa/ Moxi	1	0	0	0
Cip/ moxi	1	1	0	0
Cip	4	4	0	0

Cip = ciprofloxacin; Oflo= Ofloxacin; pef= pefloxacin; Spa= Spafloxacin; Moxi = moxifloxacin; Levo= levofloxacin; ATCC= American Typed; EC=*E. coli*

Table 8(b): Antibiotic Profile of the Resistant *E. coli* Isolates and their Plasmid Genes

Number of Antibiotics Resistant To	Number of Strains Showing Pattern	Qnr Gene Present	Efflux Gene Present	AMG Gene Present
One Antibiotic (Cip Only)	4	4	0	0
Two Antibiotics	3	1	0	0
Three Antibiotics	5	3	2	0
Four Antibiotics	11	10	4	1
Five Antibiotics	42	32	22	2
Six Antibiotics	27	19	8	1

4. Discussion

The results of the susceptibility testing gives a picture of quinolone resistance in the study area. Five of the 6 quinolone drugs were resisted by the greater majority (>90 %) of the *E. coli* isolates.

The frequency of the PMQR genes 73(80.2 %) obtained in this study is much higher than what was found among *E. coli.* in Lome, Togo, the frequency of 67.03 % was reported by Salah *et al.* (2019).[14]. Much lower frequencies were obtained in many other places; in Mexico, it was reported in 32.1 % ;[15] in the United Kingdom (UK) in 35.2 %.[16] In China in 43.6 %,[17] while in Niger, it was reported in 44.4 %.[18] In the Republic of Korea, the frequency was even lower, 5 %.[19] Similarly, the frequency of the *qnr* genes obtained here varied from what was reported in the other countries. In Mexico, *qnr* A was obtained in clinical isolates of *E coli* in 22.7 %., qnr B in 20.9 % and qnr S in 6.4 %. In Iran, Firoozeh *et al.*, (2014) obtained 22.2 % of qnr A and 14.3 % of qnr B from 63 ciprofloxacin-resistant isolates. While similar results of low qnr A and B were said to have been obtained in Jamaica, UK and Spain; in Japan, a lower frequency of 6.5 % was obtained of qnr A, while qnr S and B were not found.[20] This is a clear contradiction of the current study where qnr S and qnr B were prevalent and qnr A not detected. In Korea, Pallecchi *et al*. (2009)[21] found qnr B dominant while qnr A was not found. Qnr S and qnr B as found out in this study, often occurred together in the same isolates with a prevalence of 12 %. The prevalence of aac (6) lb-cr (4 %) found in our study is quite low compared to the prevalence of 34.5 % found in the UK by Ciesielczuk *et al.* (2013) and 18 % detected in Southern Nigeria

among species of Gram-negative bacteria. However, their presence indicates their availability in our society and their importance in reducing the activity of Ciprofloxacin by N-acetylation at the amino nitrogen on its piperazine substituent. [22]

The frequency of the efflux pump genes obtained in this study, 41(18.55 %), is very similar to the percentage frequency of 18.7 % obtained by Ogbolu *et al*., (2016)[23] in Southern Nigeria. However, the frequency of *OqxB* and *QepA* is by far higher than what Ciesielczuk *et al* (2013)[16] obtained in the UK. By implication, these genes contribute to the flushing out of the quinolone antibiotics from the cell membrane of the organism, thereby contributing to the high resistance of the *fluoroquinolones*.

5. Conclusion

We report a very high frequency of the Plasmid-mediated quinolone resistance genes in multi-resistant clinical isolates of Escherichia coli. Qnr S is the leading gene, while Qnr A was not detected. The plasmid genes, therefore, contribute to the high quinolone resistance being recorded in our society since more of the genes were detected in those isolates that resisted five and six of the quinolone drugs. The fact that these genes are carried on the Plasmid may facilitate their easy spread to other bacterial species. Whole-genome sequencing will be required to expose the molecular mechanisms of quinolone antibiotic resistance, which will enable better interventions in antimicrobial resistance.

References

[1] Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. *In Pathogens and Global Health*. 2015;109: 309-18. doi: 10.1179/2047773215Y.0000000030.

[2] Zaman S B, Hussain M A, Nye R, Mehta V, Mamun K T, Hossain N. A Review on Antibiotic Resistance: Alarm Bells are Ringing. *Cureus. 2017;* 9: e1403. doi: 10.7759/cureus.1403.

[3] WHO, "Antimicrobial resistance. Global report on surveillance.," World Health Organization. , 2014. [Online]. https://doi.org/10.1007/s13312-014-0374-3.

[4] Penders J, Stobberingh E E, Savelkoul P H M, Wolffs P F G. The human microbiome as a reservoir of antimicrobial resistance. *In Frontiers in Microbiology*, 2013. https://doi.org/10.3389/fmicb.2013.00087.

[5] Etienne M, Lefebvre E, Frebourg N, Hamel H, Pestel-Caron M, Caron F et al. Antibiotic treatment of acute uncomplicated cystitis based on rapid urine test and local epidemiology:Lessons from a primary care series. BMC Infect Dis. 2014; 14:137. doi: 10.1186/1471-2334-14-137.

[6] Martín-Gutiérrez G, Rodríguez-Martínez J M, Pascual Á, Rodríguez-Beltrán J, Blázquez J. Plasmidic qnr genes confer clinical resistance to ciprofloxacin under urinary tract physiological conditions.

Antimicrobial Agents and Chemotherapy. 2017; 61: e02615-16. https://doi.org/10.1128/AAC.02615-16.

[7] Pham T D M, Ziora Z M, Blaskovich M A T. MedChemComm Quinolone antibiotics. *Med. Chem. Commun. 2019;* 10:1719–1739. DOI: https://doi.org/10.1039/C9MD00120D.

[8] Sárközy G. Quinolones: A class of antimicrobial agents. *Veterinarni Medicina. 2001;* 46: 9-10. 257–274.

[9] Tarchouna M, Ferjani A, Marzouk M, Guedda I Boukadida J. Prevalence of plasmid-mediated quinolone resistance determinants among clinical isolates of Escherichia coli in a Tunisian hospital. *International Journal of Current Microbiology and Applied Sciences. 2015;* 4:195–206. DOI: 10.4236/aim.2021.1110041.

[10] Todorović D, Velhner M, Grego E, Vidanović D, Milanov D, Krnjaić D, Kehrenberg C. Molecular Characterization of Multidrug-Resistant Escherichia coli Isolates from Bovine Clinical Mastitis and Pigs in the Vojvodina Province, Serbia. *Microbial Drug Resistance. 2017;* 24: 95–103. DOI: 10.1089/mdr.2017.0016.

[11] Rodríguez-Martínez J M, Cano M E, Velasco C, Martínez-Martínez L, Pascual Á. Plasmid-mediated quinolone resistance: An update. *Journal of Infection and Chemotherapy. 2011;* 17:149–182. DOI: 10.1007/s10156-010-0120-2.

[12] Aldred K J, Kerns R J, Osheroff N. Mechanism of quinolone action and resistance. *Biochemistry. 2014;* 53:1565–1574. doi: 10.1021/bi5000564.

[13] Howe R A, Andrews J M. BSAC standardized disc susceptibility testing method (version 11). *Journal of Antimicrobial Chemotherapy. 2012;* 67: 2783–2784. DOI: 10.1093/jac/dks391.

[14] Salah F D, Soubeiga S T, Ouattara A K, Sadji A Y, Metuor-Dabire A, Obiri-Yeboah D, Banla-Kere A, Karou S, Simpore J. Distribution of quinolone resistance gene (qnr) in ESBL-producing Escherichia coli and Klebsiella spp. in in Lomé, Togo. *Antimicrobial Resistance and Infection Control. 2019;* 8: 1-8. DOI: https://doi.org/10.1186/s13756-019-0552-0.

[15] Ramírez-Castillo F Y, Moreno-Flores A C, Avelar-González F J, Márquez-Díaz F, Harel J, Guerrero-Barrera A L, An evaluation of multidrug-resistant Escherichia coli isolates in urinary tract infections from Aguascalientes, Mexico: Cross-sectional study. *Annals of Clinical Microbiology and Antimicrobials.* 2018; 17: 34. DOI: 10.1186/s12941-018-0286-5.

[16] Ciesielczuk H, Hornsey M, Choi V, Woodford N, Wareham D W. Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. *Journal of Medical Microbiology. 2013:* 62; 1823–1827. DOI: 10.1099/jmm.0.064428-0.

[17] Yang T, Zeng Z, Rao L, Chen X, He D, Lv L, Wang J, Zeng L, Feng M, Liu J H. The association between occurrence of plasmid-mediated quinolone resistance and ciprofloxacin resistance in Escherichia coli isolates of different origins. *Veterinary Microbiology.* 2014; 170; 89-96. https://doi.org/10.1016/j.vetmic.2014.01.019.

[18] Moumoni A et al. Quinolone Resistance (qnr) genes in fecal carriage of extended Spectrum beta-lactamases producing Enterobacteria isolated from children in Niger. *Current Reasearch in Microbiology and Biotechnology. 2017;* 5:953–957.

[19] Hong B K, Chi H P, Chung J K, Kim E C, Jacoby G A, Hooper D C. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. *Antimicrobial Agents and Chemotherapy*. 2009; 53: 639–645. DOI: https://doi.org/10.1128/AAC.01051-08.

[20] Firoozeh F, Zibaei M, Soleimani-Asl Y. Detection of plasmid-mediated qnr genes among the quinolone-resistant Escherichia coli isolates in Iran. *Journal of Infection in Developing Countries. 2014;* 8: 818–822. DOI: 10.3855/jidc.3746.

[21] Pallecchi L, Riccobono E, Mantella A, Bartalesi F, Sennati S, Gamboa H, et al. High prevalence of qnr genes in commensal enterobacteria from healthy children in Peru and Bolivia. *Antimicrobial Agents and Chemotherapy. 2009;* 56:26 DOI: 10.1128/AAC.01722-08.

[22] Robicsek A, Strahilevitz J, Jacoby G A, Macielag M, Abbanat D, Chi H P et al. Fluoroquinolone-modifying enzyme: A new adaptation of a common aminoglycoside acetyltransferase. *Nature Medicine. 2006;* 12: 83–88. https://doi.org/10.1038/nm1347.

[23] Ogbolu D O, Alli A O, Anorue M C, Daini O A , Oluwadun A. Distribution of plasmid-mediated quinolone resistance in Gram negative bacteria from a tertiary hospital in Nigeria. *Indian Journal of Pathology and Microbiology. 2016;* 9:322–3. DOI: 10.4103/0377-4929.188108.