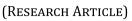


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Molecular Characterization of Plasmid-Mediated Quinolone resistance in Urinary *Escherichia coli* from some Healthcentres in Nasarawa-West Senatorial District, Nasarawa State, Nigeria

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Abstract

The most common bacteria associated with urinary tract infections (UTIs) worldwide *Escherichia coli*. To treat UTIs, Fluoroquinolones are widely used especially Ciprofloxacin. The honey moon did not last long due to the development of resistance acquired by the bacteria as a result of resistance gene. This study investigated ciprofloxacin resistant urinary *E. coli* resistance via plasmid-mediated quinolone resistance (PMQR) genes rather than the chromosomal mutation of patients attending some Healthcentres in Nasarawa-West Senatorial District, Nasarawa State, Nigeria. Ninety-Two (92) 42% confirmed ciprofloxacin urinary resistant *E. coli* from patients attending the three (3) study Centres were screened for the presence of PMQR resistant genes (*aac(6')-lb-cr, oqxA, oqxB, qnrA, qnrB, qnrS*) using polymerase chain reaction (PCR) method. The screening detected the presence of QnrA 4(44.4%), QnrB and QnrS both with 5(55.6%) occurrence, meaning *aac(6')-lb-cr*, QnrVC, GyrA, ParC and others were not detected. It is clear that these isolates harbored some PMQR genes both singly and in combination. This is a strong warning to both the prescribers and users of Antibiotic of the consequences of negligence of strict adherence to the usage of antimicrobials, since resistance to antibiotics has led to death of great number of People worldwide.

Keywords: Escherichia coli; Urine; Quinolones; Resistance; PMQR.

1. Introduction

Escherichia coli (*E. coli*) are Gram negative rod-shape facultative anaerobic bacteria belonging to the family Enterobacteriaceae. This organism is widely known as the most common etiological agent of Urinary tract infections (UTIs) causing about 75-80% of the UTIs in both hospital and community setting ^[1,2,3]. The treatment and control of UTIs are accomplished through therapy with different classes of antibiotics namely; β -lactam, fluoroquinolones, aminoglycosides and sulfamethoxazole/trimethoprim ^[4] and among the antibiotics mentioned, fluoroquinolones is widely used as empirical treatment of complicated and non-complicated UTIs ^[5,6]. Fluoroquinolones are synthetic, targeting enzymes involved in bacterial DNA replication ^[7, 8]. The emergence of strains of Enterobacteriaceae resistance to fluoroquinolones due to their continual use or misused has been reported in several studies ^[5, 6].

It has been agreed worldwide that Antimicrobial resistance poses serious potential threat to human health around the world. A review published in 2016 on Antimicrobial Resistance, that, by the year 2050, an estimate of as many as 10 million people could die annually from Antimicrobial Resistance. ^[9].

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The preliminary mechanism of fluoroquinolones resistance in bacteria is traditionally mediated by the mutation of chromosomal genes encoding DNA gyrase, topoisomerase IV, regulatory efflux pumps, and/or poring giving rise to new strains of the same species [8]. Recent reports indicate that quinolone resistance in Enterobacteriaceae may be due to plasmid resistance genes namely: oqxAB, qnrA, qnrB, qnrS, aac(6')-Ib-cr, and qepA ^[1,10]. The plasmid-mediated quinolone resistance (PMQR) genes namely: qnrA, qnrB, and qnrS code for proteins belonging to the pentapeptide repeat family interacting with DNA gyrase and topoisomerase IV enzymes to prevent the interaction of the quinolone with the enzymes [11]. The PMQR gene; aac(6')-lb-cr captured as a variant of aminoglycoside acetyltransferase, diminishes the fluoroquinolone activity by adding an acetyl group to this antimicrobial agent ^[12]. The quinolone efflux pump gene (*gepA*), is a proton-dependent transporter, which causes hydrophilic quinolone resistance. especially to norfloxacin, ciprofloxacin, and enrofloxacin^[13].

Plasmid-mediated guinolone resistance (PMOR) determinants have been recognized worldwide with a thoroughly high prevalence among extended-spectrum β -lactamases- (ESBLs-) producing and non-ESBL producing Enterobactericeae ^[10, 14]. Therefore, the research is meant to screen for the presence of PMQR gene and their level of spread within the study senatorial district.

2. Materials and Methods

Target gene	Primer	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	References
qnrA	QnrA-F	ATTTCTCACGCCAGGATTTG	580	[29]
	QnrA-R	ACGATGCCTGGTAGTTGTCC		
qnrB	QnrB-F	GATCGGCAAAGGTTAGGTGA	264	[29]
	QnrB-R	CGTTCTCACGCTCTGACATT		
qnrS	QnrS-F	GACCGGAATCCGATTTCTTC	428	[29]
	QnrS-R	CCAGGCCTTACGCTAACTTC		
qnrVC	QnrVC-F	GCTGATGATGATGATGATGATG	600	[29]
	QnrVC-R	CGGCGGCGGCGGCGGCGGCGG		
gyrA	GyrA-F	CAAATTCGCCCTCGAAACCCT	368	[29]
	GyrA-R	GCGGCGGCGGCGGCGGGGC		
parC	ParC-F	GCCCGTGCAGCGGCGCAT	219	[29]
	parC-R	GCGGCGGCGGCGGCGGGCGGGC		

Table 1 Primers and their amplicon sizes for detection of resistance gene in *Escherichia coli*

F= Forward; R= Reverse; bp = Base pair; DOI 10.1186/s13756-017-0249-1

2.1. PMQR gene E. coli Isolates

2.1.1. Isolation of Escherichia coli

Isolates of *E. coli* were obtained from the urine samples of patients with suspected cases of Urinary tract infections as follows; With the aid of a wire loop, the urine sample was streaked on MacConkey Agar plate and incubated at 37°C for 24 h. Pinkish colonies that grew on MacConkey agar were further inoculated on Eosin Methylene Blue agar and incubated at 37°C for 24 h. Greenish metallic sheen colonies that grew on the Eosin Methylene Blue agar plate were selected as presumptive E. coli.

2.1.2. Confirmation of Escherichia coli

Gram Staining

The Gram staining of the organism was carried out as earlier described by Cheesbrough ^[16]. Briefly, a smear of three (3) pure colonies of the organism was made on a drop of normal saline on a clean grease free slide and allowed to air-dry. The slide was passed twice through the flame to heat fix, then flooded with crystal violet solution for 30 sec and rinsed under slow running tap water. The washed slide was decolorized briefly with acetone, then immediately rinsed under

slow running tap water and counter-stained with safranin solution for 60 sec. The slide was again rinsed under slow running tap water, then allowed to air dry and then examined under x100 oil immersion objective.

2.1.3. Biochemical Tests

Indole Test

The indole test was carried out as follow; three (3) pure colonies of suspected organism was inoculated into 5 ml of peptone water in Bijou bottles and incubated at 37°C for 24 h. Few drops of Kovac's reagent were added to 24 h culture of the suspected. The formation of red ring at the top indicated an indole positive reaction.

Methyl Red Test/Voges-Proskauer Test

Methyl red/Voges-Proskauer test for suspected organism was carried out as follows; three (3) pure colonies of the suspected organism was inoculated into 10 ml of MR/VP medium in Bijou bottles and incubated at 37°C for 72 h. The 72 h culture was divided into two portions. To the first portion, some few drops of methyl red indicator were added and formation of red colour indicated methyl red positive. To the second portion, ten drops of 10% potassium hydroxide were added, followed by some drops of beta-naphthol. Formation of pinkish red colour indicated Voges-Proskauer positive.

Citrate Test

The Citrate test was carried out as follow; three (3) pure colonies of suspected organism were picked using sterile straight wire and stabbed on Simmons' citrate agar slant and incubated at 37°C for 72 h. Formation of green colour will indicate citrate positive.

Nine (9) confirmed *E. coli* isolated from urine of patients with suspected UTIs from some Healthcentres (General Hospital, Keffi – GHK; General Hospital Garaku – GHG and Medical Centre Mararaban-gurku – MCM-g) in Nasarawa-West Senatorial District, Nasarawa State, Nigeria that were resistant to ciprofloxacin were obtained and used for this study. The isolates were maintained at 4°C on nutrient agar (Oxoid Ltd, Basingstoke, UK) slants in a Refrigerator/Freezer (Model PRN 1313 HCA, BEKO, Germany); and sub-cultured on MacConkey agar (Oxoid Ltd, Basingstoke, UK) at 37°C for 24 h to obtain pure colonies before use for experiments.

2.2. Molecular Detection of Quinolones resistance genes

2.2.1. DNA extraction

The bacterial DNA was extracted by a method as earlier described by ^[17] with minor modification. Ten (10) milliliters of an overnight broth culture of the bacterial isolate in 1mL Luria Bertani (LB) were spun at 14000rpm for 3 min. The supernatant was discarded, and the harvested cell pellet was resuspended in 1 mL sterile distilled water and transferred into 1.5 mL centrifuge tube and centrifuged at 14000rpm for 10 min. The supernatant was discarded carefully. The pellet was resuspended in 100 μ L of sterile distilled water by vortexing. The tube was centrifuged again at 14000 g for 10 min, and the supernatant was discarded carefully. The cells were re-suspended in 500 μ l of normal saline and heated at 95°C for 20 min. The heated bacterial suspension was cooled on ice for 10mins and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20°C for other downstream reactions

2.2.2. DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. 2μ of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button ^[18].

2.2.3. DNA Amplification by Polymerase Chain Reaction (PCR) for quinolones resistance genes

The multiplex polymerase chain reaction (mPCR) for qnrA, qnrB, qnrS, qnrVC, gyrA and parC was performed in 45 μ l total volume using the ABI 9700 Applied Biosystems thermal cycler, containing 2.5 μ l MgCl2 (50 mM), 2.5 μ l 10 × buffer, 0.9 μ l of each primer (2.7 μ M), 8 mM of dNTPs, 0.5 μ l Taq DNA polymerase (Zymo research, USA) and 5 μ l of DNA template. Amplification was carried out in the following program: an initial denaturation step at 95°C for 5 minutes,

followed by 40 denaturation cycles at 94°C for 1 min, annealing at 55° for 30s and extension at 68°C for 30s, and a final extension step at 68 °C for 10 minutes.

2.3. Study Area

2.3.1. Study Centres

The study was carried out in the following health facilities in Nasarawa State, Nigeria, namely: General Hospital Garaku (GHG), General Hospital Keffi (GHK), and Medical Centre Mararaban-gurku (MCM-g). Figures 1-4 shows respectively, Map of Nigeria showing the Map of Nasarawa State located in Central Nigeria; Map of Nasarawa State and its thirteen Local Government Areas; Map of Nasarawa State showing the three (3) Senatorial Districts; Nasarawa-West Senatorial Districts showing the three (3) Local Governments where the three health facilities are located. The choice of the three study Centres is premise on them being the most populated towns within the Nasarawa-West Senatorial Districts with good patients turn out as well as serving as referral Centres by the nearby towns and villages' health facilities because of availability of qualify health professionals as well as equipment and facilities.

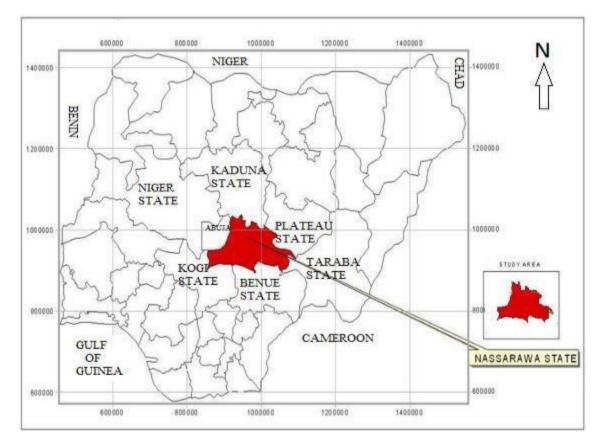


Figure 1 Map of Nigeria showing the Map of Nasarawa State located in Central

Nigeria (Source: https://www.researchgate.net/figure/Map-of-Nigeria-Showing-Nasarawa-State-the- studied-Area_fig1_316622675)

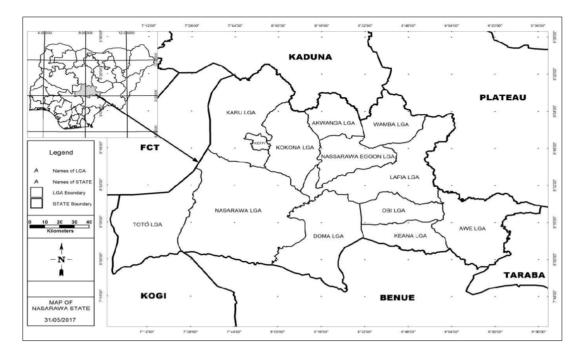


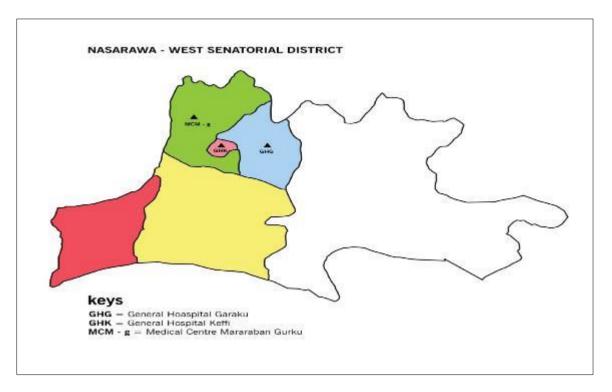
Figure 2 Map of Nasarawa State and its thirteen Local Government Areas

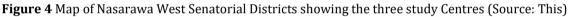
(Source: https://www.researchgate.net/figure/Map-of-Nasarawa-State_fig1_324538039)



Figure 3 Map of Nasarawa State showing the three Senatorial Districts

(Source: <u>https://en.wikipedia.org/wiki/2023 Nigerian Senate elections in Nasarawa State#/media/</u>File:2023_Nasarawa_Senate_preelection_situation.png)





2.3.2. Study Population

The targeted population in the Nasarawa-West Senatorial District in this research was about 716,802 being the last population figure (Nigeria 2006 Census - Nasarawa State – ^[19] using General Hospital Garaku (GHG), General Hospital Keffi (GHK) and Medical Centre Mararaban- gurku (MCM-g) as study Centres. See Table 3.2 ^[20]

 Table 2 Nasarawa-West Senatorial Zones, Nasarawa State, Nigeria

NASARAWA -WEST SENATOTIAL DISTRICT	716,802	
KARU	205,477	
KEFFI	92,664	
KOKONA	109,749	
NASARAWA	189,835	
тото	119,077	

https://nasarawastate.gov.ng/map-of-nasarawa-state/

2.3.3. Sample Determination and Size

The sample size for this research was determined using the formula as provided by Cochran, which is considered appropriate in situations with large populations as in this research (Population greater than 10,000) (https://www.statisticshowto.com/probability-and-statistics/find-sample-size/).

$$n_0 = \frac{Z^2 p q}{e^2}$$

The Cochran formula is:

Where:

 n_0 = the sample size

e = the desired level of precision (i.e. the margin of error),

p = assuming half of the population possess the investigated character. This gives us maximum variability. So, p = 0.5.

q = 1 – p

z-value = 1.96 (Z value is found in the Z- table. Which for 95% confidence level, that is probability of 0.05, gives us 1.96 on the Z-table)

Substituting the values in the formula:

Z² = 1.96 x 1.96 = 3.8416

P = 0.5

q = 1 - 0.5 = 0.5

 $e = 0.5 \ge 0.0025$

$$n0 = \frac{3.8416 \, x \, 0.5 \, x \, 0.5}{0.0025}$$

$$n0 = \frac{0.9604}{0.0025}$$

n0 = 384.16 = 384

One hundred and twenty-eight (128) of urine samples were collected from each collection Centre, namely General Hospital Garaku (GHG), General Hospital Keffi (GHK), and Medical Centre Mararaban-gurku (MCM-g) being study Centres in Nasarawa-West Senatorial District.

2.4. Ethical Approval

The ethical approval was obtained from the Research and Ethical Committee of the Nasarawa State Ministry of Health, Nasarawa State, Nigeria.

2.5. Subject Selection (Eligibility criteria)

2.5.1. Inclusion criteria

Inclusion Strategy

The target patients were males and females attending the selected health facilities.

Exclusion Strategy: This study excluded female adults who are on their monthly menstruation whose urine may contain blood stains. Infants too were excluded. Patients who have taken antibiotics within the week of sampling were also exempted.

3. Results

3.1. Isolation and Identification of Escherichia coli

The Pinkish colonies on MCA and metallic sheen on EMB which was gram negative and Indole positive, methyl red positive, ONPG positive, Nitrate positive, Lysine positive, Ornithine positive, and other biochemical test were all observed inferring the presence of *E. coli* as shown in Table 3

Table 3 Cultural, Morphological and Biochemical Characteristics of Escherichia coli from Healthcentres of Garaku, Keffi
and Maraban-gurku townships, Nasarawa State, Nigeria

Cultural Characteristi	Morphological Characteristics		Biochemical Characteristics									Inferen ce			
cs	Gram Reactio n	Morpholo gy	In d	M r	V p	C t	TD A	ONP G	Ly s	OR N	U r	N t	H ₂ S	Ma l	
Pinkish colonies on MCA and Greenish	-	Rod Shape	+	+	-	-	-	+	+	+	-	+	-	-	E. coli
Metallic Sheen on EMB Agar															

Key: + = Positive; - = Negative; IND = Indole; MR = Methyl Red; VP = Voges-Proskauer; CT = Citrate; TDA = Tryptophan Deaminase, ONPG = Ortho-Nitrophenyl-β-galactoside, LYS = Lysine; ORN = Ornithine,UR = Urease, NT = Nitrate, H₂S = Hydrogen Sulphide; Mal = Malonate

3.2. Occurrence of Escherichia coli

The occurrence of *E. coli* isolates in relation to the total sample size of 384 was 92(24%) as shown in Figure 5. At the GHK, the occurrence was 28 (21.9%), GHG had 25(19.5%) occurrence while MCM-g had 39(30.5%).

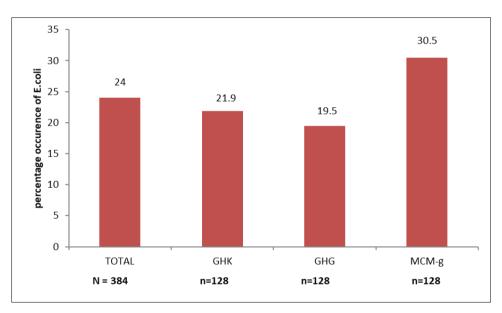


Figure 5 Antibiotics Resistance Profile of *Escherichia coli* isolated from Healthcentres in Keffi, Garaku and Mararaban-gurku, Townships Nasarawa State, Nigeria

3.3. Antibiotic Resistance Profile

Antibiotics Resistance profiles of the *E. coli* isolated from urine of patients attending health facilities in the three Hospitals (GHK, GHG and MCM-g) are shown in Table 4. The isolates were generally more resistant to Ampicillin (AMP) with 75.0%. This was followed by Streptomycin (S) 70.7% which was then closely followed by Cefotaxime (CTX) 68.5% but less resistant to Ciprofloxacin (9.8%) followed by Gentamicin 17.4%.

	Disk Content	No (%) of Resistance					
	(µg)	GH	GH	Med. Centre M/Gurku n=39	Total		
ANTIBIOTICS		Keffi	Garaku				
		n =28	n = 25		n = 92		
Amoxicillin/Clavulanate (AMC)	30	5 (17.9)	11 (44.0)	16 (41.0)	32 (34.8)		
Ampicillin (AMP)	30	15 (53.6)	19 (76.0)	35 (89.7)	69 (75.0)		
Ceftazidime (CAZ)	30	7 (25.0)	12 (48.0)	15 (38.5)	34 (37.0)		
Ciprofloxacin (CIP)	5	0 (0.0)	2 (8.0)	7 (17.9)	9 (9.8)		
Gentamicin (CN)	10	1(3.6)	4(16.0)	11 (28.2)	16 (17.4)		
Cefotaxime (CTX)	30	15 (65.2)	14 (56.0)	34 (37.2)	63 (68.5)		
Imipenam (IPM)	10	7 (25.0)	7 (28.0)	11 (28.2)	25 (27,2)		
Nitrofurantoin (NIT)	300	6 (21.4)	9 (36.0)	4 (10.3)	19 (20,7)		
Streptomycin (S)	10	16 (57.1)	18(72.0)	31 (79.5)	65 (70.7)		
Co-trimoxazole (SXT)	25	11 (39,3)	11 (44.0)	23 (59.0)	45 (49.0)		

Table 4 Antibiotics Resistance Profile of *Escherichia coli* isolated from Healthcentres in Keffi, Garaku, and Mararaban

 gurku townships, Nasarawa State, Nigeria

GH= General Hospital; Med. Centre= Medical Centre; M/Gurku= Mararaban-gurku

3.4. Detection of plasmid-mediated Quinolone Resistance genes (QnrA, QnrS and QnrB).

Figure 6 showed the detection of three (3) fluoroquinolone resistance gene (QnrA, QnrS and QnrB) in the isolate (FREC). Some isolates showed the presence of more than one resistance gene on them. For example, isolates 8, 13, 20 and 38 all had two (2) each of the PMQR genes with QnrA/QnrB, QnrB/QnrS, QnrA/QnrS and QnrB/QnrS respectively being present in those samples. Samples 13 from General Hospital Keffi and 38 from General Hospital Garaku had same type PMQR genes of QnrS/QnrB. Isolate 35 from Medical Centre Mararaban-gurku had the three Quinolone resistance genes (QnrA/QnrS /QnrB).

Lane 8, 20 and 35 from Medical Centre Mararaban-gurku and lane 48 from General Hospital Keffi represent the expression of QnrA (580bp). While lane 7, 20 and 35 from Medical Centre Mararaban-gurku, while 13 from General Hospital Keffi and 38 from General Hospital Garaku represents the expression of QnrS (428bp) while lane 5 and 38 from General Hospital Garaku and 8 and 35 from Medical Centre Mararaban-gurku and 13 from General Hospital, Keffi represents the expression QnrB (264bp).

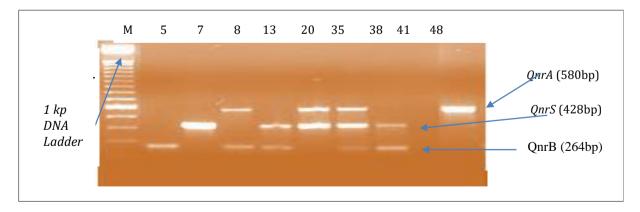


Figure 6 Agarose gel electrophoresis of the amplified quinolones resistance genes of Escherichia coli isolates. Lane 8, 20, 35 and 48 represent the expression of the QnrA (580bp) gene; Lane 7, 13, 20, 35, and Lane 38 represent the expression of the QnrS (428bp) gene; Lane 5, 8, 35 and Lane E38 represent the expression of the QnrB(264bp) gene while Lane M represents 1kp DNA molecular ladder.

3.5. Percentage occurrence of PMQR gene present in *Escherichia coli* isolated from Healthcentres in Keffi, Garaku, and Mararaban-gurku townships, Nasarawa State, Nigeria

The result showed the presence of three (3) PMQR with QnrB and QnrS having the highest percentage occurrence of 55.6% each while QnrA being the least with 44.4%. see table 3.3.

Table 5 Percentage occurrence of PMQR gene present in *Escherichia coli* isolated from Healthcentres in Keffi, Garaku,and Mararaban- Gurku townships, Nasarawa State, Nigeria

PMQR Gene	Samples	% Occurrence			
QnrA	8,20,35,48	4(44.4%)			
QnrB	5, 8, 13, 35, 38	5(55.6%)			
QnrS	7,13, 20, 35, 38	5(55.6%)			

4. Discussion

Escherichia coli is one of the most common etiological agents of both community and hospital acquired urinary tract infections worldwide ^[21]. The observed percentage occurrences of *E. coli* from urine of the patients was actually expected, in agreement with the study previously described by Odongo ^[22], Giri ^[23]. The percentage occurrences of *E. coli* from urine of the patients observed in this study 24% was a little lower than 28.7% as reported by Mouanga ^[24]and 40% as reported by Ojezele ^[25], 59.3% by Giri ^[23]. The isolation of *E. coli* from urine of the patients is an indication that this organism may be responsible for the UTIs.

The Antibiotic Resistance Profile of *E. coli* is expected as antibiotic resistance is now a common phenomenon that is known to be responsible for 1.27 million global deaths directly in 2019 and contributed to 4.95 million deaths about 10million for deaths ^[26]. Although, the resistance to ciprofloxacin (being the antibiotic of interest in this research being a quinolone) was low (9.8%) in comparison to other antibiotics used in this study such as the ones mentioned above, it is however still lower when compared to 18.8% as reported by Silva ^{[27].}

Plasmid-mediated quinolone resistance genes are well documented to contributing to Antibiotic resistance especially the ESBL- producing isolates. As reported by Jomehzadeh [^{28]} in South-West Iran, 85% of all his isolates harbored at least one PMQR gene, the most frequent one being aac(6')-Ib-cr variant (47.4%). Coexistence of aac(6')-Ib-cr variant and qnrB were the most broadly distributed genotype among quinolone resistance isolates. Notably, none of the isolates contained the qnrC, qnrD, and qepA genes. Of the 300 *E. coli* isolates from water samples, 66% were positive for PMQR genes. Of these, the majority of isolates were positive for *qnrS* (82%, n = 164) followed by *aac(6')*-*lb*-cr (9% n = 17), *oqxAB* (7%, n = 13) *qnrB* (6%, n = 11) and *qepA* (4%, n = 8). None of the isolates was positive for *qnrA*, *qnrC* & *qnrD*. ^[29]. Though the *aac(6')*-*lb*-cr strain of the PMQR gene being the most frequent, as reported by Jomehzadeh ^[28] and Amin ^[29]was not isolated, the next frequent strain of QnrB was indeed found to be most frequent with 55% occurrence as reported by Jomehzadeh ^[28] QnrS shares the same percentage occurrence of 55% in agreement with Amin ^[29]

The absence of qnrC, qnrD, and qepA genes reflected the position of Jomehzadeh ^[28] meaning they are less found on isolates.

From the result, three (3) Plasmid-mediated quinolone resistance genes (PMQR) were confirmed to be present in the samples. In general, all the 9 samples that were analyzed for the presence of the PMQR gene had two (2) or more present except sample 5 and 7 from GHG and MCM-g that had one PMQR gene each QnrB and QnrS respectively. Sample 8 from MCM-g has two of the PMQR gene of QnrA and QnrB present. Sample 13 and 38 from GHK and GHG respectively both have QnrB and QnrS genes. Likewise, sample 20 from GHG has 2 of the PMQR genes QnrS and QnrA. While sample 35 had all the three (3) PMQR genes QnrA, QnrB and QnrS.

5. Conclusion

This study so far investigated the presence of *E. coli* in urine of patients that attended three health facilities at Keffi (GHK), Garaku (GHG) and Mararaban-gurku (MCM-g) townships in Nasarawa State, Nigeria with percentage occurrence of 24%. The study also investigated the antibiotic resistance profiles, *E. coli* isolated from the urine of the patients where the isolates were less resistant to ciprofloxacin, Gentamicin and Nitrofurantoin with percentage resistance of 10.6%,

18.8% and 22.4% respectively. Three PMQR: QnrA, QnrB and QnrS were identified with QnrB and QnrS having equal and highest percentage occurrence (55.6%) with QnrA having the lowest percentage occurrence (44.4%). Other Strains (*qnrA*, *qnrC* & *qnrD*) of PMQR gene were not isolated. This research findings would help drum it loudly to both prescribers as well as users of antimicrobial of the need to be more conscious and deliberate to avoid medications misuse and abuse for the PMQR genes are here with us.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest.

Statement of ethical approval

The ethical approval was obtained from the Research and Ethical Committee of the Nasarawa State Ministry of Health, Nasarawa State, Nigeria.

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Author's Contributions

The study was designed by Eko KO, Ngwai YB, Igbawua IN and Nkene I., conducted the experiments. Ngwai YB, Ishaleku D supervised the study. Myself (Eko KO) prepared the manuscript. All authors proof-read and approved the article.

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