

Detection of QnrS genes in *E. coli* from Clinical isolates in Bayelsa State.

ABSTRACT

Escherichia coli (*E. coli*), is a Gram-negative rod known to be a member of the normal flora of both humans and animals. *E. coli* belong to the family of Enterobacteriaceae and a causative agent of urinary tract infections (UTIs) such as pyelonephritis, cystitis, and pneumonia. It is characterized by its oxidase activity, motility, lactose, glucose and sucrose fermentation property. It grows well at a temperature of 37°C and a pH of 6.0-7.0. Resistance occurs when an antibiotic is no longer effective in inhibiting the growth of and killing bacteria. Quinolone are a group of synthetic wide-spectrum antibiotic that are used in the treatment of several infections caused by members of the Enterobacteriaceae especially *E. coli*. Quinolone resistance gene, such as qnrA, qnrB and qnrS are the three main classes of qnr determinants which have been identified in several members of Enterobacteriaceae family such as *E. coli* and *K. pneumoniae*. Plasmid-mediated quinolone resistance is mediated by the genes (qnr) encoding proteins that belong to the pentapeptide repeat family and protect DNA gyrase and topoisomerase IV against quinolone compounds. This study was to detect the presence of qnrS gene in *E. coli* from clinical isolates using standard microbiology methods. These methods were based on macroscopic and microscopic characteristics, biochemical testing, API 20E, antimicrobial susceptibility testing with Gentamycin, Cefuroxime, Augmentin, septrin, Streptomycin, Ceporex, Nalidixic acid, Ciprofloxacin, Ofloxacin, Nitrofurantoin, and Meropenem and Polymerase chain Reaction (PCR) technique. A total fifty (50) clinical isolates were collected from Niger University Teaching Hospital, Okolobiri. These isolates were from urine, blood, stool, sputum, wound swab and High vaginal swab from both males and females. Out of the 50 isolates, 18(36%) were from males while 32(64%) were from females. Of the 50 isolates from these samples 15(30%) was *K. pneumoniae*, 11(22%) *P. aeruginosa*, 8(16%) *C. freundii*, 9(18%) *E. coli* and 7(14%) *P. mirabilis*. Out of the 50 isolates, 9(18%) *E. coli* were recovered and subjected to PCR amplification using specific primers after extraction of DNA from the isolates, 8(88.8%) were positive to qnrS gene. Susceptibility rates of *E. coli* to antibiotics was highest in Cefuroxime and Augmentin 9(100%), Gentamycin and Ofloxacin 5(55.55%) and Ciprofloxacin 1(11.11%) respectively. In conclusion, this study reveals a high prevalence of qnrS gene among *E. coli* from clinical isolates from NDUTH, Okolobiri. Therefore, more studies should be done for early detection of resistance quinolone gene that could help in early diagnosis and modification of quinolone with a wider spectrum of activity and improve treatment.

Key Words: Resistance genes, Quinolone, Enterobacteriaceae

INTRODUCTION

Escherichia coli (*E. coli*), is a common human and animal pathogen commonly implicated in urinary tract infections (UTIs) such as cystitis and pyelonephritis.² *E. coli* are Gram-negative, non-sporulating, a non-fastidious, motile, and facultative anaerobic rods belonging to the family Enterobacteriaceae. *E. coli* is a member of the normal flora in the gastrointestinal tract of human and warm blood animals. *E. coli* is oxidase negative, lactose, glucose, and sucrose fermenting. It grows at a temperature of 37°C and a pH of 6.0-7.0, while some diarrheagenic *E. coli* strains can tolerate a pH of 2.0. *E. coli* easily grows in the laboratory on MacConkey agar producing lactose-fermenter rose pink colonies. Eosin Methylene Blue medium is appropriate for the isolation of *E. coli* producing distinctive colonies with a characteristic green metallic sheen that differentiate it from any Enterobacteria.¹⁷ *E. coli* is a bacterium that is found almost everywhere and has been isolated from different sources. UTIs, which are recognized as the most commonly reported nosocomial infections, account for up to 35% of infections associated with the health-care system¹⁵.

Antibiotic resistance (ABR) is currently identified as one of the biggest threats not only to global health but also to food security and development. Resistance occurs when the antibiotics are no longer effective at inhibiting the growth or killing the bacteria¹⁹. Quinolones are a group of synthetic broad-spectrum antimicrobial agents that are helpful in the treatment of several infections caused by gram-negative bacteria, especially *E. coli*. However, over

the past few years, the gram-negative bacteria have increasingly turned to be resistant to quinolones.⁸ Several genes and systems are involved in quinolone resistance Enterobacteriaceae isolates

Bacteria acquire resistance to quinolones in two ways; one is the chromosomal mutation in the gene *gyrA* and *parC* which encode the quinolone targets DNA gyrase and topoisomerase and acquisition of plasmid containing genes for quinolone resistance. Chromosomal mutation gives high-level of resistance to quinolones and it is transmitted vertically while plasmid mediated quinolone resistance (PMQR) confers low-level of resistance, it is transmitted horizontally among distantly related organisms making their spread much faster than that of chromosomal mutation.⁷

Quinolone resistance gene (*qnrS*), a member of PMQR (plasmid mediated quinolone resistant) determinants, was first discovered in a strain of *Klebsiella pneumoniae* (*K.pneumonia*). The first member of this family (*qnrS*) is *qnrA* that was discovered in the USA in 1998. *Qnr* genes such as *qnrA*, *qnrB*, and *qnrS* are three types of the main classes of *qnr* determinants which have been identified in several members of Enterobacteriaceae family such as *E.coli* and *K.pneumonia*. However, over the past few years, some other classes of the gene namely *qnrC* and *qnrD* have been discovered⁸ this family has been classified into four generations based on their antimicrobial activity.

The most popular quinolone antibiotics are Nalidixic Acid, Ciprofloxacin, and Levofloxacin as members of the first, second, and third generations, respectively^{5, 6}. Resistance to quinolone is often linked to amino acid substitutions in the quinolone-resistance-determining regions of DNA gyrase (*gyrA* and *gyrB*) DNA topoisomerase IV (*parC* and *ParE*) subunits, leading to target modification. Inappropriate and unnecessary administration of these antibiotic has led to an increase in the development options¹³. Early detection of the gene that is resistance to quinolone could help in early diagnosis and modification of quinolone with a wider spectrum of activity and improve treatment.

METHODOLOGY

Study Area and Subject

The study was undertaken at Niger Delta University Teaching hospital, Okolobiri, Yenagoa Bayelsa State because of the presence of large number of patients. A total of fifty clinical samples were obtained from the Microbiology Department of NDUTH Okorobiri. Samples obtained were Urine, nose swab, ear swab, throat swab, wound swab, high vaginal swab and wound swab of both male and female outpatients and inpatients.

2.2 Ethical Consideration

Consents were obtained from the ethical committee of the Niger Delta University Teaching Hospital (NDUTH) Okolobiri. The consents of the patients were also sought for and obtained before the samples were obtained from them.

2.3 Collection of samples

A total of fifty clinical samples were obtained from the Microbiology Department of NDUTH Okorobiri. Samples obtained were Urine, nose swab, ear swab, throat swab, wound swab, high vaginal swab and wound swab of both male and female outpatients and inpatients.

Sample processing

Isolation

With the aid of sterile wire loop, the isolates were cultured aseptically on MacConkey agar, nutrient agar and Luria-Bertani (LB) Broth respectively and incubated at 37°C for 24 hours.

Identification

After 24 hours of incubation, the cultivated colonies were identified macroscopically and microscopically for pigmentation, size, texture, and degree of opacity and recorded.

Gram Staining

On a clean grease free glass slide, a drop of normal saline was placed and a colony of the test isolates was picked with the aid of a sterile wire loop and emulsified on the drop of saline. The slide was allowed to air dry and was heat fixed by passing the slide over a Bunsen flame 3 times. The smear was stained with crystal violet for 1 minute and rinsed with tap water. The slide was flooded with lugol's iodine for 1 minute and rinsed with tap water. It was then rapidly decolorized with acetone alcohol for few seconds and rinsed with tap water. The smear was counter stained with neutral red for 2 minutes and rinsed with tap water. The back of slide was cleaned dry with cotton wool and allowed to air dry. A drop of immersion oil was placed on the smear and it was view under the microscope using 100x objective lens.

Biochemical test

Oxidase :A pieces of filter paper was placed in a petri dish and soaked with oxidase reagent. With an applicator stick, a colony of the test organism was smeared on the filter paper and observed for color change within few seconds. No blue purple was observed indicating Oxidase negative test.

2.8 Identification of bacterial isolates (API 20E)

An incubator tray was placed on a flat surface and 5ml of sterile distilled water was distributed into the tray to create a humid chamber. The API 20E strip was placed in the tray. Using a sterile disposable applicator stick, a single colony from an isolate plate was emulsified in 5ml sterile distilled water. This was adjusted to 0.5 McFarland's standard. With a sterile disposable pipette, both the tube and cupule of the test CIT, VP, and GEL were filled with the bacterial suspension, while only the tubes were fill for others. An anaerobic environment was created in the test ADH, LDC, ODC, URE, and H₂S by filling with mineral oil. The tray was labeled with identification number and the lid placed. It was then incubated for 24 hours at 37°C. It was read by observing for color change by comparing with the API reading scale (Color chart).

Standardization of Bacteria Isolates

Isolated colonies from pure culture plates were sub-cultured into peptone water, and incubated for 12 hours. Turbidity was then adjusted by dilution with sterile peptone water until visually comparable with a MacFarland's 0.5 standard. The MacFarland's 0.5 standard was prepared by adding Barium Chloride (BaCl) with Tetraoxosulphate VI acid (H₂SO₄). H₂SO₄(1%) was prepared by measuring out 1ml of H₂SO₄ with the aid of a measuring cylinder and was added to a measured 99mls of distilled water in another beaker. Then, 99.4mls of the preparation was transferred into a clean beaker with the aid of sterile pipette.

The 0.6ml of Barium chloride solution was then added to 99.4mls of H₂SO₄ Given final volume of 100mls of Barium sulphate. The absorbance of the turbidity standard was verified using a spectrophotometer at 625nm and an Absorbance of -0.08 to 0.10 for the 0.5 Mac farland standard was read.

Antimicrobial Susceptibility Test

The antibiotic susceptibility testing of the pure isolates was carried out by agar diffusion method (Kirby Bauer) using commercially available disc to determine the drug sensitivity and resistance pattern of the isolates. With a sterile disposable applicator stick, a single colony from an isolate plate was emulsified in 5ml sterile distilled water. This was adjusted to 0.5 McFarland's standard. This was carried out according to the standard disc diffusion technique as described by Clinical Laboratory Standard Institute (CLSI). Paper discs (Abtek Biologicals) containing the antibiotics: The antibiotics included ciprofloxacin (CPX) 10mcg,

Gentamicin (CN) 10mcg, Augmentin (AU) 30mcg, Amoxicillin (AM) 30mcg, Erythromycin (E) 30mcg, Ofloxacin 10mcg, Tetramycin 30mcg, Cloxacilin 30mcg and Nitrofuratoin 30mcg .

A sterile cotton swab was dipped into the respective bacterial stock solution, and excess fluid from the swab was removed by pressing the swab against the wall of the tube. The Mueller-Hinton (MH) agar entire surface was then swabbed with the bacterial suspension, and allowed to air dry for 15 min. Antibiotic discs impregnated with a defined quantity of antimicrobial agent was placed and then layered aseptically on the Mueller-Hinton agar surface. The Plates were then incubated at 37°C overnight and the zones of inhibition (ZI) recorded. Interpretation of results were based on guidelines of the Clinical and Laboratory Standards. A concentration gradient of the antibiotic forms by diffusion from the disc and growth of the test organism is inhibited at a distance from the disc forming zone of inhibition. Zone of inhibition was measured with meter rule, which determines if the antibiotic used was resistance, intermediate, or sensitive to the isolates.

2.11 Molecular Analysis

DNA Extraction

The boiling method was used for the extraction of DNA from clinical isolates, which was inoculated into Luria Bertani (LB) medium in a small tube (2ml Eppendorf tube) and centrifuged in a Denville 260D brushless micro centrifuges at 1400 rpm for 4 minutes in a micro centrifuge. The supernatant was discarded using a micropipette and 1ml of 0.5 normal saline was added to the sediments and the tube was vortexed on eltech XH-B Vortex. The tube was incubated in the heating block at 95°C for 20 minutes; the tube was then taken to the freezer for fast cooling for 4 minutes. The tube was further centrifuged at 14000 rpm for 3 minutes and 500 µl of the supernatant was transferred into 1.5ml eppendorf tube and stored in the freezer at -2°C for further analysis.

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 µl of sterile distilled water and blanked using normal saline. 2 µl 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 “measure” button. DNA concentration was measured in ng/µl (5 – 100 ng/ µl) while purity level was determined at 260/280, and absorbance (1.5 – 2.0).

The PCR Amplification of qnrS Gene

The qnrS resistant gene allelic variant were amplified respectively using ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix include: the X2 Dream Taq polymerase master mix supplied by Inquababiotec, South Africa (Taq polymerase, dNTPs, MgCl), the primers at a concentration of 0.5M and 80ng of the extract DNA as a template. Touch-down PCR (Applied Biosystem Thermal Cycler) was used to detect the Integrons genes of the isolates. It was performed in a 20ml reaction mixture containing 2X master mix (Taq polymerase, dNTPs, MgCl², and Buffer). The forward and reverse primers at a concentration of 0.4N molar supplied by Inquaba Biotech in South Africa, Water and DNA extract as template were added. The PCR tube were arranged on ice rack, 19 µl of the cocktail was pipette into each of the labeled tubes and 1 µl of the DNA template from isolates were pipette into the labeled tubes containing the cocktail respectively.

The genes were amplified using the set PCR condition was as follow: initial denaturation (98°C for 30sec), followed by 35 cycles of denaturation (98°C for 10sec). Annealing (63°C for 30 seconds); initial extension (72°C for 6 min) and final extension (72°C for 10min).

The sequence of the primers

qnrS: 5'GCAAGTTCATTGAACAGGCT-3'

qnrS : 3'TCTAAACCGTCGAGTTCGGCG-5' 438bp

Agarose Gel Electrophoresis

One gram of Agarose powder was weighed on a weighing balance and poured into a conical flask with 100ml of already prepared 1X TAE buffer. It was mixed to dissolve very well for few seconds and then placed on microwave at 80 - 95 °c for 3-5minutes to dissolve properly. The dissolved gel powder in a conical flask was allowed to cool for few seconds. Combs were placed in the casting tray on one end to enable the shape of the gel. The gel was poured into the casting tray having combs placed on and allowed to solidify within 30 minutes. The Agarose polymerizes into a gel as it cools, the combs was removed from the gel to form wells for sample. The DNA amplicon coloured with tracking dye (ethidium bromide) was pipette into the wells. The tray was placed into a chamber that generates electric current through the gel. The negative electrode was placed on the side nearest to the sample, the positive electrode placed on the other side. DNA has a negative charge and was drawn to the positive electrode at 125volt, 500miliamps, at 25minutes, small DNA molecule was able to travel faster through the gel and visualized with a UV transilluminator. One well called the DNA ladder contains DNA fragments of known sizes. This ladder was used alongside with the plasmid samples to determine the size.

RESULTS

Table 1a and 1b Shows the distribution of bacterial isolate by age and gender. In male less than 20 years of age, a total of 6(33.33%) were recovered, 4(66.66%) were *K. Pneumonia*, and 2(33.33%)*E. coli*. Males within the age of 21-30years, a total of 1(5.55%) isolate were recovered, 1(100%)*C.freundii*. Between age 31 - 40, a total of 4(22.22%) isolates were recovered, 2(50%) *P.aeruginosa*, and 2(50%) *P. mirabilis*. Those within 41 - 50 years, a total of 4(22.22%) isolates were recovered, 1(25%) *K. pneumonia*, 1(25%) *P.aeruginosa*, and 2(50%) *C. freudii*. Within age 51-60years,a total of 1(5.55%) isolate were recovered,only *E. coli* 1(100%) was obtained. Age above 60years, a total of 2(11.11%) isolates recovered, 1(50%) *P. aeruginosa*, and 1(50%) *E. coli*. For females below 20 years of age, 9(28.13%) isolates were recovered, out of which 2(22.22%) were *K.pneumonia*,2(22.22%)*P. aeruginosa* 1(11.11%) *P. mirabilis*, 1(11.11%) *C. freudii* and 3(33.33%) *E.coli*. Within 21 -30 years, a total of 4(12.50%) isolates were recovered, 1(25%) *K. pneumonia*,1(25%) *P. aeruginosa*, and 2(50%) *P. mirabilis*. Between 31 - 40 years, 6(18.75%) isolates were recovered,2(33.33%)*K.pneumonia*,1(16.66%)*P.mirabilis*,2(33.33%) *C. freudii* and 1(16.66%) *E.coli*. Females within 41 -50 years, a total of 1(3.13%) isolate were recovered, 1(100%) were *E.coli*.Within 51 -60 years, a total of 2(6.25%) isolate were recovered, 1(50%) were *K. pneumonia* and 1(50%) *C. freudii*. Those above than 60 years a total of 10(31.25%) isolates were recovered, 4(40%)*K.pneumonia*,4(40%)*P. aeruginosa*, 1(10%) *P.mirabilis*,and 1(10%)*C.freudii*

Table 1a. Distribution of Specimen by Age/Gender (Male)

Age Range	K. pneumonia	P. aeruginosa	P. mirabilis	C. Freundii	E. coli	Total%
<20	4(66.66%)	0	0	0	2(33.33%)	6(33.33%)

21-30	0	0	0	1(100%)	0	1(5.55%)
31-40	0	2(50%)	2(50%)	0	0	4(22.22%)
41-50	1(25%)	1(25%)	0	2(50%)	0	4(22.22%)
51-60	0	0	0	0	1(100%)	1(5.55%)
>60	0	1(50%)	0	0	1(50%)	2(11.11%)
Total	8(33.33%)	5(20.83%)	2(11%)	3(16.66%)	4(22.22%)	18(100%)

Table 1b. Distribution of Specimen by Age/Gender (Female)

Age range	K. pneumonia	P. aeruginosa	P. mirabillis	C. Freundii	E. coli	Total%
<20	2(22.22%)	2(22.22%)	1(11.11%)	1(11.11%)	3(33.33%)	9(28.13%)
21-30	1(25%)	1(25%)	2(50%)	0	0	4(12.50%)
31-40	2(33.33%)	0	1(16.66%)	2(33.33%)	1(16.66%)	6(18.75%)
41-50	0	0	0	0	1(100%)	1(3.13%)
51-60	1(50%)	0	0	1(50%)	0	2(6.25%)
>60	4(40%)	4(40%)	1(10%)	1(10%)	0	10(31.25%)
Total	10(31.25%)	7(21.88%)	5(15.63%)	5(15.63%)	5(15.63%)	32(100%)

Out of the 17(34%) urine specimens, 7(41.18%) were obtained from males, while 10(58.82%) from females. Of the sputum 5(10%), 4(80%) were obtained from males while 1(20%) from females. Of the 5(10%) blood sample, 3(60%) were from males while 2(40%) were from females. A total of 2(4%) stool were collected from 2(100%) females. Of the 8(16%) wound swab, 4(50%) were collected from both males and females. A total of 13(26%) HVS samples, 13(26%) were obtained from females. A sum total of 50 samples were collected, 18(36%) were gotten from males and 32(64%) from females.

Table 2 Shows the distribution of specimen by gender.

SPECIMEN	MALE%	FEMALE%	TOTAL%
Urine	7(41.18%)	10(58.82%)	17(34%)
Sputum	4(80%)	1(20%)	5(10%)
Blood	3(60%)	2(40%)	5(10%)
Stool	0	2(100%)	2(4%)
Wound swap	4(50%)	4(50%)	8(16%)
High Vaginal Swap	0	13(100%)	13(26%)
Total	18(36%)	32(64%)	50(100%)

Table 3 Shows the distribution of bacterial isolates by specimen.

50 isolates were recovered. A total of 17(34%) were isolated from urine samples 6(35.29%) yielded *K.pneumonia*, 1(5.88%) *P. aeruginosa* 7(41.18%) *C. freudii*, 1(5.88%) *E. coli* and 2(11.76%) *P.mirabilis*. 5(10%) were isolated from sputum samples 4(80%) *P. aeruginosa*, and 1(20%) *E. coli*. 5(10%) were isolated from blood samples, 3(60%) yielded *K.pneumonia*, 1(20%) *P. aeruginosa*, and 1(20%) *E. coli*. A total of 2(4%) isolated from stool sample yielded 1(50%) *P. aeruginosa* and 1(50%) *P. mirabilis*. A total of 8(16%) wound swab out of which yielded 1(12.5%) *K. pneumonia*, 2(25%) *P.aeruginosa*, 3(37.5%) *E. coli* and 2(25%) *P.mirabilis*. 13(26%) HVS samples were isolated, 5(38.46%) *K.pneumonia*, 2(15.38%) *P.aeruginosa*, 1(7.69%) *C.freudii*, 3(23.07%) *E.coli* and 2(15.38%) *P. mirabilis*. In total, 15(30%) *K.pneumonia*, 11(22%) *P. aeruginosa*, 8(16%) *C.freudii*, 9(18%) *E.coli* and 7(14%) *P. mirabilis* were recovered.

Table 3: DISTRIBUTION OF BACTERIAL ISOLATES BY SPECIMEN

Specimen	K. Pneumonia %	P. Aeruginosa %	C. Freundii %	E. Coli %	P. Mirabillis %	Total
Urine	6(35.29%)	1(5.88%)	7(41.18%)	1(5.88%)	2(11.76%)	17(34%)
Sputum	0	4(80%)	0	1(20%)	0	5(10%)
Blood	3(60%)	1(20%)	0	1(20%)	0	5(10%)
Stool	0	1(50%)	0	0	1(50%)	2(4%)
Wound swap	1(12.5%)	2(25%)	0	3(37.5%)	2(25%)	8(16%)
HVS	5(38.46%)	2(15.38%)	1(7.69%)	3(23.07%)	2(15.38%)	13(26%)
Total	15(30%)	11(22%)	8(16%)	9(18%)	7(14%)	50(100%)

Table 4 shows the reaction of *E. coli* against the various biochemical reagents by the API 20E. Table 5 reveals that *E. coli* express high rate of resistance to cefuroxime 9(100%) and augmentin, 5(55.55%) to gentamycin and ofloxacin, and 1(11.11%) to ciprofloxacin respectively. The rate of resistant in this study differs from study by Salah *et al.*, (2019) which shows a resistant rate of 93.46% to ciprofloxacin.

Table 4: API 20E (Analytical Profile Index) of Bacterial

Escherichia coli

TEST	ONPG	ADH	LDC	ODC	CI	H ₂ S	U	T	IN	VP	G	GL	M	IN	SO	RH	SAC	MEL	AMY	ARA	OX
COL	Y	Y	0	0	C	C	Y	Y	P	C	C	Y	Y	B	Y	Y	B	B	B	Y	C
OUR														G			G	G	G		

RES	+		+	+	-	-	+		+	+	+	-		+	-	-	-	+	-		
ULT																					
SCO	1	0	4	1	0	0	0	0	4	0	0	4	1	0	4	1	0	0	0	2	0
RE																					
PRO		5			1		4			4			5		1					2	
FILE																					

ONPG(O-nitrophenyl-b-D-galactopyranoside)=Yellow(Y)-positive, Colourless (C)-negative; ADH(Adenine dehydrogenase)=Orange(O)-positive, Yellow(Y)-negative; LDC(Lysine decarboxylase)=Orange(O)-positive, Yellow (Y); ODC(Ornithine decarboxylase)=Orange(O)-positive, Yellow (Y)-negative; CIT(Citrate)=Blue green(BG)-positive, Yellow (Y)-negative; H₂S(hydrogen sulfide)=Black deposit (BD)-positive, Colourless (C)-negative; URE(urease)=Red(R)-positive, Yellow (Y)-negative; TDA(Tryptophan deaminase)=Brown(Br)-positive, Yellow (Y)-negative; IND(Indole)=Red(R)-positive, Yellow (Y)-negative; VP(Voges-Proskauer)=Pink(P)-positive, Colourless (C)-negative; GEL(Gelatinase)=Black deposit (BD)-positive, Colourless (C)-negative; Sugars(Glucose, Mannose, Inositol, Sorbitol, Rhamnose, Sucrose, Melibiose, Amygdalin and Arabinose)=Yellow (Y)-positive, Blue (B)-negative; OX(Oxidase)=Violet(V)-positive, Colourless (C)-negative.

Table 5 Antimicrobial Susceptibility Test

Isolates	GEN R%	CXM R%	OFL R%	AUG R%	NIT R%	CIP R%	MRP R%	S R%	CEP R%	NA R%	SXT R%
K.pneu n=15	12(80)	11(73.33)	9(60)	15(100)	10(66.66)	9(60)	7(87.5)	10(66.66)	15(100)	15(100)	15(100)
P.aerug n=11	10(90.90)	11(100)	7(63.64)	11(100)	10(90.90)	4(36.37)	8(73.72)	8(72.72)	10(90.90)	11(100)	11(100)
P.mirab n=7	5(71.45)	7(100)	4(57.14)	7(100)	6(85.71)	5(71.45)	2(28.59)	7(100)	7(100)	7(100)	7(100)
C.freue n=8	8(100)	7(87.5)	5(62.5)	8(100)	8(100)	6(75)	7(87.5)	7(87.5)	8(100)	8(100)	7(87.5)
E.coli n=9	5(55.55)	9(100)	5(55.55)	9(100)	0	1(11.11)	0	0	0	0	0
Total	40(80)	45(90)	30(60)	50(100)	34(68)	25(50)	24(48)	32(64)	40(80)	41(82)	40(80)

Keys

GEN-Gentamycin, CXM-Cefuroxime, OFL-Ofloxacin, AUG-Augmentin, NIT-Nitrofurantoin, CIP-Ciprofloxacin, MRP-Meropenem, S-Septin, CEP-Ceporex, NA-Nalidixic acid, SXT-Streptomycin.

Table 6 shows the distribution of specimen by using pie chart with urine having the highestpercentage,34%,followed by wound swab and stool 16%, HVS 14%, and the lowest been sputum and blood 10%.

Figure 1 Pie Chart Representing Sample Distribution

vine=17,,Sputum=5,,B100d=5,St001=8,Wound swab=8,High vagina swab=7

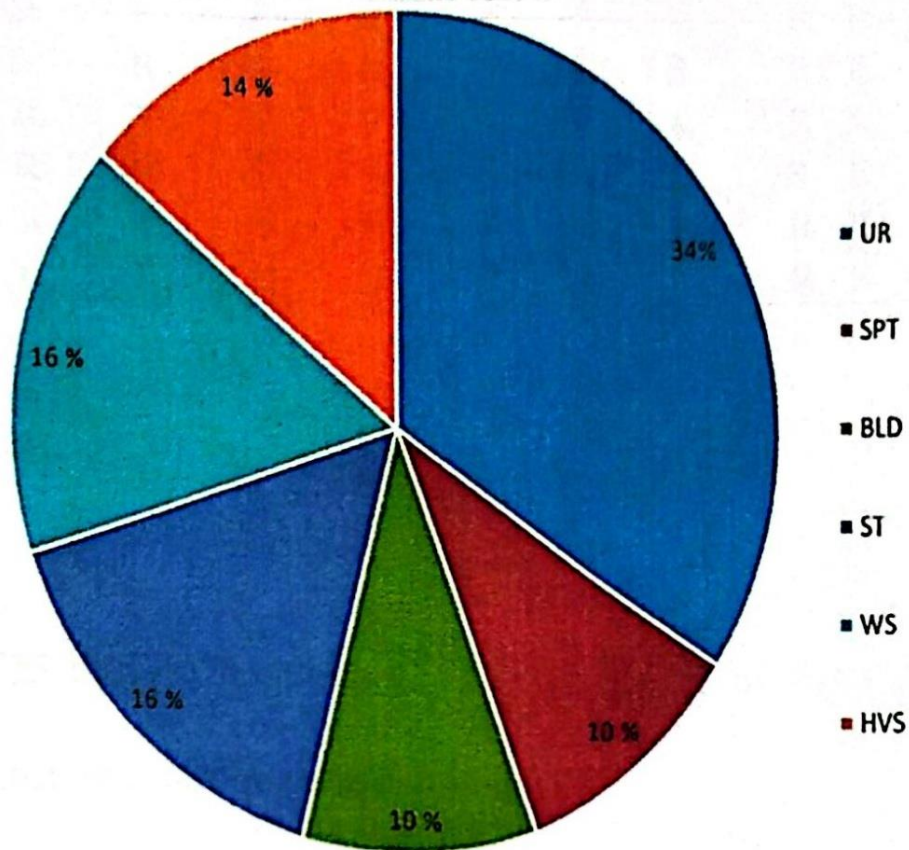


Table 7 shows the prevalence of qnrS gene identified among the isolate which were susceptible to gentamycin, ciprofloxacin, ofloxacin, nitrofurantoin, meropenem, septrin and ceporex respectively. Plate 1-4, shows the agarose electrophoresis for qnrS gene in *E. coli*. The present of the qnrS gene was indicated by positive band in lanes as represented by the numbers. Lane S represents the 100bp molecular ladder.

Specimen	GEN	CXM	OFL	AUG	NIT	CIP	MRP	S	CEP	NA	SXT	QnrS
Sp1	S	R	R	R	R	S	S	R	S	R	R	
Wd11	S	R	S	R	R	S	S	R	R	R	R	
Wd31	S	R	R	R	R	S	R	S	R	R	R	
Wd37	R	R	S	R	R	R	R	R	R	R	R	
Hvs19	S	R	S	R	S	S	S	RR		R	R	
Hvs25	R	R	R	R	R	R	R	R	R	R	R	
Hvs32	R	R	S	R	R	R	R	S	R	R	R	
BLD26	R	R	R	R	R	R	R	R	R	R	R	
Ur15	R	R	R	R	R	R	S	R	R	R	R	

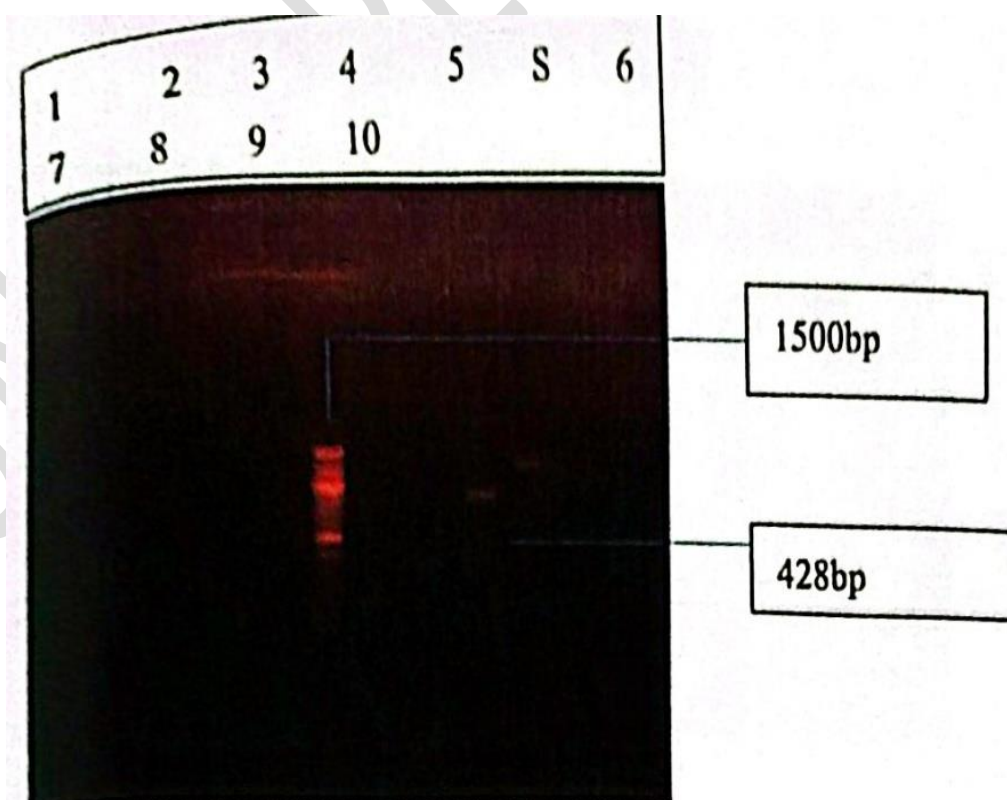


PLATE 1: Agarose gel electrophoresis of *QnrS* gene of some selected bacterial isolates. Lane 1, 7 and 8 represents the *QnrS* gene bands (428bp). Lane S represents the 100bp Molecular ladder.

11 12 13 14 15 16 17 18 S 19 20 21 22

23 24 25 26



PLATE 2: Agarose gel electrophoresis of *QnrS* gene of some selected bacterial isolates. Lane 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25 represents the *QnrS* gene bands (428bp). Lane S represents the 100bp Molecular ladder.

27 28 29 30 31 32 33 34 S 35 36 37 38 39 40 41 42

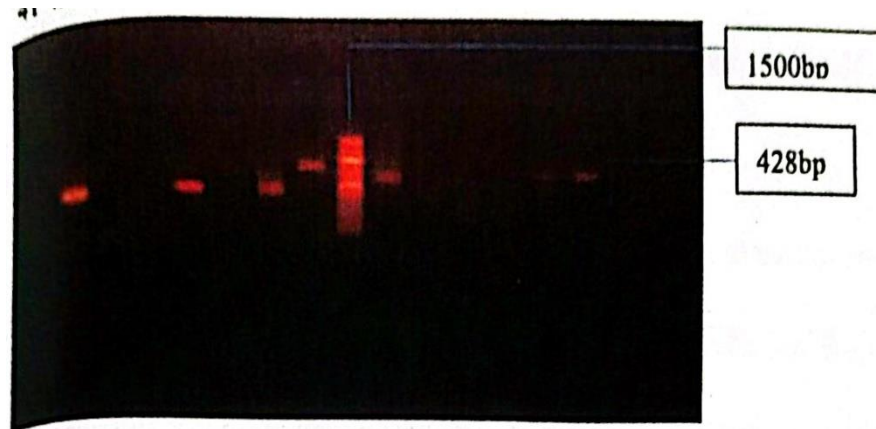


PLATE 3: Agarose gel electrophoresis of *QnrS* gene of some selected bacterial isolates. Lane 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 39 and 40 represents the *QnrS* gene bands (428bp). Lane S represents the 100bp Molecular ladder.

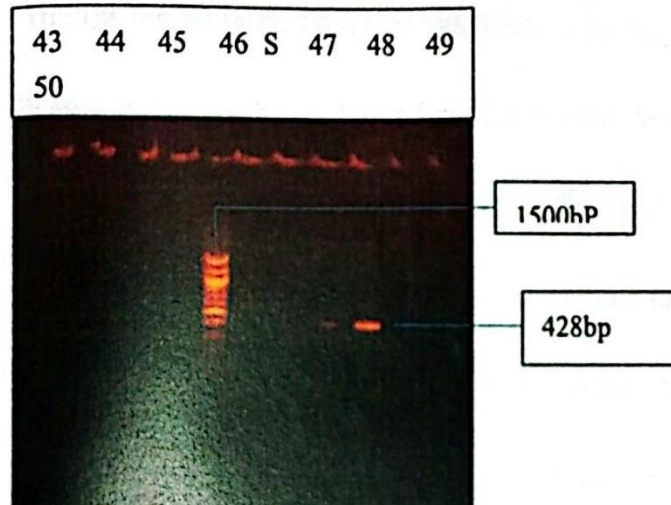


PLATE4:Agarose gel **electrophoresis** of QnrS gene of **some selected** bacterial isolates. Lane 43, 44,46,47,48,49 and 50**represents** the QnrS gene bands (428bp). Lane S **represents** the100bp Molecular ladder.

DISCUSSION

The spread of antimicrobial resistance in bacteria due to antimicrobial use has become a public and global health concern. *E. coli* strain are used as indicator bacteria for antimicrobial resistance among the Enterobacteriaceae. They are known to be pathogenic for several infectious diseases¹. *E. coli* may resist a variety of antibiotics and act in different ways to transfer antibiotic resistance genes to other strain bacteria¹². The quinolone resistant gene, qnrS in *E. coli* was detected in eight samples out the fifty samples collected. From this study, it was observed that the qnrS gene seems to be more resistance against qnr gene, which is in correlation with other study by Amin³.

Among the isolates, based on distribution by age and gender, a total of 4(22.22%) were isolated from the male and 5(15.63%) from the female. Male and female less than 20 years differ slightly in percentage,with the male showing 2(33.33%) and 3(33.33%) for the females respectively. Between 21-30 years, no *E. coli* were isolated from both male and female. From 31-40 years, male had no isolate,while the female had 1(16.66%) isolate. Age range between

41-50 years, no isolate was found in male, while 1(100%) *E. coli* were isolate from female. Between 51-60 years, male 1(100%) while female had no isolate. Age 60 years and above, male 1(100%) while female had no isolate. This is in agreement with study by Odonga¹⁴ with a prevalence of *E. coli* being higher among the females (11.5%) compared with the males (8.3%) form urine isolate.

Based on distribution by specimen, a total of 18(36%) from different specimen were isolated from the males and 32(64%) from the females. The prevalence *E. coli* was high in females 10(58.82%)while 7(41.18%) males from urine which is in conformity with study by⁹ with the females having (58%) and males (42%). This result is also consistent with other studies reporting a higher prevalence of *E. coli* in UTI in females²⁰. The reason for the high prevalence of this microorganism in females is that the urethra of females are short and this shortens the distance to be moved by bacteria to the bladder and sexual activities, which increases the inoculation of bacteria into the bladder⁹. These predisposing factors of UTI are accelerated by poor hygiene and low socioeconomic status²⁰. Alteration in the vaginal microflora may play a major role in encouraging the colonization of the vagina with coliforms¹⁴. For the sputum sample, 4(80%) from males while 1(20%) from females. This correlate with¹⁸ which reveal greater number of *E coli* from male 30(66.8) than females 12(33.2%). More *E. coli* were isolated from males 3(60%) than females 2(40%%) in blood. This study disagrees with study by¹⁰, with male 1(25%) and females 3(75%). Wound swab was the same for both males and females 4(50%) which also disagrees with¹⁰, with the females 8(44.4%) and males 10(55.6%). In contrast also in a study conducted in Pakistan, the incidence of *E. coli* isolates from wound swabs was the same in both males and females¹¹. It is difficult to explain this variation, and further studies with larger sample sizes are needed to explore the reason.

E. coli express high rate of resistance to cefuroxime 9(100%) and augmentin,5(55.55%) to gentamycin and ofloxacin, and 1(11.11%) to ciprofloxacin respectively. The rate of resistant in this study differ from study by¹⁶. These differences in the sensitivity pattern of antibiotics could be attributed to the time difference between the two studies, population variations, and significant differences in the sample sizes and types,

Conclusion

E.coli is one of the most studied bacteria among the *Enterobacteriaceae* family. Various studies have identified *E. coli* to be implicated in many diseases, especially those of the urinary tract.The present of different resistance gene such as qnrS identified in *E. coli* have reduced the effect of drugs such as quinolone targeted at this bacterium. QnrS gene is a plasmid mediated quinolone resistant (PMQR) gene whose action is against DNA gyrase and topoisomerase. This present study detects the prevalence of qnrS gene in *E. coli* among the various clinical isolate. Therefore, the detection of qnrS gene is a threat to treatment options and pharmaceutical product.The use of inappropriate and unnecessary administration of antibiotics should be avoided. Also, laboratory diagnosis should be done to select the drug of choice before

administration of antibiotics. Pharmaceutical industries should modify the already existing quinolones to improve treatment. Patient should visit the hospital for proper diagnosis and avoid self-medication and drug abuse.

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