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PREVALENCE OF Aac (6')-ib-cr and QepA GENES AMONG QUINOLONE RESISTANT Escherichia coli ISOLATES FROM URINE SAMPLES IN YENAGOA METROPOLIS, BAYELSA STATE, NIGERIA

***Oluwayemisi Agnes Olorode, Ofonime M. Ogba, Edith D. Johnson**

Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

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ABSTRACT

Quinolone resistance are commonly chromosomally encoded but plasmid mediated quinolone resistance has been reported. Can *Escherichia coli* strains harbor Quinolone resistance genes? This study was carried out between January, 2019 and October 2019, to determine the presence of aminoglycoside acetyltransferase (aac(6')-ib-cr) and quinolone efflux pumps (QepA) genes in quinolone resistant *Escherichia coli* strains from urine samples in Bayelsa state, Nigeria. A total of 250 midstream urine samples collected (from the patients with Urinary Tract Infection cases attending Niger Delta Teaching Hospital, Okolobiri and Federal Medical Centre, Yenagoa) and examined using Cysteine Lactose Electrolyte Deficient and Eosin Methylene Blue agar, include 164(65.6%) females and 86(34.4%) males. Pure isolates of *Escherichia coli* were subjected to antibiotics susceptibility testing using modified Kirby Bauer disc diffusion method and Mueller Hilton agar. Out of 250 samples analyzed 22/250 (8.8%) were positive to *Escherichia coli* 13 (59.1%) females and 9(40.9%) males. Twelve (12) quinolone resistant isolates were subjected to genomic DNA extraction by boiling method to determine the presence of aac(6')-ib-cr and qepA genes using an AB19700 applied Bio-system Thermo-cycler.. Ofloxacin was the most potent drug with percentage susceptibility of 64, this was followed by Streptomycin 54.5%; Pefloxacin 41%, Septrin 36%, Ciprofloxacin 32%; Gentamycin 27%, Augmentin 13.6%, Ceporex 12%, Nalidixic acid 4.5% and Ampicillin 4.5%. The isolates were highly resistant to quinolone class of antibiotics, Nalidixic acid, with percentage resistance of 95.5, followed by Ciprofloxacin 68%, Pefloxacin 59% and Ofloxacin 36%. Out of twelve (12) quinolone resistant *Escherichia coli* isolates subjected to molecular analysis, 6 (50%) and 1(8.3%) expressed the presence of aac(6')-ib-cr on 480 base pair and QepA genes on 800 base pair respectively giving a summation of 7(58.3%) isolates in all. Statistical analysis showed that there is a significant difference in the potency of the antibiotics used in this study at P= .05, but no significant difference between the resistant genes detected which made the null hypothesis acceptable at P<0.05. In conclusion, these findings have revealed the difficulties clinician might encounter in the treatment of urinary tract infection caused by *Escherichia coli* using quinolone class of antibiotics.

Keywords: Urinary Tract Infection*; Escherichia coli*; Quinolone Resistance genes aac(6')-ib-cr and QepA Genes; Antimicrobial drugs.

INTRODUCTION

Escherichia coli is a gram negative, non-spore forming microorganism known to cause range of infections including Urinary Tract Infection (UTI); an infection in any part of the urinary system which comprises of the kidneys, ureters, bladder and urethra caused by microorganism according to Mayo clinic, (2018); it is said to exist when a significant number of microorganisms, usually greater than 10^5 cells per millilitre of urine, are detected in properly collected mid-stream " clean catch"urine. Symptomatic UTIs can be classified based on severity which range from urosepsis syndrome, pyelonephritis affecting the kidney to cystitis, infection of the bladder (Foxman, 2014; Smelov *et al*., 2016). Uropathogenic *Escherichia coli* (UPEC), a micro biota of the gut that prevents colonization of the intestinal pathogenic bacteria and due to its symbiotic relationship it is expelled into the environment with faecal matter. UPEC is the main cause of 80% to 90% of community-acquired UTIs (Foxman,2014; Flores-Mirelesetal., 2015). Four main UPEC phylo -groups (A, B1, B2, and D) have been detected on the basis of genomic Pathogenicity Islands (PAI) and the expression of virulence factors, such as adhesins, toxins, surface polysaccharides, flagella, and iron-acquisition systems (Bien et al., 2012). Usually many of these virulence factors are required for UPEC to cause UTI (Hannan *et al*., 2012). However, besides UPEC, UTI can be caused by Klebsiella pneumoniae (about 7%), Proteus mirabilis (about 5%), and *Pseudomonas aeruginosa*, Enterococcusfaecalis, Enterobactercloacae, Streptococcus bovis, and the fungus *Candida albicans* (Hof, 2017). Quinolone resistance genes can be chromosomally or plasmid mediated. Clinically, quinolone class of antibiotics is mostly employed to treat UTI caused by *Escherichia coli*; among these antimicrobial drugs, Ciprofloxacin is the frequently used fluoroquinolones and has shown an excellent activity against pathogens commonly encountered in UTIs. Quinolones exact their antibacterial effect by preventing bacterial DNA from uncoiling and duplicating Cottell *et al.,* 2011.The aim of this study is to detect (using Polymerase Chain Reaction technique) the quinolones resistant genes (aac(6')-ib-cr and qepA) in *E. coli* isolated from the mid- stream urine samples of patients with complaints of Urinary Tract Infection after antibiotic susceptibility testing is carried out.

***Corresponding Author: Dr Oluwayemisi Agnes Olorode,**
Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

MATERIALS AND METHODS

Study Area

The study area includes Federal Medical Center (FMC) Yenagoa and Niger Delta University Teaching Hospital, NDUTH, Okolobiri all in Yenagoa Local Government Area, the state capital of Bayelsa. Yenagoa is geographically located at lat 4°55`29`N, Long 6°15`51`E. It has an area of 706km² and a population of 1,704,515 at the 2006 census (NPC, 2006).

Sample Size

Taro Yamane formula was used to calculate the sample size.

Sample collection and Culture

Two hundred and fifty (250) urine samples obtained from the department of Medical Laboratory Science in FMC, Yenagoa and

NDUTH, Okolobiri were immediately taken to the laboratory for culture using Cysteine Lactose Electrolyte Deficient Agar and Eosin Methylene Blue and incubated at 37^{0}C for 24hrs.

Molecular analysis

DNA extraction (Boiling Method)

Overnight broth culture of the bacterial isolate in Luria Bertani (LB) was transferred into 1.5ml eppendorf tube and was spun at 14000rpm for 3 min in a micro-centrifuge. The supernatant was discarded and 1000µl of 0.5% normal saline was added to the sediment and was vortexed on Hitech XH-B vortexer. The cells were re-suspended in 500ul of normal saline and heated at 95^{0}C for 20 min. The heated microbial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20^{o}C for other downstream reactions.

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. Two micro litre 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

Amplification of Aac(6')-ib-cr gene

The aac((6')-ib-cr genes from the isolates were amplified using the aac(6')-ib-cr (F: 5'- TTGCGATGCTCTAGGAGTGGCTA-3') and aac(6')-ib-cr R: (5'-CTCGAATGCCTGGCGTGTT-3') primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mixture included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and 25ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 59ºC for 40 seconds; extension, 72ºC for 50 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 25 minutes and visualized on a blue light transilluminator for a 480bp product size.

Amplification of QepA gene

The Qep A gene from the isolates were amplified using Qep A F- (5'-CTGCAGGTACTGCGTCATG-3'), and QepA-R(5'-CGTGTTACTGAAGTTCTTC-3').'
Primers on a ABI 9700 Applied Bio systems thermal cycler at a final volume of 15 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4uM and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 54ºC for 40 seconds; extension, 72ºC for 50 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator for a 800bp product size

RESULT

Twenty two {13 (59.1%) females; 9(40.9%) males} urine samples out of two hundred and fifty 250 {164(65.6% females); 86(34.4% males)} collected and analyzed were positive to *Echerichia coli* within the age range of 1 to 80 years. The examined patients had urinary tract infection with female preponderance over male. Among the test quinolone class of antibiotics in this study *Escherichia coli* showed the highest resistance to Nalidixic acid 95.5% followed by Ciprofloxacin 68%; Pefloxacin 59%; and the least was Ofloxacin 36%. A total of twelve (12) quinolone resistant *Escherichia coli* subjected to molecular analysis revealed the presence of plasmid mediated quinolone resistance genes aminoglycoside acetyltransferase (aac(6')-ib-cr) 6(50%) and quinolone efflux pumps (QepA) 1(8.3%).

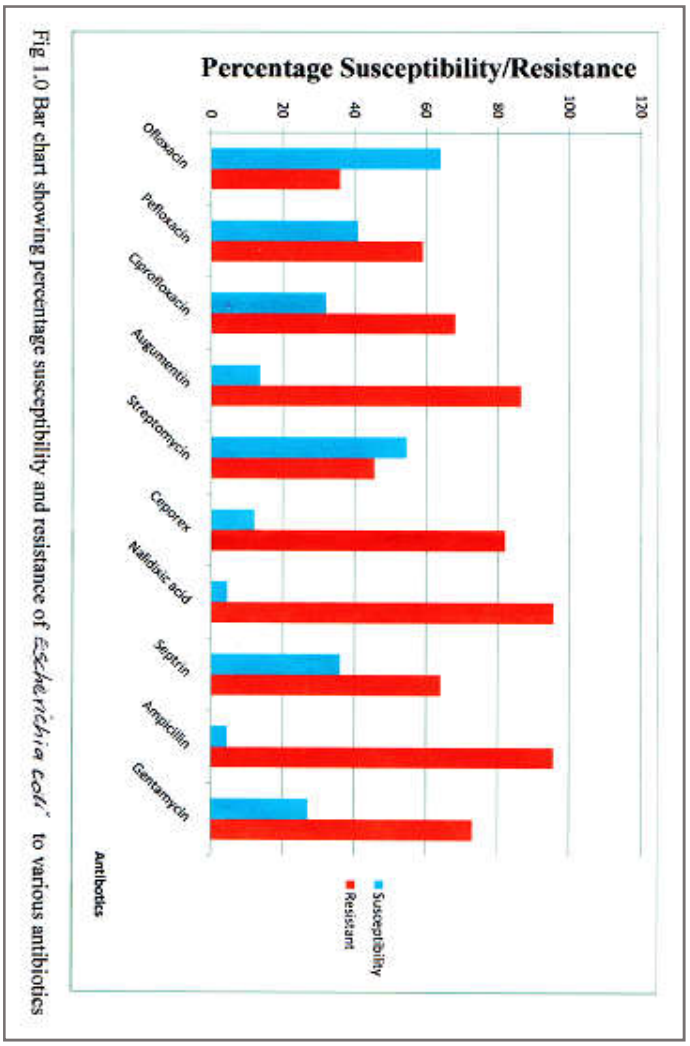

Fig 1.0 Bar chart showing percentage susceptibility and resistance of *Escherichia coli* to various antibiotics

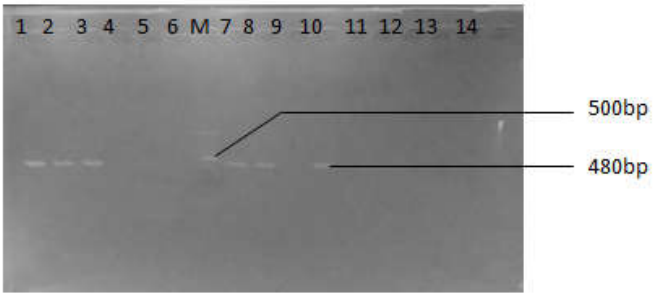

Fig: 4.0

Agarose gel electrophoresis of the aac (6')-ib-cr gene of some selected bacterial isolates. Lane 1, 2, 3, 7, 8 and 10 represents the aac (6')-ib-cr gene bands (480bp). Lane M represents the 100bp Molecular ladder.

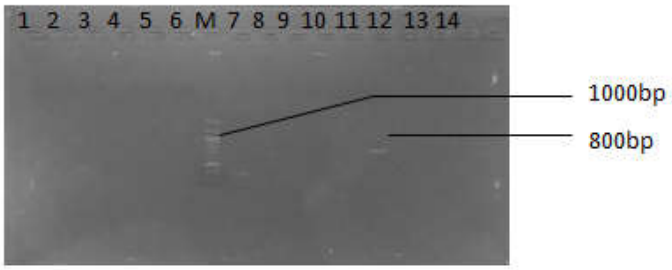

Fig: 5.0

Agarose gel electrophoresis of qepA gene of some selected bacterial isolates. Lane 12 represents the qepA gene bands (800bp). Lane M represents the 100bp Molecular ladder.

$$X^2 = \frac{(O-E)^2}{E}$$

Antibiotics	Observed(O)	Expected(E)	O-E	$(O-E)^2$	$X^2=\frac{(O-E)^2}{E}$	Σx^2
Ofloxacin	64	28.91	35.09	1231.31	42.59	
Pefloxacin	41	28.91	12.09	146.17	5.06	
Ciprofloxacin	32	28.91	3.09	9.55	0.33	
Augmentin	13.6	28.91	15.31	234.39	8.11	
Gentamycin	27	28.91	1.91	3.65	0.13	
Streptomycin	54.5	28.91	25.59	654.85	22.65	
Ceporex	12	28.91	-16.91	285.95	9.89	
Nalidixic acid	4.5	28.91	-24.41	595.85	20.61	
Septrin	36	28.91	7.09	50.27	1.74	
Ampicillin	4.5	28.91	-24.41	595.85	20.61	154.08

Research hypothesis: there is a significant difference between the potency of test antibiotics use on *Escherichia coli* at P = 0.05 level of significance. Null hypothesis is discarded.

Table 2.0: Statistical Analysis depicting acquisition of the Resistance genes in *Escherichia coli isolates*

$$X^2 = \frac{(O-E)^2}{E}$$

Sample	Observed(O)	Expected(E)	O-E	$(O-E)^2$	$X^2=\frac{(O-E)^2}{E}$	Σx^2
Ur13	1	1	0	0	0	
Ur18	1	1	0	0	0	
Ur44	1	1	0	0	0	
Ur63	0	1	-1	1	1	
Ur85	0	1	-1	1	1	
Ur97	0	1	-1	1	1	
Ur98	0	1	-1	1	1	
Ur99	1	1	0	0	0	
Ur100	1	1	0	0	0	
Ur102	0	1	-1	1	1	
Ur105	1	1	0	0	0	
Ur106	1	1	0	0	0	5

Null hypothesis: There is no significant difference in the acquisition of aac(6')-ib-cr and qepA genes at P = 0.05 level of significance. Research hypothesis is not accepted.

DISCUSSION

Out of 250 urine samples consisting of 86(34.4%) males and 164(65.6%) females collected and examined, 22 {9, (40.9%)} males; {13, (59.1%)} females were positive to *Escherichia coli*. This shows that urinary tract infection is more prevalent in females than in males and could be attributed to the close proximity of the urogenital to the anus and greater length of urethra in their male counterpart (Grupta *et al*., 2005). *Escherichia coli* infection was more prevalent in patients between ages of 21 and 30 years, this could be attributed to their active sexual life style. The result from this study contradicts other reports which stated that Ciprofloxacin is the most effective quinolone in the treatment of urinary infection as recorded by Stamm *et al* 2001. Antibiotics resistance is a growing problem and some of these are due to the indiscriminate use of antibiotics by humans (Cottell *et al*., 2011). Findings in this study showed that Ofloxacin is the most effective quinolone drug with 36% resistance and 63% suceptibility for the treatment of Urinary tract infection caused by *E. coli* in Bayelsa state, Nigeria. Out of the 12 Quinolone Resistant *E coli* isolates subjected to molecular analysis to detect Quinolone, Aac(6')-ib-cr gene was expressed in six 6(50%) isolates while qep A gene was detected in one 1(8.3%) isolate. This agrees with the study of Hassan *et al.,* (2011) who reported the prevalence of aac(6')-ib-cr gene(45.9%) and qep A (5.7%) in *E.coli* isolated from urine samples in Turkey and Ezeh *et al.* (2017) who reported the prevalence of Aac(6')-ib-cr (70%) and qep A (70%) from uropathogens in Zaira. Statistical analysis showed that there is a significant difference in the potency of the antibiotics used in this study at P>0.05, likewise there is no significant difference among the resistant gene, which made the null hypothesis acceptable at P<0.05; this shows that there are other resistant genes apart from the study genes that can also confer resistance to quinolone class of antibiotics.

CONCLUSION

This study has revealed Aminoglycoside Acetyltransferase (Aac(6')-ib-cr) and Quinolone Efflux Pump (Qep A) to be prevalent genes conferring resistance to quinolones antibiotics in the treatment of urinary tract infection caused by *E.coli.* The presence of these genes can limit therapeutic option and this is a great concern in clinical practice.

Recommendation

There is an incidence of Plasmid Mediated Quinolone Resistance among the studied population, therefore; It is necessary to monitor the spread of quinolone resistant genes in Escherichia coli and to ensure careful antibiotics use among the study population.

Acknowledgement

We acknowledge the staff and the management of Federal Medical Centre, Yenagoa and Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State for permitting us to collect the samples.

Authors' contributions

OAO & DEJ designed and conducted the experiment; OMO analyzed the data. All authors read and approved the final manuscript. Authors declare that verbal consent was obtained from the patients for the publication of this article.

Ethical Approval

All authors hereby declare that the individual patients' consent was sought and approved. The ethical committee of the Federal Medical Centre, Yenagoa and Niger Delta University Teaching Hospital approved the collection of the sputum samples.

Competing Interest: None

Source of Funding: Self funding

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