Molecular Detection of Plasmid-Mediated Quinolone Resistance in Ciprofloxacin-Resistant *Escherichia coli* from Urine of Patients attending Garki Hospital, Abuja, Nigeria

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ABSTRACT

Urinary tract infections (UTIs) caused by *Escherichia coli* (*E. coli*) is common worldwide; and its successful treatment using antibiotics is limited by acquisition of resistance by the bacteria. This study investigated the occurrence of plasmid-mediated quinolone resistance (PMQR) genes in ciprofloxacin-resistant *E. coli* from urine of patients with suspected cases of UTIs attending Garki Hospital Abuja (GHA), Nigeria. A total of 8 confirmed ciprofloxacin-resistant *E. coli* was screened for carriage of PMQR genes using polymerase chain reaction (PCR) method. The occurrences of the PMQR genes detected were in the order: *aac*-(6')-*Ib-cr* (87.5%) > *qnrB* (50.0%) > *qnrS* (37.5%) > *oqxAB* (12.5%) > *qnrA* (0.0%). *qnrB* and *qnrS* did not exist alone, but in combination with other genes; *aac*-(6')-*Ib-cr* existed both alone and in combination with others; the most prevalent patterns of existence were *aac*-(6')-*Ib-cr* alone and *aac*-(6')-*Ib-cr* + *qnrB* + *qnrS* at 25.0% each. This study has shown that the ciprofloxacin-resistant *E. coli* harbored *aac*-(6')-*Ib-cr*, *qnrB*, *qnrS* and *oqxAB* PMQR genes, with *aac*-(6')-*Ib-cr* being the most prevalent. The genes were present either alone or in combination with one another. This has implication for the clinical application of fluoroquinolones to treat UTI in the study location and environs.

Keywords: Antibiotic, *Escherichia coli*, hospital, molecular, plasmid-mediated.

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I. INTRODUCTION

Urinary tract infections (UTI) have been reported as a major problem worldwide [1]. *Escherichia coli* (*E. coli*), a member of the bacteria family, Enterobacteriaceae, is widely reported as one of the major causes of urinary tract infections (UTIs) both in outpatients and hospitalized patients.

Quinolones or fluoroquinolones (FQs) are widely used in the therapy of UTIs because of their efficacy against a broad spectrum of gram-positive and, in particular, gram-negative bacteria, lesser side effects and convenient oral dosages [2]-[6]. However, the extensive use of these agents for multiple clinical

indications in human or veterinary medicine has led to the development of bacterial resistance to quinolones over time [2]. Fluoroquinolone-resistance in gram-negative bacteria is in fact a global issue [6].

Bacterial resistance to quinolones results from: mutations in genes encoding DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) associated with the quinolone-resistance determining regions (QRDRs); carriage of the plasmid-mediated quinolone resistance (PMQR) genes; and reduced accumulation of drugs or chemicals due to active efflux pump activity [6]-[8].

Plasmid-mediated quinolone resistance, which has been reported in clinical and environmental isolates around the world, appears to be spreading [9], [10]. Plasmid-mediated quinolone resistance was first reported in 1998 in the United States in a multi-resistant urinary *Klebsiella pneumoniae* that could transfer low level resistance to nalidixic acid, ciprofloxacin, and other quinolones to a variety of gram-negative recipients [1]. However, PMQR is now being reported in other gram-negative as well as and gram-positive bacteria [10].

Three mechanisms of PMQR have been discovered. First, it was shown to be encoded by *qnr* gene, later named *qnrA*, as other distantly related *qnr* determinants (namely *qnrB*, *qnrC*, *qnrD*, *qnrS* and *qnrVC*) were found which code for proteins of the pentapeptide repeat family that protects DNA gyrase and topoisomerase IV from quinolone inhibition [9], [10]. The second plasmid-mediated mechanism involves acetylation of quinolones with an appropriate amino nitrogen target (such as ciprofloxacin and norfloxacin) by *aac(6')-Ib-cr* gene encoding a variant of the common aminoglycoside acetyltransferase, AAC(6')-Ib-cr; the third mechanism is enhanced efflux produced by plasmid genes *qepA* and *oqxAB* for pumps QepA and OqxAB respectively [10].

Plasmid-mediated quinolone resistance in *E. coli* has been reported globally [11]-[17]. In Nigeria, few reports on PMQRs are available [18], [19]. However, no specific report is available on the study area. This study therefore, investigates the occurrence of PMQR genes in ciprofloxacin-resistant *E. coli* isolated from the study location to provide a possible basis for the presence of the observed ciprofloxacin resistance. This study has far reaching implication as fluoroquinolones are drugs of choice for the treatment of urinary tract infections, of which *E. coli* is a common etiological agent.

II. MATERIALS AND METHODS

A. Bacterial Isolates

Eight confirmed ciprofloxacin-resistant *E. coli* (CREC) isolated from urine of patients with suspected UTIs attending Garki Hospital Abuja, Nigeria (GHA) were screened in this study. The isolates, which were obtained from a recent study [20], have antibiotic resistance profiles as shown in Table 1. The isolates were maintained on nutrient agar (Oxoid Ltd, Basingstoke, UK) slants at 4°C 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 in experiments.

B. DNA Extraction

The DNA of the isolates was extracted using boiling method as described by [21]. Briefly, following the purification, one pure colony of the ciprofloxacin resistant isolates, was inoculated into 2 ml of Luria-Bertani (LB: Oxoid Ltd., Basingstoke, UK) broth and incubated at 37°C for 18 h. Exactly 200 µl of the LB culture was then transferred into Eppendorf tube and centrifuged in a microcentrifuge (Model 5417R:

Touch plate Super Mixer, CAT. No. 1291, Lab-line Instrument Inc., USA) at room temperature at 3200 rpm for 2 min. The supernatant was discarded leaving the cells in the tube. The cells were washed twice with washing buffer. Exactly 0.5 ml of sterile phosphate buffer was added to the pellet and vortexed for 5 sec. It was then heated at 90°C for 10 min. It was then cooled down rapidly by freezing for 10 min. It was then centrifuged at 3200 rpm for 1 min to separate the DNA and the cells debris. 300 µl of the supernatant containing the DNA was then transferred into 2 ml Eppendorf tube and stored at -10°C for further use.

C. Amplification of Target Genes

The DNA amplification of target PMQR genes in the CREC isolates was carried out using singleplex PCR method by modification of the method earlier described by [21] using primers with sequences and expected amplicon sizes for each gene as listed in Table 2. Briefly, the reaction was carried out in 25 µl reaction volume in artificial tubes which is made up of 5 µl master mix, 2.4 µl primer (0.4 µl each of forward and reverse primers), 0.5 µl of MgCl₂ (Sigma Chemicals, England), 1.5 µl of DNA template and 15.6 µl of nuclease-free water. The reaction tubes were placed in the holes of the thermo cycler (Model TC-312, Techne, England) and its door was closed. The *oqxA*, *oqxB*, *qnrA*, *qnrB*, and *qnrS* genes were amplified under the following conditions: Initial denaturation at 94°C for 5 min followed by 32 cycles of amplification at 94°C for 45 sec each, annealing at 53°C for 45 sec, with final extension at 72°C for 5 min.

The amplification condition for detection of *aac(6')-Ib-cr* gene was carried out as follows: initial denaturation at 95°C for 5 min, annealing at 59°C for 40 sec and initial extension at 70°C for 30 sec and with final extension at 72°C for 5 min [21].

D. Agarose Gel Electrophoresis of Amplified Genes

The amplified genes were separated on 1.5% agarose gel in agarose gel electrophoresis at 12.0 V for 20 min. The separated genes were observed under U-V light to determine their base pair using 1500 bp DNA ladder as a standard.

III. RESULT AND DISCUSSION

The detection of PMQR genes in the CREC isolates is as given in Table 3. The order of occurrences of the genes was: *aac(6')-Ib-cr* (87.5%) > *qnrB* (50.0%) > *qnrS* (37.5%) > *oqxAB* (12.5%) > *qnrA* (0.0%). The patterns of existence of the genes detected were as shown in Table 4. *qnrB* and *qnrS* did not exist alone, but in combination with other genes; *aac(6')-Ib-cr* existed both alone and in combination with others; the most prevalent patterns of existence were *aac(6')-Ib-cr* alone and *aac(6')-Ib-cr* + *qnrB* + *qnrS* at 25.0% each. The distribution of the genes in relation to the isolates, as presented in Table 5, shows that isolate No.

7 contained all the genes detected. Representative agarose gel electrophoresis is as shown for *qnrB* (Plate 1), *qnrS* (Plate 2), *aac(6')-Ib-cr* (Plate 3) and *oqxAB* (Plate 4).

TABLE 1: Primers and their expected amplicon sizes for detection of plasmid-mediated quinolone resistance genes in the *Escherichia coli* isolates

S/N	Target Genes	Sequence	Amplicon Size (bp)	References
1	<i>qnrA</i> (F)	5'-CAGCAAGAGGATTCTCAG-3'	630	[22]
	<i>qnrA</i> (R)	5'-AATCCGGCAGCACTATTACTC-3'		
2	<i>qnrB</i> (F)	5'-GGCTGTCAGTTCTATGATCG-3'	488	[22]
	<i>qnrB</i> (R)	5'-GAGCAACGATGCCTGGTAG-3'		
3	<i>qnrS</i> (F)	5'-GCAAGTTCATTGAACAGGGT-3'	428	[23]
	<i>qnrS</i> (R)	5'-TCTAAACCGTCGAGTTCGGCG-3'		
4	<i>oqxAB</i> (F)	5'-CCGCACCGATAAAATTAGTCC-3'	313	[22]
	<i>oqxAB</i> (R)	5'-GGCGAGGTTTTGATAGTGGA-3'		
5	<i>aac-(6)-Ib-cr</i> (F)	5'-TTGGAAGCGGGACGGAM-3'	260	[24]
	<i>aac-(6)-Ib-cr</i> (R)	5'-ACACGGCTGGACCATA-3'		

F= Forward; R= Reverse

TABLE 2: Antimicrobial Resistance Pattern of ciprofloxacin-resistant *Escherichia coli* from urine of patients with suspected urinary tract infections attending Garki Hospital, Abuja Nigeria

Antimicrobial Resistance Pattern	No. of Isolates
AMC,S,CTX,CAZ,FOX,IPM,CIP,AMP	1
AMC,S,CTX,CAZ,FOX,CIP,AMP	1
AMC,S,SXT,FOX,CN,CIP,AMP	1
AMC,S,SXT,CTX,CAZ,FOX,CN,IPM,CIP,AMP	1
S,SXT,CTX,CAZ,FOX,CIP,AMP	1
S,FOX,CN,CIP,AMP	1
S,SXT,CTX,CAZ,CIP,AMP	1
CTX,CN,CIP,AMP	1
TOTAL	8

No. = Number; AMC= Amoxicillin/Clavulanic Acid; AMP= Ampicillin; CTX= Cefotaxime; CAZ= Ceftazidime; CIP= Ciprofloxacin; FOX= Cefoxitin; CN=Gentamicin; IPM= Imipenem; S= Streptomycin; SXT= Sulphamethoxazole/Trimethoprim [20].

TABLE 3: Occurrence of plasmid-mediated quinolone resistance genes in ciprofloxacin-resistant *Escherichia coli* from urine of patients attending Garki Hospital Abuja, Nigeria

Quinolone Resistance Genes	No. (%) of <i>Escherichia coli</i> (n= 8)
<i>QnrA</i>	0(0.0)
<i>QnrB</i>	4(50.0)
<i>QnrS</i>	3(37.5)
<i>OqxAB</i>	1(12.5)
<i>aac-(6)-Ib-cr</i>	7(87.5)

TABLE 4: Pattern of existence of the plasmid-mediated quinolone resistance genes in the ciprofloxacin-resistant *Escherichia coli* from urine of patients attending Garki Hospital, Abuja, Nigeria

Quinolone Resistance Genes	No. (%) of <i>Escherichia coli</i> (n= 8)
<i>qnrB</i> alone	1(12.5)
<i>qnrS</i> alone	0(0.0)
<i>oqxAB</i> alone	0(0.0)
<i>aac-(6)-Ib-cr</i> alone	2(25.0)
<i>aac-(6)-Ib-cr</i> + <i>qnrB</i>	1(12.5)
<i>aac-(6)-Ib-cr</i> + <i>qnrS</i>	1(12.5)
<i>aac-(6)-Ib-cr</i> + <i>qnrB</i> + <i>qnrS</i>	2(25.0)
<i>aac-(6)-Ib-cr</i> + <i>qnrB</i> + <i>qnrS</i> + <i>oqxAB</i>	1(12.5.0)

No. = Number

TABLE 5: Distribution of plasmid-mediated quinolone resistance genes in ciprofloxacin-resistant *Escherichia coli* from urine of patients in Garki Hospital Abuja, Nigeria

Isolate	Antimicrobial Resistance Pattern	Presence of PMQR Genes					PMQR Genes
		<i>qnrA</i>	<i>qnrB</i>	<i>QnrS</i>	<i>oqxAB</i>	<i>aac-(6')-Ib-cr</i>	
1.	AMC,S,CTX,CAZ,FOX,IPM,CIP,AMP	-	+	-	-	+	<i>aac-(6')-Ib-cr + qnrB</i>
2.	AMC,S,CTX,CAZ,FOX,CIP,AMP	-	-	-	-	+	<i>aac-(6')-Ib-cr</i>
3.	AMC,S,SXT,FOX,CN,CIP,AMP	-	-	+	-	+	<i>aac-(6')-Ib-cr + qnrS</i>
4.	AMC,S,SXT,CTX,CAZ,FOX,CN,IPM,CIP,AMP	-	+	-	-	-	<i>qnrB</i>
5.	S,SXT,CTX,CAZ,FOX,CIP,AMP	-	-	-	-	+	<i>aac-(6')-Ib-cr</i>
6.	S,FOX,CN,CIP,AMP	-	+	+	-	+	<i>aac-(6')-Ib-cr + qnrB + qnrS</i>
7.	S,SXT,CTX,CAZ,CIP,AMP	-	+	+	+	+	<i>aac-(6')-Ib-cr + qnrB + qnrS + oqxAB</i>
8.	CTX,CN,CIP,AMP	-	+	+	-	+	<i>aac-(6')-Ib-cr + qnrB + qnrS</i>

AMC= Amoxicillin/Clavulanic Acid; AMP= Ampicillin; CTX= Cefotaxime; CAZ= Ceftazidime; CIP= Ciprofloxacin; FOX= Cefoxitin; CN=Gentamicin; IPM= Imipenem; S= Streptomycin; SXT= Sulphamethoxazole/Trimethoprim

Plate 1: Agarose gel electrophoresis of the amplified *qnrB* genes from the *E. coli* isolates. Lanes 1, 4, 5, 6, 7 and 8 represent the *qnrB* band, Lane M represents the 1500bp molecular ladder, while other lanes show no bands.

Plate 2: Agarose gel electrophoresis of the amplified *qnrS* genes from the *E. coli* isolates. Lanes 3, 4, 6, 7 and 8 represent the *qnrS* band, Lane M represents the 1500bp molecular ladder, while other lanes show no bands.

Plate 3: Agarose gel electrophoresis of the amplified *aac (6')-Ib-cr* genes from the *E. coli* isolates. Lanes 1, 2, 3, 5, 6, 7 and 8 represent the *aac-(6')-Ib-cr* band, Lane M represents the 1500bp molecular ladder, while other lanes show no bands.

Plate 4: Agarose gel electrophoresis of the amplified *oxqAB* genes from the *E. coli* isolates. Lanes 7 represent the *oxqAB* band, Lane M represents the 1500bp molecular ladder, while other lanes show no bands.

Fluoroquinolones are now listed by the WHO as Critically Important Antimicrobials (CIA) for the treatment of human infections [25]. Ciprofloxacin is a fluoroquinolone with a range of indications, including urinary tract infections frequently caused by *E. coli* [3], [26], [27]. In the decade after its introduction all resistance reported in Enterobacteriaceae was associated with chromosomal mutations [28]. Recently, PMQR has become a frequent component of clinical resistance [3], [29]. Antimicrobial resistance to FQs mediated either by chromosomal mutations or transmissible plasmids is thus a major public health challenge. This study investigated the presence of PMQR genes in ciprofloxacin-resistant *E. coli* isolated from suspected UTI patients attending Garki Hospital, Abuja, Nigeria.

The detection of *qnrB*, *qnrS*, *oxqAB* and *aac-(6')-Ib-cr* PMQR genes in the CRECs from urine of suspected UTI patients is in tandem with other studies which have reported different prevalences of PMQR genes in CRECs from urine of humans [30]-[33]. Plasmid-mediated quinolone resistance genes are known to mediate transmissible resistance to fluoroquinolones [9], [10], [34].

The observed higher prevalence of *aac-(6')-Ib-cr* gene (87.5%) compared with any of the *qnr* PMQR genes is not surprising as it is often more common than *qnr* genes [2], [22], [35]-[38]. Our study has also shown a higher prevalence

of *qnrB* (50.0%) compared with *qnrS* (37.5%) in agreement with already known fact that of the *qnr* varieties, *qnrB* seems somewhat more common than *qnrA* or *qnrS*, which are more common than *qnrD* [39]. The detection of *oxqAB* gene in *E. coli* is not new as it has been found on plasmids in clinical isolates of *E. coli* in other studies [40]-[42]. The *OqxAB* multidrug resistance mechanism was initially identified from *E. coli* strains recovered from swine manure [43]. The observed low prevalence of the *oxqAB* gene (12.5%) is in contrast with a study in Iran by [43] which reported a high prevalence of 69.1%.

The presence of *qnr* genes in clinically relevant species of both gram-positive and gram-negative bacteria may foster quinolone resistance development as several classes of *qnr* genes have been identified that reduce susceptibility to fluoroquinolones [29], [44]. The Qnr proteins bind to DNA gyrase and topoisomerase IV and protect the enzymes from inhibition by quinolones [45], [46]. Although plasmid-borne *qnr* genes contribute to resistance development, the presence of a plasmid-borne *qnr* gene alone is insufficient to confer clinical resistance [47].

The presence of the *aac(6')-Ib-cr* gene in the isolates suggests its possible involvement in the ciprofloxacin resistance observed in the isolate containing the gene. This gene has been implicated in FQ resistance in *E. coli* in

Nigeria and a few other African countries. The association of *aac(6')-Ib-cr* gene with ESBL CTXM-15 has been reported [35], [48]–[51], making its detection of serious clinical importance. The gene encodes a variant of the common aminoglycoside acetyltransferase, AAC(6')-Ib-cr, which catalyzes the acetylation of quinolones with an appropriate amino nitrogen target (such as ciprofloxacin and norfloxacin) [52].

The *oqxAB* gene detected in the isolates contributed to the ciprofloxacin resistance as the gene mediates enhanced efflux by encoding the efflux pump OqxAB [10]. The detection of *oqxAB* gene is significant clinically as linkage of the gene with genes for the extended beta-lactamase CTX-M-14 and other plasmid-mediated CTX-M alleles has been noted [53].

IV. CONCLUSION

The CREC harbored PMQR genes with occurrences in the order: *aac(6')-Ib-cr* (87.5%) > *qnrB* (50.0%) > *qnrS* (37.5%) > *oqxAB* (12.5%). *qnrA* was not detected in any of the isolates. *qnrB* and *qnrS* did not exist alone, but in combination with other genes; *aac(6')-Ib-cr* existed both alone and in combination with others; the most prevalent patterns of existence were *aac(6')-Ib-cr* alone and *aac(6')-Ib-cr* + *qnrB* + *qnrS* at 25.0% each. The occurrence of *qnrB*, *qnrS*, *oqxAB* and *aac(6')-Ib-cr* PMQR genes observed in the CREC isolates is indication that these genes may have contributed to the resistance by the isolates to ciprofloxacin. We thus recommend prudent use of fluoroquinolones both in human and veterinary medicine since there is already established strong correlation between the consumption and an increase in the resistance.

REFERENCES

- [1] R. M. Akova, F. Baquero, J. Carlet, M. Cavaleri, S. Coenen, J. Cohen, D. Findlay, I. Gyssens, O. E. Heuer, G. Kahlmeter, H. Kruse, R. Laxminarayan, E. Liébana, L. López-Cerero, A. MacGowan, M. Martins, J. Rodríguez-Baño, J. M. Rolain, C. Segovia, B. Sigauque, E. Tacconelli, E. Wellington, J. Vila, Corrigendum to "The global Threat of Antimicrobial Resistance: Science for Intervention" *New Microbes New Infect*, 2015, 6: pp 22–29
- [2] J. Ma, Z. Zeng, Z. Chen, X. Xu, X. Wang, Y. Deng, D. Lü, L. Huang, Y. Zhang, J. Liu, High Prevalence of Plasmid-mediated Quinolone Resistance Determinants *qnr*, *aac(6')-Ib-cr*, and *qepA* among ceftiofur-resistant *Enterobacteriaceae* isolates from companion and food-producing animals. *Antimicrob Agents Chemother*, 2009, 53: pp 519–524.
- [3] L. S. Redgrave, S. B. Sutton, M. A. Webber, L. J. Piddock. Fluoroquinolone Resistance: Mechanisms, Impact on Bacteria, and Role in Evolutionary Success. *Trends Microbiol*, 2014, 22: pp 438–445.
- [4] K. J. Aldred, R. J. Kerns, N. Osherooff. *Biochemistry* Mar 18; 2014, 53(10): pp 1565–1574.
- [5] M. Röderova, D. Halova, I. Papousek, M. Dolejska, M. Masarikova, V. Hanulik, V. Pudova, P. Broz, M. Htutou-Sedlakova, P. Sauer, J. Bardon, A. Cizek, M. Kolar, I. Literak, Characteristics of Quinolone Resistance in *Escherichia coli* Isolates from Humans, Animals, and the Environment in the Czech Republic. *Front Microbiol*, 2016, 7: pp 2147.
- [6] J. C. Yeh, D. Y. Lo, S. K. Chang, C. C. Chou, H. C. Kuo, Prevalence of Plasmid-mediated Quinolone Resistance in *Escherichia coli* Isolated from Diseased Animals in Taiwan. *J Vet Med Sci*, 2017, 79(4): pp 730–735.
- [7] G. A. Jacoby, Mechanisms of Resistance to Quinolones. *Clin Infect Dis*, 2005, 41(2): pp 120–126.
- [8] S. J. Osei, D. G. Amoako, Genomic and Phenotypic Characterisation of Fluoroquinolone Resistance Mechanisms in *Enterobacteriaceae* in Durban, South Africa. *PLoS ONE*, 2017, 12(6)
- [9] R. Zhang, T. Ichijo, Y. L. Huang, J. C. Cai, H. W. Zhou, H. Yamaguchi, High Prevalence of *qnr* and *aac(6')-Ib-cr* Genes in Both Water-Borne Environmental Bacteria and Clinical Isolates of *Citrobacter freundii* in China. *Microbes Environment*; 2012, 27: pp 158–163.
- [10] G. A. Jacoby, J. Strahilevitz, D. C. Hooper, Plasmid-mediated quinolone resistance. *Microbiol. Spectr.* 2014, 2(2).
- [11] M. Wang, J. H. Tran, G. A. Jacoby, Y. Zhang, F. Wang, D. C. Hooper, Plasmid-mediated Quinolone Resistance in Clinical Isolates of *Escherichia coli* from Shanghai, China. *Antimicrob Agents Chemother*, 2003, 47(7): pp 2242–8.
- [12] V. Cattoir, P. Nordmann, J. Silva-Sanchez, P. Espinal, L. Poirel, ISEc1-mediated Transposition of *qnrB*-like Gene in *Escherichia coli*. *Antimicrob Agents Chemother*, 2008, 52, pp 2929–2932.
- [13] S. S. Namboodiri, J. A. Opintan, R. S. Lijek, M. J. Newman, I. N. Okeke, Quinolone resistance in *Escherichia coli* from Accra, Ghana. *BMC Microbiology*, 2011, 11: pp 44.
- [14] D. Tausova, M. Dolejska, A. Cizek, L. Hanusova, J. Hrusakova, O. Svoboda, G. Camlik, I. Literak, *Escherichia coli* with Extended-spectrum β -lactamase and plasmid-mediated quinolone Resistance Genes in Great Cormorants and Mallards in Central Europe. *J Antimicrob Chemother*, 2012, 67: pp 1103–1107.
- [15] A. Bartoloni, L. Pallecchi, E. Riccobono, A. Mantella, D. Magnelli, T. Di Maggio, A. L. Villagran, Y. Lara, C. Saavedra, M. Strohmeyer, F. Bartalesi, C. Trigo, G. M. Rossolini, Relentless Increase of Resistance to Fluoroquinolones and Expanded-Spectrum Cephalosporins in *Escherichia coli*: 20 years of Surveillance in Resource-limited Settings from Latin America. *Clin Microbiol Infect*, 2013, 19, pp 356–361.
- [16] C. H. Kim, E. H. Choi, J. Y. Sung, C. E. Oh, H. B. Kim, E. C. Kim, H. J. Lee, Prevalence of Plasmid-Mediated Quinolone Resistance Genes and Ciprofloxacin Resistance in Pediatric Bloodstream Isolates of *Enterobacteriaceae* over a 9-year period. *Jpn J Infect Dis*, 2013, 66: pp 151–154.
- [17] I. Jamborova, M. Dolejska, J. Vojtech, S. Guenther, R. Uricariu, J. Drozdowska, I. Papousek, K. Pasekova, W. Meissner, J. Hordowski, A. Cizek, I. Literaka. Plasmid-Mediated Resistance to Cephalosporins and Fluoroquinolones in Various *Escherichia coli* Sequence Types Isolated from Rooks Wintering in Europe. *Applied and Environmental Microbiology*, 2015, 81(2): pp 648–657.
- [18] D. Fortini, K. Fashae, A. Garcí'a-Ferna'ndez, L. Villa, A. Carattoli, Plasmid-mediated Quinolone Resistance and β -lactamases in *Escherichia coli* from Healthy Animals from Nigeria. *J Antimicrob Chemother*; 2011, 66: pp 1269–1272.
- [19] E. T. Sumrall, E. B. Gallo, A. O. Aboderin, A. Lamikanra, I. N. Okeke, Dissemination of the Transmissible Quinolone-Resistance Gene *qnrS1* by IncX Plasmids in Nigeria. *PLoS ONE*, 2014, 9(10): pp 1–10.
- [20] M. O. Eghieye, S. M. Jodi, B. E. Bassey, I. H. Nkene, R. H. Abimiku, Y. B. Ngwai, Antimicrobial Resistance Profile of *Escherichia coli* Isolated from Urine of Patients in Selected General Hospitals in Abuja Municipal, Nigeria. *Asian Journal of Advanced Research and Reports* 2018, 2(2): pp 1–10.
- [21] L. A. Porteus, J. L. Armstrong, R. J. Seider, L. S. Watrud, An effective Method to extract DNA from Environmental Samples for Polymerase Chain Reaction Amplification and DNA Finger Print Analysis. *Current microbiology*, 1994, 29: pp 301–307.
- [22] H. Ciesielczuk, M. Hornsey, V. Choi, N. Woodford, D. W. Wareham, Development and Evaluation of a Multiplex PCR for Eight Plasmid-mediated Quinolone-resistance Determinants, 2013, 62(12): pp 1823–1827.
- [23] V. Cattoir, L. Poirel, V. Rotimi, C. J. Soussy, P. Nordmann, Multiplex PCR for Detection of Plasmid-mediated Quinolone Resistance *qnr* Genes in ESBL-producing Enterobacterial Isolates. *J Antimicrob Chemother*, 2007, 60: pp 394–397.
- [24] D. W. Wareham, I. Umoren, P. Khanna, N. C. Gordon, Allele-specific Polymerase Chain Reaction (PCR) for Rapid Detection of the *aac(69)-Ib-cr* Quinolone Resistance Gene. *Int J Antimicrob Agents*, 2010, 36: pp 476–477.
- [25] WHO. World Health Organization, Critically Important Antimicrobials for Human Medicine. *Clin. Infect. Dis*, 2017, 48.
- [26] W. Castro, M. Navarro, C. Biot, Medicinal Potential of Ciprofloxacin and its Derivatives. *Future Med Chem*, 2013, 5: pp 81–96.
- [27] WHO. Antimicrobial Resistance: Global Report on Surveillance 2014. Geneva, Switzerland: WHO, 2014.
- [28] D. C. Hooper, Mechanisms of action and resistance of older and newer fluoroquinolones. *Clin Infect Dis*, 2000, 31(2): pp 24–8.
- [29] J. M. Rodríguez-Martínez, J. Machuca, M. E. Cano, Plasmid-mediated Quinolone Resistance; *Drug Resist Update*; 2016, 29: pp 13–29.
- [30] H. Nazik, B. Bektöre, B. Öngen, M. İktaç, M. Özyurt, N. Kuvat, O. Baylan, H. Keküllüoğlu, T. Haznedaroğlu, F. H. Keleşoğlu, Plasmid-

- Mediated Quinolone Resistance Genes in *Escherichia coli* Urinary Isolates from Two Teaching Hospitals in Turkey: Coexistence of TEM, SHV, CTX-M and VEB-1 Type β -lactamases. *Tropical Journal of Pharmaceutical Research*, 2011, 10(3): pp. 325-333
- [31] W. Pasom, A. Chanawong, A. Lulitanond, C. Wilailuckana, S. Kenprom, P. Puang-Ngern, Plasmid-mediated Quinolone Resistance Genes, *aac(6')-Ib-cr*, *qnrS*, *qnrB*, and *qnrA*, in Urinary Isolates of *Escherichia coli* and *Klebsiella pneumoniae* at a Teaching Hospital, Thailand. *Jpn J Infect Dis*, 2013, 66(5): pp 428-32.
- [32] C. Inwezerua, N. Mendonça, V. Calhau, S. Domingues, O. E. Adeleke, G. J. Da Silva, Occurrence of Extended-Spectrum β -lactamases in Human and Bovine Isolates of *Escherichia coli* from Oyo state, Nigeria. *J Infect Dev Ctries*, 2014, 8(6): pp 774-779.
- [33] L. Zhao, J. Zhang, B. Zheng, Z. Wei, P. Shen, S. Li, L. Li, Y. Xiao, Molecular Epidemiology and Genetic Diversity of Fluoroquinolone-Resistant *Escherichia coli* Isolates from Patients with Community-Onset Infections in 30 Chinese County Hospitals. *Journal of Clinical Microbiology*, 2015, 53 (3): pp 766-770.
- [34] J. M. Blair, M. A. Webber, A. J. Baylay, D. O. Ogbolu, L. J. Piddock, Molecular Mechanisms of Antibiotic Resistance. *Nat. Rev. Microbiol.* 2015, 13; pp 42–51.
- [35] J. D. Pitout, Y. Wei, D. L. Church, D. B. Gregson, Surveillance for plasmid-mediated quinolone resistance determinants in Enterobacteriaceae within the Calgary Health Region, Canada: the Emergence of *aac(6')-Ib-cr*. *J Antimicrob Chemother*, 2008, 61: pp 999–1002.
- [36] E. Ruiz, Y. Saenz, M. Zarazaga, R. Rocha-Gracia, L. Martinez-Martinez, G. Arlet, C. Torres, *qnr*, *aac(6')-Ib-cr* and *qepA* genes in *Escherichia coli* and *Klebsiella* spp.: Genetic Environments and Plasmid and Chromosomal Location. *J Antimicrob Chemother*, 2012, 67: pp 886–897.
- [37] C. H. Park, A. Robicsek, G. H. Jacoby, D. Sahm, D. C. Hooper, Prevalence in the United States of *aac(6')Ib-cr* Encoding a Ciprofloxacin-Modifying Enzyme. *Antimicrob Agents Chemother*, 2006, 50: pp 3953–3955.
- [38] J. Yang, Y. Luo, J. Li, Y. Ma, C. Hu, S. Jin, L. Ye, S. Cui, Characterization of clinical *Escherichia coli* Isolates from China Containing Transferable Quinolone Resistance Determinants. *J Antimicrob Chemother*; 2010, 65: pp 453–459.
- [39] L. Poirel, V. Cattoir, P. Nordmann, Plasmid-mediated Quinolone Resistance; Interactions Between Human, Animal, and Environmental Ecologies. *Front. Microbiol.*, 2012, 3: pp 24.
- [40] A. Norman, L. H. Hansen, Q. She, S. J. Sørensen, Nucleotide Sequence of pOLA52: a Conjugative IncX1 Plasmid from *Escherichia coli* Which Enables Biofilm Formation and Multidrug Efflux. *Plasmid.*, 2008, 60: pp 59–74
- [41] H. B. Kim, M. Wang, C. H. Park, E. C. Kim, G. H. Jacoby, D. C. Hooper, *oqxAB* Encoding a Multidrug Efflux Pump in Human Clinical Isolates of Enterobacteriaceae. *Antimicrob Agents Chemother.*, 2009, 53: pp 3582–3584.
- [42] J. Zhao, Z. Chen, S. Chen, Y. Deng, Y. Liu, W. Tian, X. Huang, C. Wu, Y. Sun, Z. Zeng, J. H. Liu, Prevalence and Dissemination of *oqxAB* in *Escherichia coli* Isolates from Animals, Farmworkers, and the Environment. *Antimicrob Agents Chemother*; 2010, 54: pp 4219–4224.
- [43] L. H. Hansen, S. J. Sørensen, H. S. Jørgensen, L. B. Jensen, The prevalence of the OqxAB Multidrug Efflux Pump Amongst olaquinox-resistant *Escherichia coli* in pigs. *Microb. Drug Resist.* 2005, 11: pp 378–382.
- [44] D. C. Hooper, G. H. Jacoby. Mechanisms of Drug Resistance: Quinolone Resistance. *AnnNYAcadSci* 1354: pp 12–31.
- [45] J. H. Tran, G. A. Jacoby, D. C. Hooper, Interaction of the plasmid-encoded quinolone resistance protein QnrA with *Escherichia coli* topoisomerase IV. *Antimicrob Agents Chemother*; 2005, 49: pp 3050–2.
- [46] J. H. Tran, G. A. Jacoby, D. C. Hooper, Interaction of the plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA Gyrase. *Antimicrob Agents Chemother*; 2005b, 49: pp 118–25.
- [47] L. Garoff, K. Yadav, D. Hughes, Increased Expression of Qnr is Sufficient to Confer Clinical Resistance to Ciprofloxacin in *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*, 2018, 73(2); pp 348–352.
- [48] G. L. Jones, R. E. Warren, S. J. Skidmore, V. A. Davies, T. Gibreel, M. Upton, Prevalence and Distribution of Plasmid-Mediated Quinolone Resistance Genes in Clinical Isolates of *Escherichia coli* Lacking Extended-Spectrum β -lactamases. *J Antimicrob Chemother*; 2008, 62:1245–1251.
- [49] P. J. Baudry, K. Nichol, M. DeCorby, P. Lagace-Wiens, E. Olivier, D. Boyd, M. R. Mulvey, D. J. Hoban, G. G. Zhanel, Mechanisms of resistance and mobility among multidrug-resistant CTX-M-producing *Escherichia coli* from Canadian intensive care units: the 1st report of QepA in North America. *Diagn Microbiol Infect Dis*; 2009, 63: pp 319–326.
- [50] E. Literacka, B. Bedenic, A. Baraniak, J. Fiett, M. Tonkic, I. Jajic-Bencic, M. Gniadkowski, *blaCTX-M* genes in *Escherichia coli* strains from Croatian Hospitals are Located in New (*blaCTX-M-3a*) and Widely Spread (*blaCTX-M-3a* and *blaCTX-M-15*) Genetic structures. *Antimicrob Agents Chemother.*; 2009, 53: pp 1630–1635.
- [51] M. Perilli, C. Forcella, G. Celenza, P. Frascaria, B. Segatore, C. Pellegrini, G. Amicosante, Evidence for *qnrB1* and *aac(6')-Ib-cr* in CTX-M-15-producing Uropathogenic Enterobacteriaceae in an Italian Teaching Hospital. *Diagn Microbiol Infect Dis*; 64: pp 90–93.
- [52] S. Sabtcheva, M. Kaku, T. Saga, Y. Ishii, T. Kantardjiev, High Prevalence of the *aac(6')-Ib-cr* Gene and its Dissemination Among Enterobacteriaceae Isolates by CTX-M-15 Plasmids in Bulgaria. *Antimicrob Agents Chemother*; 2009, 53: pp 335–336.
- [53] B. T. Liu, Q. E. Yang, L. Li, J. Sun, X. P. Liao, L. X. Fang, S. S. Yang, H. Deng, Y. H. Liu, Dissemination and Characterization of Plasmids Carrying *oqxAB-bla CTX-M* genes in *Escherichia coli* Isolates from Food-Producing Animals. *PLoS One.*; 2013, 8.