Molecular Characterization of Ciprofloxacin Resistant *Escherichia coli* from Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. Author JAO designed the study. Author IMA performed laboratory analysis of clinical samples, statistical analysis and wrote the protocol and the first draft of the manuscript. Authors JAO and MJN managed the analysis of the study and literature searches. Author PPA assisted in the laboratory analysis of samples. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: This study aimed to characterize ciprofloxacin-resistance genes in clinical *Escherichia coli* isolates obtained from a six-month antimicrobial resistance (AMR) surveillance from Ghana.
Methods: Eighty-three of 440 archived *E. coli* isolates were confirmed by biochemical reactions and resistance profiles by the disc diffusion method. These isolates were cultured from urine (42), stool (23), vaginal swabs (12), wounds (5) and heart valve (1) during AMR surveillance. Minimum Inhibition Concentration (MIC) by E-test method was performed on all *E. coli* isolates that were resistant to ciprofloxacin by the disc diffusion method. Additionally, all isolates with reduced MIC to ciprofloxacin (>32 μg/ml) were selected for molecular assays. Three chromosomal and nine plasmid-mediated resistance genes were screened in all Ciprofloxacin resistant *E. coli* (CRE) by polymerase chain reaction (PCR). Randomly selected amplified genes were commercially sequenced and analyzed.

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Results: In total, 47/83 (56.6%) *E. coli* isolates were resistant to ciprofloxacin and 29 (61.7%) had MIC values greater than 32 μg/ml. Chromosomal mediated genes (*gyr*A, *gyr*B and *par*C) were present in all 29 CRE isolates (100%). Distribution of the plasmid-mediated genes were as follows; *qnr*A 16/29 (55.1%), *qnr*B 16/29(55.1%), *qnr*C 22/29(75.8%), *qnr*S 26/29(89.6%), *qep*A 5/29(17.2%) and *oqx*B 19/29(65.5%). Genes encoding for altered aminoglycoside acetyltransferase [*aac*(6')*1bcr*] were also present in all 29 CRE isolates. The majority (72.4%) of the CRE isolates had *gyr*A mutations at codons 83 and 87. In *par*C, the mutations were at codons 71 and 80. Five isolates had mutations at codon 56 and four each had mutations at positions 79 and 80.

Conclusion: In this study, fluoroquinolone resistance genes were identified in all CRE isolates, mostly with putative mutations in the Quinolone Resistance Determining Region (QRDR). These chromosomal and plasmid-mediated genes may be widespread in Ghana and associated with CRE from the AMR surveillance. Although new mutations points were identified in *par*C, they may not be linked to the CRE.

ABBREVIATION

MIC   : *Minimum Inhibitory Concentration*
PCR   : *Polymerase Chain Reaction*
PMQR : *Plasmid Mediated Quinolone Resistant*
QRDR : *Quinolone Resistance Determining Region*
RND   : *Resistance Nodulation-cell Division*
CRE   : *Ciprofloxacin Resistant E. coli*

1. INTRODUCTION

Antimicrobial agents have played a significant role in alleviating illness and death associated with infectious diseases in animals and humans but selection pressure exerted by the antimicrobial use has been a major factor responsible for the emergence and spread of drug-resistance among pathogenic and commensal bacteria [1]. Antimicrobial resistant organisms are difficult to treat, requiring costly and sometimes toxic alternatives (CDC, 2010). Most resistance is human related, being due to drug misuse or use of substandard drugs [2]. Antimicrobial resistance also occurs by intrinsic resistance where the bacteria specie has the innate ability to resist activity of a particular antimicrobial agent through its inherent structural or functional characteristics with attributes due to: 1. Lack of affinity of the drug for the bacterial target 2. Inaccessibility of the drug into the bacterial cell 3. Extrusion of the drug by efflux pump 4. Production of enzymes to inactivate the drug [3] or acquired resistance where the bacteria acquire foreign genetic material either through horizontal gene exchange among *E. coli* either through the transfer of broad host range plasmids, or through the movement of transposons [4].

Fluoroquinolones have excellent bactericidal activities against a broad range of bacteria, including *Escherichia coli* [5] and ciprofloxacin is commonly prescribed due to its availability in oral and intravenous preparations [6]. This antibiotic is well absorbed from the gastrointestinal tract after oral administration.

Antimicrobial resistance in Gram-negative bacteria especially *E. coli* has increased in recent times [7] and the emergence and diffusion of multi-drug resistant strains of *E. coli* is complicating treatment of several infections in both hospital and community settings [8].

Several surveillance studies in Ghana have shown that *E. coli* resistance to ciprofloxacin has increased in recent years. However, the mechanisms of resistance expressed by such isolates in Ghana are not well understood. Also, there is limited information on the contribution of genetic elements for this clinically significant resistance in Ghana. This study aimed at identifying genes associated with resistance to ciprofloxacin in clinical *E. coli* isolates. Fluoroquinolone resistance is often caused by mutations in the quinolone-resistance determining regions (QRDRs) of two principal target enzymes, DNA gyrase, which is partially encoded by subunit genes, *gyrA* and *gyrB*, and DNA topoisomerase IV, encoded by *parC* and *parE* [9]. Plasmid Mediated Quinolone Resistant genes (PMQR) was discovered by Martinez-Martinez et al. [10] from a multiresistant strain of Klebsiella pnuemoniae isolated from urine sample. These genes are likely to originally come from aquatic organisms and code for pentapeptide repeat proteins. These proteins are known to reduce

susceptibility to quinolones by protecting the complex of DNA and DNA gyrase or topoisomerase IV enzymes from the inhibitory effect of quinolones [11].

2. MATERIALS AND METHODS

2.1 Study Design

A cross sectional study was conducted at the bacteriology Department of Microbiology, School of Allied Health Sciences, University of Ghana located at Korle-Bu Teaching Hospital (KBTH).

2.2 Sample Size and Collection

A total of 440 archived samples were obtained from the Department of Microbiology, School of Biomedical and Allied Health Sciences, University of Ghana of which 83 *E. coli* isolates were confirmed phenotypically by biochemical tests and resistance profiles using the disc diffusion method.

Archived *E. coli* isolates obtained from 18 study laboratories across Ghana during laboratory-based surveillance of antimicrobial resistance (AMR) by Opintan, Newman, Arhin et al., [12] were stored in the bacteriology section of the Department of Microbiology.

2.3 Specimen Processing

2.3.1 Resuscitation and identification of archived *Escherichia coli* isolates

Archived isolates were cultured onto Eosin Methylene Blue Agar (Oxoid, UK) and incubated at 37°C for 24 hours. Dark purple flat colonies with a metallic sheen colour indicated the presence of *E. coli.* Purity plating was then performed using EMB agar (Oxoid, UK).

Lactose fermenting colonies observed after overnight incubation were identified by standard laboratory methods (indole, citrate, urease utilization, and sugar fermentation test) and confirmed with a Minibact-E (Statens Serum Institute, Denmark) test following the manufacturer's instructions.

2.3.2 Antimicrobial susceptibility testing

E. coli isolates that were successfully identified were tested for their susceptibility against 10 antimicrobial agents using the Kirby-Bauer method on Mueller Hinton agar which included ciprofloxacin. After 18 hours of incubation, zones of inhibition were measured in millimeters and recorded. Interpretation of the results was done following CLSI 2016 breakpoint for antibiotics. *E. coli* ATCC 25922 was used as quality control in all tests. Isolates that were resistant to ciprofloxacin were further tested for Minimum Inhibitory Concentration (MIC) using the E-test (bioMerieux SA, France).

2.4 DNA Extraction

DNA was extracted from *E. coli* isolates with reduced ciprofloxacin susceptibility (MIC > 32 µg/L). Bacteria cells were grown overnight in 3 ml Trypticase Soy broth. The overnight broth culture was transferred into Eppendorf tubes and cells were pelleted by centrifugation. This procedure was carried out using the Wizard genomic DNA purification kit (Promega, U.S.A) following the manufacturer's instructions.

2.5 PCR Screening for Resistant Genes

DNA extracts from the *E. coli* isolates were amplified using polymerase chain reaction (PCR) to determine chromosomal resistant genes (*gyr*B, *par*C and *gyr*A) and plasmid-mediated genes proteins (*QnrA QnrB QnrC QnrD* and *QnrS*), *aac*(6')*lb*, *qep*, *oqx*B, and 1-*QnrB* genes. The protocol for PCR was adopted from Rezazadeh, Baghchesaraei and Peymani [13] with slight modifications, and the primers used are shown in Table 1.

2.6 Sequencing of PCR Products

Amplicons of the genes found in the quinolone resistance determining region, *gyr*A and *par*C were commercially sequenced at Inqaba (South Africa) using AB1 3500XL terminator. The sequences were analyzed and compared to wild type *E. coli* K12 substrain MG 1566 using the CLC Genomic Workbench (Qiagen, Aarhus, Denmark) software. Twenty-nine of each of the *gyr*A and *par*C and 2 amplicons each of 1-*qnr*B, *qnr*B, *qnr*S, and *aac*(6')1b were sequenced.

2.7 Statistical Analysis

Data was analyzed using SPSS version 23 and presented as frequency (percentages).

Table 1. Chromosomal and plasmid mediated resistant genes

Primers	Sequence 5' – 3'	Size (pb)	References
gyrA Fw	ACGTACTTAGGCAATGACTGG	190	Everett, Jin, Ricci et al., [14]
gyrA Rev	AGAAGTCGCCGTCGATAGAAC		
parC Fw	TGTATGCGATGTCTGAACTG	256	Everett, Jin, Ricci et al., [14]
parC Rev	CTCAATAGCAGCTCGGAATA		
Adk Fw	ATTCTGCTTGGCGCTCCGGG	583	Wirth, Falush, Lan et al., [15]
Adk Rev	CCGTCAACTTTCGCGTATTT		
recA Fw	CGCATTCGCTTTACCCTGACC	720	Wirth, Falush, Lan et al., [15]
recA Rev	TCGTCG AAATCTACGGACCGGA		
gyrB Fw	TCGGCGACACGGATGACGGC	203	Everett, Jin, Ricci et al., [14]
gyrB Rev	ATCAGGCCTTCACGCGCATC		
16S rRNA Fw	ATGCCGCGTGTATGAAGAA	1465	Lane [16]
16S rRNA Rev	GGTAACGTCAATGAGCAAAGG		
QnrA Fw	GGATGCCAGTTTCGAGGA	516	Wang, Guo, Xu et al., [17]
QnrA Rev	TGCCAGGCACAGATCTTG		
QnrB Fw	GGMATHGAAATTCGCCACTG	932	Jacoby, Cattoir, Hooper et al., [18]
QnrB Rev	TTTGCYGYYCGCCAGTCGAA		
QnrC Fw	GGGTTGTACATTTATTGAATC	447	Wang, Guo, Xu et al., [17]
QnrC Rev	TCCACTTTACGAGGTTCT		
QnrD Fw	CGAGATCAATTTACGGGGAATA	644	Cavaco, Hasman, Xia et al., [19]
QnrD Rev	AACAAGCTGAAGCGCCTG		
QnrS Fw	TCGACGTGCTAACTTGCG	417	Wang, Guo, Xu et al., [17]
QnrS Rev	GATCTAAACCGTCGAGTTCGG		
QepA Fw	TGGTCTACGCCATGGACCTCA	780	Périchon, Courvalin and Galimand [20]
qepA Rev	TGAATTCGGACACCGTCTCCG		
OqxB Fw	ATCGGTATCTTCCAGTCACC	541	Şahinturk, Arslan, Büyükcangaz et al., [21]
oqxB Rev	ACTGTTTGTAGAACTGGCCG		
aac(6')lb Fw	TTGCGATGCTCTATGAGTGGCTA	482	Park, Robicsek, Jacoby et al., [22]
aac(6')lb Rev	CTCGAATGCCTGGCGTGTTT		
1-QnrB Fw	GATCGTGAAAGCCAGAAAGG	469	Wang, Guo, Xu et al., [17].
1-QnrB Rev	ACGATGCCTGGTAGTTGTCC		

3. RESULTS

From the 440 archived isolates received from the laboratory-based surveillance of antimicrobial resistance,140 (32.8%) lactose fermenting bacteria were successfully revived out of which 83 were *E. coli* isolates by standard laboratory methods and Minibact-E. Antimicrobial susceptibility testing carried out on the 83 *E. coli* isolates revealed that 47 (56.6%) were resistant to ciprofloxacin by Kirby-Bauer disc diffusion method. Out of the 47 isolates that were resistant to ciprofloxacin by Kirby Bauer method, 29 had reduced susceptibility by MIC (>32 µg/l). The resistant isolates were obtained from urine (22), wounds (2), vaginal swabs (3), a heart valve (1) and stool (1).

All of the 29 isolates had the chromosomal genes; *gyr*A, *gyr*B, *par*C, housekeeping genes, *adk,* and plasmid-mediated resistance gene, *aac* (6') *lb.*

3.1 Determination of Mutations in QRDR

Nucleotide sequences of the QRDR of the *gyr*A subunit for the 29 *E. coli* isolates revealed that a single mutation at codon 83 (Serine TCG →Leucine TTC) was present in seven isolates. Nucleotide number 248 of *gyr*A altered from C to T, which then changed the amino acid serine into leucine. Double mutations in *gyr*A (Serine TCG 83 to Leucine TTC and Aspartate GAC 87 to Asparagine AAC) were also observed in 21 of the *E. coli* isolates. In one sample, a double mutation at position 87 changed Aspartate (GAC) to Tyrosine (TAC). Other mutations were detected but did not result in changes in the amino acids (Table 3).

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Table 2. QRDR and PMQR genes determined by PCR from E. coli with reduced susceptibility to ciprofloxacin

Isolate	MIC	Source	QRDR						PMQR								
			*gyr*A	*gyr*B	*par*C	*adk*	*RecA*	*16S rRNA*	*QnrA*	*QnrB*	*QnrC*	*QnrD*	*QnrS*	*oqxB*	*QepA*	*Aac*(6')	*1-QnrB*
2130	>32	MSU	+	+	+	+	-	+	-	+	+	-	-	-	-	+	+
9640	>32	MSU	+	+	+	+	-	+	-	+	+	-	-	-	-	+	-
6969	>32	MSU	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+
662	>32	MSU	+	+	+	+	-	+	-	+	-	-	-	-	-	+	-
8517	>32	MSU	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-
2562	>32	W. S	+	+	+	+	-	+	-	+	+	-	+	-	+	+	-
459	>32	W. S	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-
15356	>32	HVS	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-
31753	>32	MSU	+	+	+	+	-	+	-	+	+	-	+	+	-	+	+
7430	>32	HVS	+	+	+	+	-	+	-	-	-	-	+	+	-	+	-
11659	>32	MSU	+	+	+	+	-	+	+	-	+	-	+	+	-	+	+
11557	>32	MSU	+	+	+	+	-	+	+	-	-	-	+	+	-	+	+
11335	>32	MSU	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+
6720	>32	MSU	+	+	+	+	-	+	+	-	-	-	+	+	-	+	+
9438	>32	MSU	+	+	+	+	-	+	+	+	+	-	+	+	-	+	+
4583	>32	H. valve	+	+	+	+	-	+	-	-	+	-	+	-	-	+	+
7130	>32	HVS	+	+	+	+	+	+	-	-	-	-	+	-	-	+	-
11530	>32	MSU	+	+	+	+	-	+	+	-	-	-	+	-	-	+	+
2860	>32	MSU	+	+	+	+	+	+	-	-	+	-	+	+	-	+	-
11568	>32	MSU	+	+	+	+	-	+	+	-	+	-	+	+	-	+	+
5992	>32	MSU	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+
10857	>32	MSU	+	+	+	+	+	+	+	-	+	-	+	+	-	+	-
5683	>32	MSU	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-
29487	>32	MSU	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
10376	>32	MSU	+	+	+	+	-	+	+	+	+	-	+	+	-	+	+
2471	>32	St. S	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
6699	>32	MSU	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
6904	>32	MSU	+	+	+	+	-	+	+	-	+	-	+	-	-	+	+
30489	>32	MSU	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+

Table 3. Alterations in *gyr*A and *par*C subunits in twenty-nine tested *E.coli* isolates compared to the reference strain *E. coli* K12 strain

Organism	Source	Region	Mutataions in QRDR			
			gyr A		*Par*C	
			83	87	71	80
E. coli K12			Ser(TCG)	Asp(GAC)	Leu(CTG)	Ser(AGC)
459	HVS	VOV	Leu(TTG)	Asn(AAC)	Pro(CCG)	Ile(ATC)
662	MSU	VOV	Leu(TTG)	Asn(AAC)		Ile(ATT)
2130	W.S	ACK	Leu(TTG)			
2471	ST.	BAS	Leu(TTG)			Ile(ATC)
2562	WS	ACK	Leu(TTG)	Asn(AAC)		Ile(ATC)
2860	MSU	ACK	Leu(TTG)		Pro(CCG)	Ile(ATC)
4583	HEA.V	WSE	Leu(TTG)			
5683	MSU	ACK	Leu(TTG)			
5992	MSU	ACK	Leu(TTG)			
6699	MSU	NOT				T-C
6720	MSU	ACK	Leu(TTG)	Asn(AAC)		
6904	MSU	NOT	Leu(TTG)	Asn(AAC)		
6969	MSU	ACK	Leu(TTG)	Asn(AAC)	Pro(CCG)	
7130	HVS	WSE	Leu(TTG)	Asn(AAC)	C-G	-
7430	HVS	WSE	Leu(TTG)	Asn(AAC)		Ile(ATC)
8517	MSU	ACK	Leu(TTG)	Asn(AAC)		Ile(ATC)
9438	MSU	ACK	Leu(TTG)	Asn(AAC)	Pro(CCG)	Ile(ATC)
9640	MSU	ACK	Leu(TTG)	Asn(AAC)		
10376	MSU	ACK	Leu(TTG)	Asn(AAC)	Pro(CCG)	Ile(ATC)
10857	MSU	ACK	Leu(TTG)	Asn(AAC)	X(CNG)	Ile(ATC)
11335	MSU	ACK	Leu(TTG)	Asn(AAC)	Pro(CCG)	Ile(ATC)
11530	MSU	ACK	Leu(TTG)	Tyr(TAC)		
11557	MSU	ACK	Leu(TTG)	Asn(AAC)	Pro(CCG)	Ile(ATC)
11568	MSU	ACK	Leu(TTG)	Asn(AAC)	Pro(CCG)	
11659	MSU	ACK	Leu(TTG)	Asn(AAC)		Ile(ATC)
15356	HVS	EAK	Leu(TTG)	Asn(AAC)		Ile(ATT)
29489	MSU	EAK	Leu(TTG)	Asn(AAC)		Ile(ATT)
30489	MSU	EAK	Leu(TTG)		C – G	
31753	MSU	UEB	Leu(TTG)	Asn(AAC)		

Note: The facilities from which the isolates were obtained ACK – Korle-Bu Teaching hospital, EAK – Eastern Regional Hospital, VOV – Volta Regional hospital, UEB – Upper east Regional hospital; BAS – Brong-Ahafo Regional hospital, NOT – Tamale Teaching hospital; Samples from which the isolates were obtained. MSU – urine, ST – stool, HEA.V – heart valve, HVS – vaginal swab, WS – wound swab

A 265-bp PCR amplicon of *par*C was also analyzed in this study. Eleven isolates each of the quinolone resistant strains had a mutation at codon 71 (T→G translating codon CTG) changing leucine to proline and 80 (G→T translating codon AGC), resulting in a substitution of isoleucine for serine. In four isolates, mutation was detected at position 79 (T→G)), where aspartate was substituted by glutamate. Four other isolates had mutations at position 84 (Glu GAA →Val GTA in three isolates and Glu GAA →Lys AAA in one isolate). Another mutation was detected in two isolates at position 89 (Met ATG →Arg AGG). Five isolates each had mutations at positions 64, 76, 82, 90, and 91.

Figs. 1 and 2 show the chromatogram of sequenced clinical *E. coli* isolates matched against reference strain K12 substrain MG 1655 for the detection of mutations in *par*C subunits.

4. DISCUSSION

The resistance to ciprofloxacin remains of great concern since the introduction of fluoroquinolones in clinical practice two decades ago [23]. This is partly attributable to its use in first-line antibiotic therapy [24]. In Africa, many formal and informal health facilities still depend on broad-spectrum antibiotics and ciprofloxacin has been the drug of choice [25]. Despite recent

reports indicating the global increase in the emergence of fluoroquinolone-resistant *E. coli* strains [26], data from Ghana is not well established.
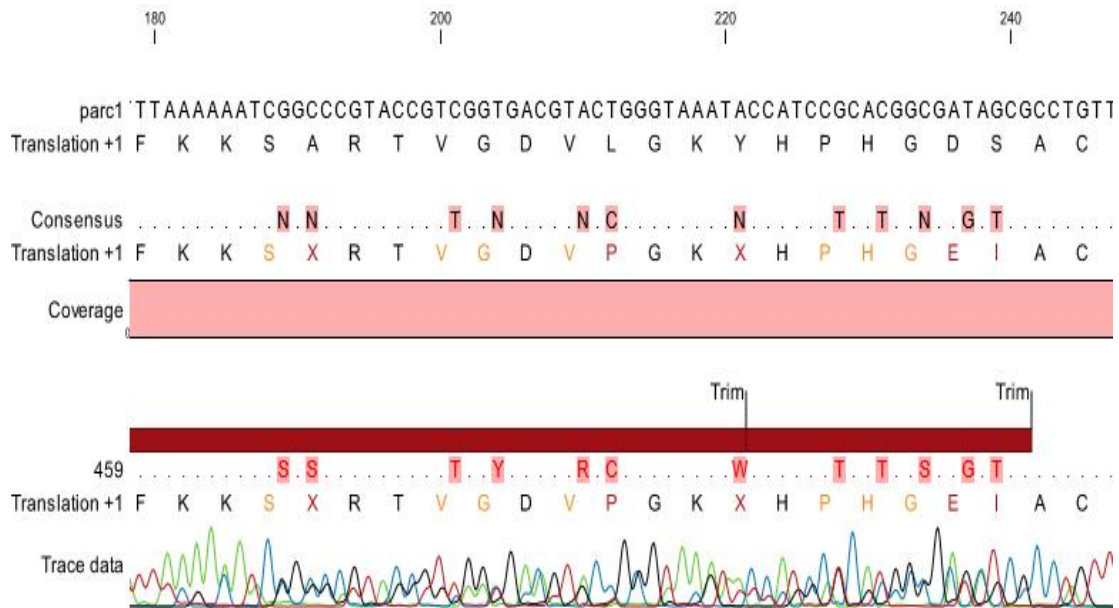
Chromatogram of *par*C subunit showing mutations at nucleotide numbers 212, 236 and 239.
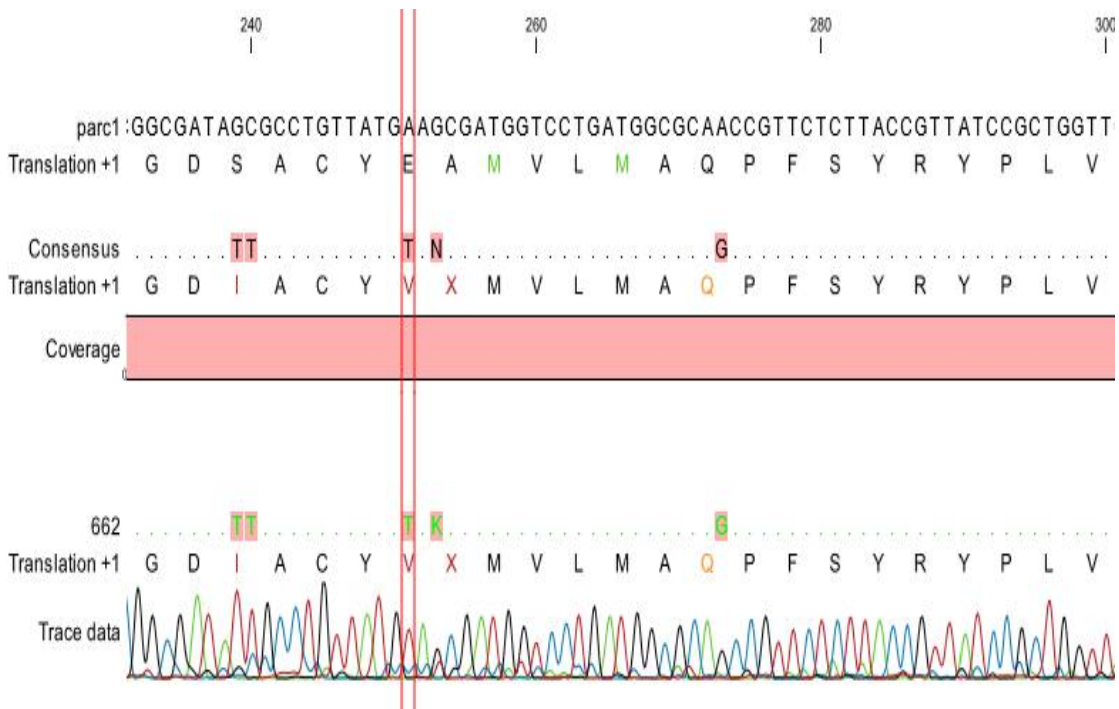
Chromatogram of *par*C subunit showing mutations at nucleotide number 251 and 293.

Fig. 1. Chromatogram of the clinical *E. coli* isolates matched against the reference strain K12 substrain MG 1655

Fig. 2. Comparison of nucleotide sequences of *gyr*A from clinical isolates and a reference strain *E. coli* K12. Dots indicate nucleotide positions identical to the corresponding reference strain sequence

The current study revealed a high frequency of plasmid- mediated resistant genes (*qnr*A, and *qnr*B, *qnr*C, *qnr*S and *oqx*B) found in the isolates, however, no *qnr*D genes were amplified. The resistant genes q*nr*S and *oqx*B have been previously isolated from *E. coli* in pigs from China [27], while isolates derived from clinical samples have only been reported in South Korea [27]. From this study, *aac*(6')*Ib* genes were found in all isolates while *qep*A was found in only 5 out of the 29 isolates. The 1-Q*nr*B gene was determined in 18 isolates. The presence of *aac(6')-Ib* in this study is higher than that found in other studies from China (51%) [28] and Korea (73.8%). In contrast, a lower prevalence of *aac*(6') *1b* was reported from the United States (32%) [22] and Iran (33.3%) [29]. Machuca, Ortiz, Recacha et al., [30] found that *aac*(6')1b-cr despite producing low-level resistance by itself, plays an important role in ciprofloxacin resistance when combined with chromosomal mutations both in vivo and in vitro. Plasmid Mediated Quinolone Resistance were first detected in 1998 from *Klebsiella pnuemoniae* in the USA [10]. Since then several other plasmid-mediated quinolone resistant genes have been reported. These include qnr proteins; qnrA, qnrB, qnrC, qnrD and qnrS whose activity is to protect the DNA from quinolone inhibition. The second plasmid-mediated resistance mechanism is also associated with acetylating quinolones using suitable amino nitrogen target site by mutants of an altered aminoglycoside acetyltransferase aac(6')-Ib. The third is an efflux mechanism created by QepAB which is a quinolone specific efflux pump and OqxAB whose activity is a multidrug efflux [11].

All isolates had high MICs (>32 µg/l) following exposure to ciprofloxacin and showed mutations in chromosomal and plasmid genes after sequencing. These findings suggest why the inappropriate use of fluoroquinolones could result in the development of resistance. The majority of the ciprofloxacin-resistant isolates contained mutations in QRDR section of *Gyr*A at amino acid 83 as has been established by [23,30,31].

Position 83 of wild-type strains contains a serine, a hydrophilic amino acid in the A subunit of gyrase which interacts with ciprofloxacin. Mutation at this position that results in an altered amino acid may induce conformational changes and hence the loss of drug-target interaction. In this current study, it is noteworthy that serine at position 83 of all mutants was replaced with a hydrophobic leucine. Yoshida, Bogaki,

Nakamura et al., [32] had reported that substitution of serine to Leucine, Tryptophan, alanine and proline at position 83 leads to a local conformation change of *gyr*A which results in the loss of drug-target interaction. Additionally, some deletions of amino acid 83 were detected (serine AGC→ A-C) which provides evidence that either an alteration or deletion of this site may lead to the loss of enzyme-drug interaction. Mutation at position 87 altered Asp-87→Asn and Asp-87→Tyr in one isolate. A change from an acidic amino acid to a basic amino acid could result in a conformational change or non-specific interactions, leading to resistance.

Findings from this current study suggest that mutations within the QRDR (positions 83 and 87) result in ciprofloxacin resistance. For expression of high-level resistance, acquisition of a second *gyr*A mutation and a *par*C mutation needs to occur and similar resistance mechanisms have been observed in *E. coli* strains Heisig [33]. In the current study, the high frequency of ciprofloxacin resistance was attributable to the presence of double mutations in both *gyr*A and a single mutation detected in *par*C genes. As many as five-point mutations were observed in *par*C in isolates 10376 and 9438 and both isolates also had 2-point mutations in *gyr*A. In the current study. All mutations were found within the mutation points in the current study which confirms observations found in other studies [23,32]. The known documented hotspots for mutation in *par*C are positions 80 and 84 [34] however in the current study, 11 isolates had mutations at position 80 and 4 isolates had mutations at position 84. In the current study, no single mutation was found in *par*C without an additional mutation in the gyrase A gene, which suggests that DNA topoisomerase IV could be a secondary target for fluoroquinolones as was observed by Soussy, Wolfson, E Y Ng et al. [35].

5. CONCLUSION

This current study revealed a high prevalence of resistant genes in *E. coli* obtained from the AMR surveillance to ciprofloxacin. The high frequency of the resistant genes especially the plasmid-mediated resistant genes in the ciprofloxacin-resistant *E. coli* suggests that these resistant genes are common in Ghana and could lead to a serious threat of outbreak of antimicrobial resistance development to complicate treatment of infections in the future. This work has also shown that the high level of resistance to

ciprofloxacin (>32 µg/ml) was attributable to the mutations in *par*C with the concomitant presence of mutations in the *gyr*A gene. Sequenced data obtained from this study has broadened our understanding of the molecular mechanism of fluoroquinolone resistance associated with *gyr*A and *par*C mutations in *E. coli* from Ghana. From the current study, it can be deduced that resistance of the *E. coli* isolates to ciprofloxacin is mainly due to the mutations in the quinolone resistance determining region (QRDR) of the drug targets (*gyr*A and *par*C). Further studies can be done to determine the level of transposons present in the *E. coli* isolates that will give rise to the mutations observed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

Ethical approval was sought from the University of Ghana, School of Biomedical and Allied Health Sciences before the commencement of the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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