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.

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**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 (gyrA, gyrB and parC) were present in all 29 CRE isolates (100%). Distribution of the plasmid-mediated genes were as follows; qnrA 16/29 (55.1%), qnrB 16/29(55.1%), qnrC 22/29(75.8%), qnrS 26/29(89.6%), qepA 5/29(17.2%) and oqxB 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 gyrA mutations at codons 83 and 87. In parC, 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 parC, they may not be linked to the CRE.

Keywords: Antimicrobial resistance; antimicrobial sensitivity testing; resistance; ciprofloxacin resistant.

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 pneumoniae 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 Methylen 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 (gyrB, parC and gyrA) and plasmid-mediated genes proteins (QnrA QnrB QnrC QnrD and QnrS), aac(6')lb, qep, oqxB, 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, gyrA and parC were commercially sequenced at Inqaba (South Africa) using ABI 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 gyrA and parC and 2 amplicons each of 1-qnrB, qnrB, qnrS, 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      | AAGAATCGCGGTGTGAAGAC                                                             |           |                                               |
| parC Fw       | TGATGCGATGCTGAACTG                                                              | 256       | Everett, Jin, Ricci et al., [14]              |
| parC Rev      | CTCAATAGCAGGCTGAAATT                                                             |           |                                               |
| Adk Fw        | ATTCGCTGCTGCGCCGG                                                               | 583       | Wirth, Falush, Lan et al., [15]              |
| Adk Rev       | CTGCTACTTCTCCTGTTTT                                                             |           |                                               |
| recA Fw       | CGAATTCGCTTTACCGCTGCC                                                        | 720       | Wirth, Falush, Lan et al., [15]              |
| recA Rev      | AGAAGTCCGATGCGGACC                                                             |           |                                               |
| gyrB Fw       | TCGGCCGACACGGATGACGCG                                                          | 203       | Everett, Jin, Ricci et al., [14]              |
| gyrB Rev      | ATCAGGCTCTCCACGCGCATC                                                          |           |                                               |
| 16S rRNA Fw   | ATGCCGCGTGTATGAAAGA                                                             | 1465      | Lane [16]                                      |
| 16S rRNA Rev  | GGTAACTCAGCAAAGG                                                                |           |                                               |
| QnrA Fw       | GGATGCCGATGCGGACC                                                              | 516       | Wang, Guo, Xu et al., [17]                   |
| QnrA Rev      | TGGCGATGCTTTCCGAGTCC                                                            |           |                                               |
| QnrB Fw       | GMATGCGATGCGGACC                                                              | 932       | Jacoby, Cattoir, Hooper et al., [18]         |
| QnrB Rev      | TTTATCGCAGCCGTGGA                                                              |           |                                               |
| QnrC Fw       | GGATTGTCATTTTACCTGGA                                                          | 447       | Wang, Guo, Xu et al., [17]                   |
| QnrC Rev      | TCCACTTCTACCGGTTT                                                              |           |                                               |
| QnrD Fw       | CGAGATCAAATGCGAGGGA                                                             | 644       | Cavaco, Hasman, Xia et al., [19]             |
| QnrD Rev      | ACAAAGCTGAGCAGGCTG                                                             |           |                                               |
| QnrS Fw       | TCGACGTGCTAAGATCCG                                                            | 417       | Wang, Guo, Xu et al., [17]                   |
| QnrS Rev      | GATCTAAACGCGATGCGG                                                             |           |                                               |
| QepA Fw       | TGGCTACGCGATGCGGCC                                                            | 780       | Périchon, Courvalin and Galimand [20]        |
| qepA Rev      | TGAATTCGCGGACTCTCCG                                                            |           |                                               |
| qoxB Fw       | ATCGATGCTTACCGGCA                                                             | 541       | Şahinturk, Arslan, Büyükcangaz et al., [21] |
| qoxB Rev      | ACTGATTGTGAGAAGCAGG                                                            |           |                                               |
| aac(6')lb Fw  | TTGCAGATGCTATGACTGGA                                                          | 482       | Park, Robicsek, Jacoby et al., [22]         |
| aac(6')lb Rev | CTGAATTCGCGGTGTGTTT                                                            | 469       | Wang, Guo, Xu et al., [17]                   |
| 1-QnrB Fw     | GATCGTGAAGGCCAGGAGG                                                            |           |                                               |
| 1-QnrB Rev    | ACGATGCGTGTGCTGTTG                                                            |           |                                               |

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; gyrA, gyrB, parC, 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 gyrA 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 gyrA altered from C to T, which then changed the amino acid serine into leucine. Double mutations in gyrA (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).
Table 2. QRDR and PMQR genes determined by PCR from E. coli with reduced susceptibility to ciprofloxacin

| Isolate | MIC | Source | QRDR | PMQR |
|---------|-----|--------|------|------|
|         |     |        | gyrA | gyrB | parc | 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    | +    | +    | +    | +    | +    | -    | -    | -    | -    | -    | +     | -    | +     | +     |
A 265-bp PCR amplicon of parC 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→C), 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 parC 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

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**Table 3. Alterations in gyrA and parC subunits in twenty-nine tested E.coli isolates compared to the reference strain E. coli K12 strain**

| Organism | Source | Region | Mutations in QRDR |
|----------|--------|--------|-------------------|
| E. coli K12 |        |        | gyr A ParC |
| 459      | HVS    | VOV    | Ser(TCG) = Asp(GAC) |
| 662      | MSU    | VOV    | Leu(TTG) = Asn(AAC) |
| 2130     | W.S    | ACK    | Leu(TTG) = Asn(AAC) |
| 2471     | ST.    | BAS    | Leu(TTG) = Asn(AAC) |
| 2562     | WS     | ACK    | Leu(TTG) = Asn(AAC) |
| 2860     | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 4583     | HEA,V  | WSE    | Leu(TTG) = Asn(AAC) |
| 5683     | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 5992     | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 6699     | MSU    | NOT    | Leu(TTG) = Asn(AAC) |
| 6720     | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 6904     | MSU    | NOT    | Leu(TTG) = Asn(AAC) |
| 6969     | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 7130     | HVS    | WSE    | Leu(TTG) = Asn(AAC) |
| 7430     | HVS    | WSE    | Leu(TTG) = Asn(AAC) |
| 8517     | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 9438     | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 9640     | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 10376    | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 10857    | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 11335    | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 11530    | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 11557    | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 11568    | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 11659    | MSU    | ACK    | Leu(TTG) = Asn(AAC) |
| 15356    | HVS    | EAK    | Leu(TTG) = Asn(AAC) |
| 29489    | MSU    | EAK    | Leu(TTG) = Asn(AAC) |
| 30489    | MSU    | EAK    | Leu(TTG) = Asn(AAC) |
| 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.
reports indicating the global increase in the emergence of fluoroquinolone-resistant *E. coli* strains [26], data from Ghana is not well established.

Chromatogram of *parC* subunit showing mutations at nucleotide numbers 212, 236 and 239.

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 gyrA 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 (qnrA, and qnrB, qnrC, qnrS and oqxB) found in the isolates, however, no qnrD genes were amplified. The resistant genes qnrS and oqxB 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′)lb genes were found in all isolates while qepA was found in only 5 out of the 29 isolates. The 1-QnrB gene was determined in 18 isolates. The presence of aac(6′)-lb 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 pneumoniae 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′)-lb. 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 GyrA 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 gyrA 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 gyrA mutation and a parC 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 gyrA and a single mutation detected in parC genes. As many as five-point mutations were observed in parC in isolates 10376 and 9438 and both isolates also had 2-point mutations in gyrA. 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 parC 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 parC 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 parC with the concomitant presence of mutations in the gyrA gene. Sequenced data obtained from this study has broadened our understanding of the molecular mechanism of fluoroquinolone resistance associated with gyrA and parC 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 (gyrA and parC). 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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