ORIGINAL ARTICLE

Mutational Analysis of Quinolone-Resistant Determining Region *gyrA* and *parC* Genes in Quinolone-Resistant ESBL-Producing *E. Coli*

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ABSTRACT

INTRODUCTION: Introduction: Co-resistance to quinolones among extended spectrum β-lactamase (ESBL)-producing *E. coli* commonly occurs in clinical settings. Quinolones act on DNA gyrase and DNA topoisomerase enzymes, which are coded by *gyrA* and *parC* genes, thus any mutation to the genes may affect the drug effectiveness. The objective of the study was to characterize *gyrA* and *parC* genes in quinolone-resistant *E. coli* isolates and correlated the mutations with their phenotypic resistance. **MATERIALS AND METHODS**: Thirty-two quinolone-resistant (QR) and six quinolone-sensitive (QS) ESBL-*E. coli* isolates were identified by antibiotic susceptibility and minimum inhibitory concentration tests. Bioinformatics analysis were conducted to study any mutations occurred in the genes and generate their codon compositions. **RESULTS**: All the QR ESBL-*E. coli* isolates were identified as multidrug-resistant bacteria. A single point mutation in the quinolone resistance-determining region (QRDR) ofgyrA, at codon 83, caused the substitution amino acid Ser83Leu. It is associated with a high level of resistance to nalidixic acid. However, double mutations Ser83Leu and Asp87Asn in the same region were significantly linked to higher levels of resistance to ciprofloxacin. Cumulative point mutations in *gyrA* and/or in *parC* were also correlated significantly ($p<0.05$) to increased resistance to ciprofloxacin. **CONCLUSION**: Together, the findings showed that the mutations in *gyrA* and *parC* genes handled the institution of intrinsic quinolone resistance in the ESBL-*E. coli* isolates. Thus, vigilant monitoring for emergence of new mutation in resistance genes may give an insight into dissemination of QR ESBL-*E. coli* in a particular region.

Keywords
quinolones, beta-Lactamases, Escherichia coli, antimicrobial resistance, hospital

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INTRODUCTION

Escherichia coli is a member of the family *Enterobacteriaceae* and is commonly associated with antibiotic resistance in many regions, thus creates concerns globally. The pathogen, whose normal habitats are the intestinal tract of humans and animals, are frequently associated with serious nosocomial as well as community-acquired infections such as pneumonia, sepsis, urinary tract infections, and several intra-abdominal infections. Although most are harmful, some strains of this species are related to a variety of antibiotic resistance genes acquired by the horizontal transfer of plasmids, pathogenicity islands, transposons, and bacteriophages.[1,2] Therefore, treatment and management of *E. coli* infections are commonly complicated by the appearance of resistance to multiple antibiotics and even to all currently known antibiotics. These multiple-drug resistant (MDR) *E. coli* strains are routinely discovered in many diagnostic laboratories by phenotypic testing.

MDR *E. coli* has commonly associated with the acquisition of extended-spectrum ß-lactamase (ESBL) determinants that give rise to the development of resistance to all ß-lactam antibiotics except

carbapenems and cephamycins. Outbreaks of ESBL-producing *E. coli* in healthcare settings have been reported by many.[3,4] Consequently, the increased prevalence of MDR *E. coli* strains has resulted in substantial usage of other antimicrobials including quinolones and fluoroquinolones (FQs). Among FQs, ciprofloxacin is one of the most used antibiotics to treat *E. coli* infection, thus resistance to the drug is on the rise. In Malaysia, the prevalence of ciprofloxacin resistance among *E. coli* isolates from all types of clinical samples is considered high, which is around 23.4% - to 24.1%.[5] Mechanism of ciprofloxacin resistance among ESBL-producing *Enterobacteriaceae* such as *Klebsiella pneumoniae* has been reported before but studies on quinolone resistance among *E. coli* are rare.[6]

As for the mechanism of action, quinolone targets DNA gyrase and DNA topoisomerase enzymes which are essential for normal bacterial growth and proliferation. The DNA gyrase subunits are encoded by *gyrA* and *gyrB* genes and are known as the primary quinolone target in gram-negative bacteria, while DNA topoisomerase IV, encoded by *parC* and *parE genes,* is a secondary target but reversely applies to gram-positive bacteria.[7] DNA gyrase is an essential regulator of DNA supercoiling and relieves topological stress arising from DNA replication complexes meanwhile, topoisomerase IV unwinds and decatenates newly replicated chromosomes following the bacterial DNA replication process. Thus, changes such as single nucleotide mutation of the genes may confer resistance to quinolone drugs by the bacterial cells.[8,9]

Genetic mechanisms of quinolone resistance may also be mediated by plasmid bearing one or multiple genes such as *qnrA, qnrB,* and *qnrC*.[10] These genes encode for proteins that protect DNA gyrase and topoisomerase IV from quinolone inhibition. Studies have shown that the quinolone-resistant bacteria may confer varying degrees of resistance either chromosomally or plasmid-mediated or in combination.[8,9] Among these resistance mechanisms, target-mediated resistance caused by specific mutations in the genes coding for subunits of DNA gyrase (*gyrA*) and topoisomerase IV (*parC*), is commonly reported worldwide.[11–14] These resistance mutations often occur in a region called the quinolone resistance determining region (QRDR) in the encoded gene, which is in close proximity to the amino-terminal domain. Although plasmids-mediated resistance may still enhance the selection of high resistance organisms, mutations in *gyrA* are the primary cause of quinolone resistance encountered in gram-negative clinical isolates.[6,12,15]

A study on antibiotic resistance among bacterial pathogens is important in the decision making of treatment intervention in a hospital setting. Many studies on the prevalence of antibiotic resistance among *E. coli* in Malaysia have been done but only a few focused on the quinolone resistance among *E. coli* clinical isolates.[16] Further investigation of the genetic mechanism of resistance would give insights into the evolutionary dynamics of clinical isolates of *E. coli* from quinolone susceptible to resistance. As local data on the genetic mechanism of quinolone resistance was scarce, we aimed to characterize the molecular mechanism for quinolone resistance being developed among ESBL-*E. coli* in Hospital Tengku Ampuan Afzan (HTAA), Pahang, Malaysia. We examined the nucleotide sequences of the DNA chromosomal *gyrA* and *parC* genes among the isolates because mutations in the genes are the main cause of quinolone resistance. Furthermore, we also studied the antibiotic susceptibility pattern of the isolated bacteria which were also identified as the MDR *E. coli.*

MATERIAL & METHOD

Bacterial Isolates

The sample size was calculated using an online application, OpenEPi (http://www.openepi.com). The sample size for a proportion or descriptive study was chosen with the desired absolute precision of 0.05 and 98% prevalence of *gyrA* gene mutation in quinolone-resistant *E. coli.* A minimum of 30 bacterial isolates was required for the mutational analysis study.

A total of 43 bacterial isolates, which were presumed as ESBL-producing *E. coli* isolates, were collected through a process convenience sampling method over 3 months (September to November 2018) from the Pathology Laboratory at HTAA, Kuantan, Pahang. Isolates were obtained from various types of clinical specimens such as urine, blood, swabs, tracheal aspirates, tissue, and

endotracheal tube which were collected from outpatients and inpatients of different wards. HTAA is a tertiary hospital in Pahang and a referral for many district hospitals within Pahang as well as certain regions of southern Terengganu.

All isolates were then labelled by number and reidentified at our laboratory by phenotypic and conventional tests such as, Gram's staining, IMViC test, combination disc test for phenotypic ESBL detection, analytical profile index (API 20E) identification system (BioMerieux, France), and applying of nalidixic acid and ciprofloxacin discs by Kerby-Bauer antibiotic sensitivity testing method, as well as determining of MICs of nalidixic acid and ciprofloxacin by applying E-test strip. Throughout the procedures, *E. coli* ATCC 25922 was used in tandem as a negative control. Bacterial isolates which were resistant to nalidixic acid or ciprofloxacin or both were identified as quinolones-resistant (QR) *E. coli*. Upon reidentification, 38 out of 43 isolates were confirmed as the ESBL *E. coli* which 32/38 were QR and 6/38 were quinolones-sensitive (QS). The other 5/43 isolates (isolates7, 8, 18, 20, and 25) were excluded from the study. The study was approved by the Medical Research Ethics Committee (MREC 18-1378, Ministry of Health, Malaysia).

Antibiotic Susceptibility and Minimum Inhibitory Concentration

Susceptibility to different antimicrobials was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) and was interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations.[17] Tested antibiotics included ampicillin (10 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), piperacillin-tazobactam (100/10 μg), gentamicin (30 μg), amikacin (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), tetracycline (30 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg) and colistin (10 μg) (Oxoid Ltd., Basingstoke, UK).

Minimum inhibitory concentrations (MICs) of nalidixic acid and ciprofloxacin were determined by E-test strips (Liofilchem s.r.l., Italy) according to CLSI guidelines. The isolates were considered resistant to nalidixic acid and ciprofloxacin if their MIC values were ≥ 32 μg/mL and ≥ 4 μg/mL, respectively. Isolates showing resistance to more than three different classes of antimicrobials were defined as MDR *E. coli* according to the previous terminology. The ESBL *E. coli* strains were identified phenotypically by a combined disc diffusion test according to the CLSI guidelines. Isolates that showed resistance to oxyimino-cephalosporins (ceftazidime and/or cefotaxime) were considered as putative ESBL *E. coli*.

Amplification of gyrA and parC gene

Genomic DNA from all bacterial samples was extracted from 5 mL of overnight grown culture using Presto™ mini gDNA bacteria kit (Geneaid Biotech Ltd, Taiwan) according to the manufacturer's protocol. The target regions of *gyrA* and *parC* genes were amplified with exTEN 2x Master Mix PCR kit (1st BASE, Apical Scientific Sdn Bhd., Malaysia), which the primer sets used and the PCR conditions were previously described by Hu et al.[18] PCR reaction mixture without DNA template was served as non-template control.

DNA sequencing and data analysis

Amplified PCR products of *gyrA* and *parC* genes were sequenced by the Sanger DNA sequencing method (DNA sequencing plus, 1st Base, Apical Scientific, Selangor, Malaysia). Mutations in QRDRs were identified by comparing the sequencing data with those of the *E. coli* K-12 strain (GenBank accession no. U00096.3) using the NCBI BLAST program (NCBI, USA), Clustal W Multiple Sequence Alignment Program (Bioedit Sequence Alignment Editor, version 7.2.5) and the Codon Code Sequence Assembly and Alignment Software (CodonCode, Corp, Centervilli, MA, USA). Statistical analyses were performed using SPSS software version 22 (SPSS Inc., Chicago, IL). Descriptive data were expressed as percentage frequency. The association between the number of amino acid mutations in the respective QRDRs and ciprofloxacin MICs was analysed by Pearson's correlation coefficient test and $p < 0.05$ was considered significant.

RESULTS

Antibiotic susceptibility profile

Thirty-eight (38) ESBL-*E. coli* isolates were obtained from urine (50%), blood (28.9%), (10.5%), tracheal aspirates (5.3%), tissue (2.6%), and endotracheal tube (2.6%), which 32/38 and 6/38 were QR and QS isolates, respectively. Antimicrobial susceptibility to 15 antibiotics (Table 1) was determined and the result showed that all 32 QR isolates were resistant to nalidixic acid, ampicillin, and tetracycline. The highest resistance rate was found to cefotaxime (96.9%) followed by ciprofloxacin (78.1%), trimethoprim-sulfamethoxazole (75%), ceftazidime (56.3%), cefepime (43.8%), and lower resistance was found to gentamicin (25%). However, no resistance was found to piperacillin-tazobactam, imipenem, meropenem, ertapenem, amikacin and colistin. In terms of quinolones antibiotics susceptibility, 7 out of 32 QR isolates were resistant to ciprofloxacin. Meanwhile, all the 6 QS isolates were 100% susceptible to piperacillin-tazobactam, carbapenem, aminoglycosides, and other quinolones used in the experiment. We also found that all the 32 QR isolates were multi-drug resistant bacteria, which most of them had resistance to 6, 7, or 8 antibiotics. The MICs of nalidixic acid were highly increased in all the QR isolates (≥256 μg/ml), and 7 of them were also had increased MIC for ciprofloxacin (≥ 4 μg/ml).

DNA sequencing analysis of gyrA and parC genes

Both *gyrA* and *parC* genes were amplified by conventional PCR. The targeted DNA was 648 bps for the *gyrA* gene and 395 bps for the *parC* gene, respectively, spanning the QRDRs.

The result of BLASTX showed a 100% identity score with *E. coli* DNA gyrase and DNA topoisomerase IV. From 32 QR ESBL-*E. coli* isolates, 96.9% (31/32) contained at least one resistant point mutation in the QRDR of *gyrA* gene with a high level of MIC of nalidixic acid (≥ 256 μg/ml). The most common mutation in the QRDR of the *gyrA* gene was the substitution of cytosine (C) with thymine (T), which

Table I. Antibiotic susceptibility profile of 38 *E. coli* isolates to commonly used antibiotics.

Antibiotic	Quinolone-resistant ESBL-*E.coli* (n=32)			Quinolone-sensitive ESBL-*E.coli* (n=6)		
	No of isolates, n(%)					
	*S	I	R	S	I	R
β-lactam						
Ampicillin	0 (0)	0 (0)	32 (100)	0 (0)	0 (0)	6 (100)
Cefotaxime	0 (0)	1 (3.1)	31 (96.9)	0 (0)	0 (0)	6 (100)
Ceftazidime	4 (12.5)	10 (31.3)	18 (56.3)	3 (50)	0 (0)	3 (50)
Cefepime	8 (25)	10 (31.3)	14 (43.8)	3 (50)	2 (33.3)	1 (16.7)
Piperacillin-Tazobactam	28 (87.5)	4 (12.5)	0 (0)	6 (100)	0 (0)	0 (0)
Imipenem	32 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)
Meropenem	32(100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)
Ertapenem	32(100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)
Aminoglycosides						
Gentamicin	24 (75)	0 (0)	8 (25)	6 (100)	0 (0)	0 (0)
Amikacin	32(100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)
Quinolones						
Nalidixic acid	0 (0)	0 (0)	32 (100)	6 (100)	0 (0)	0 (0)
Ciprofloxacin	7 (21.9)	0 (0)	25 (78.1)	6 (100)	0 (0)	0 (0)
Others						
Tetracycline	0 (0)	0 (0)	32 (100)	1 (16.7)	0 (0)	5 (83.3)
Trimethoprim-Sulfamethoxazole	7 (21.9)	1 (3.1)	24 (75)	4 (66.7)	0 (0)	2 (33.3)
Colistin	32 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)

*S, sensitive; I, Intermediate; R, Resistant

resulted in an amino acid substitution of serine (TCG) to leucine (TTG) at position 83 of the amino acid residue (96.9%) (Fig. 1). Another mutation in the QRDR of the *gyrA* gene occurred at position 87 which aspartic acid was substituted to either asparagine (71.9%) or tyrosine (3.1%).

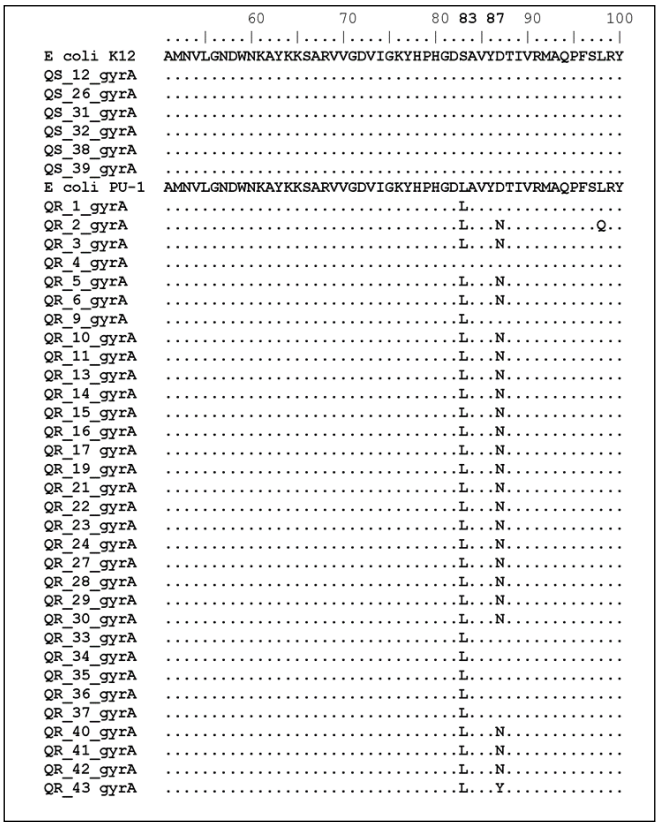

Fig 1. Missense mutations of *gyrA* gene in the quinolone-resistant (QR) ESBL-*E. coli* isolates. Partial protein sequence analysis of quinolone-resistant determination region shows amino acid changes at positions 83 and 87 when compared to the quinolone-sensitive (QS) isolates. *E. coli* K12 strain is the reference strain and represents the QS strain amino acid sequences, while *E. coli* PU-1 represents the QR isolates. The numbering of the amino acid residues was confirmed with the reference strain *E. coli* K-12 strain. (L = leucine, N = asparagine, Q = glutamine, Y = tyrosine)

Of the 32 QR ESBL-*E. coli* isolates, 26 (81.2%) contained point mutations in the QRDR of the *parC* gene (Fig. 2). Five (5) different amino acid substitutions were found in several positions inside and outside of the QRDR of the *parC* gene. The most common point mutation in the QRDR of the *parC* gene was the substitution of serine by isoleucine (78.1%). Another mutation was at codon position 84 where glutamic acid was substituted by valine (40.6%) or by glycine (12.5%). However, other resistance mutations were also found outside the QRDR of the *parC* gene such as Val144Met, (3.1%) and Asn176Thr, (3.1%). One isolate (isolate QR_4) with a high MIC of both nalidixic acid (≥ 256 μg/ml) and ciprofloxacin (32 μg/ml), was found to have no resistance-conferring mutations in neither *gyrA* nor *parC* genes. Besides the resistance-associated point mutations, several silent mutations were also found inside and outside of QRDRs in both QR and QS isolates.

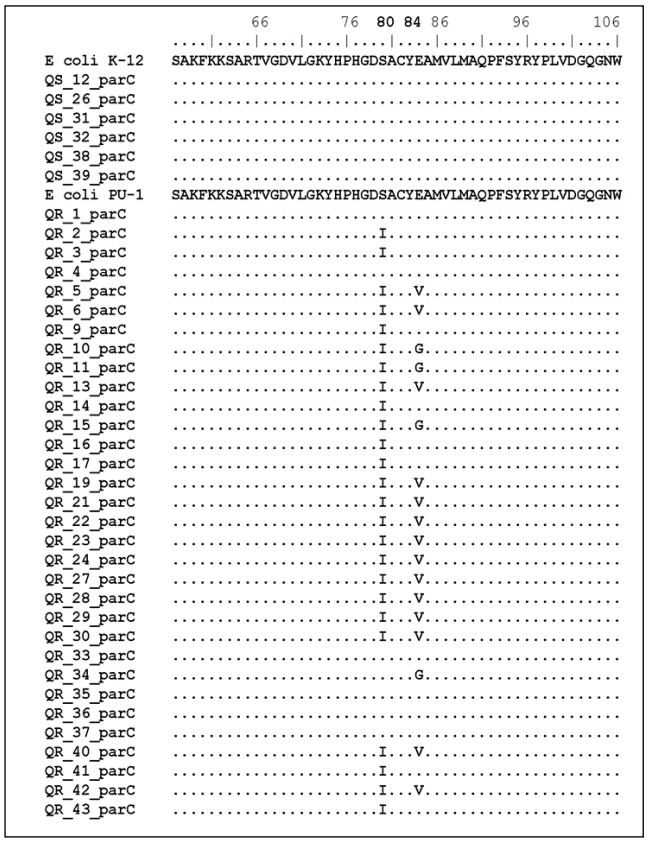

Fig 2: Missense mutations of the *parC* gene in the quinolone-resistant (QR) ESBL-*E. coli* isolates. Partial protein sequence analysis of quinolone-resistant determinant region shows amino acid changes at positions 80 and 84 when compared to the quinolone-sensitive (QS) isolates. *E. coli* K12 strain is the reference strain and represents QS strain amino acid sequences, while *E. coli* PU-1 represents the QR isolates. The numbering of the amino acid residues was confirmed with the reference strain *E. coli* K-12 strain. (I = isoleucine, V = valine, G = glycine)

Correlation between GyrA and ParC mutations and MIC of ciprofloxacin

A Pearson's correlation coefficient test result revealed that there was a moderate correlation between the number of amino acid mutations in the *gyrA* gene and MIC levels of ciprofloxacin and it was statistically significant (r=.646, p= 0.00006). Similarly, a moderate, and significant correlation was found between the number of mutations in the *parC* gene and MIC levels of ciprofloxacin (r=.504, p=.003). The test also revealed that the increase in *parC* gene mutations was strongly and significantly associated with an increase in *gyrA* gene mutations (r=.730, p= .000002) (Figure 3).

All 32 QR isolates except one revealed at least one mutation in *gyrA* and/or *parC*. The majority of the

isolates (40.6%) were shown to carry double mutations in both *gyrA* (Ser83Leu/Asp87Asn) and *parC* (Ser80Ile/Glu84Gln), which were associated with a MIC of >32 ug/ml level of ciprofloxacin MIC (Table 2). The isolates which possessed double mutations in codons 83 and 87 of *gyrA* displayed a high level of resistance to ciprofloxacin (MICs; 6-32µg/ml). Double mutations (Ser83Leu/Asp87Asn) in *gyrA* with additional mutation of types Ser80Ile or Ser80Ile/Glu84Val in *parC* also resulted in a high level of resistance to ciprofloxacin (MIC ≥32µg/ml).

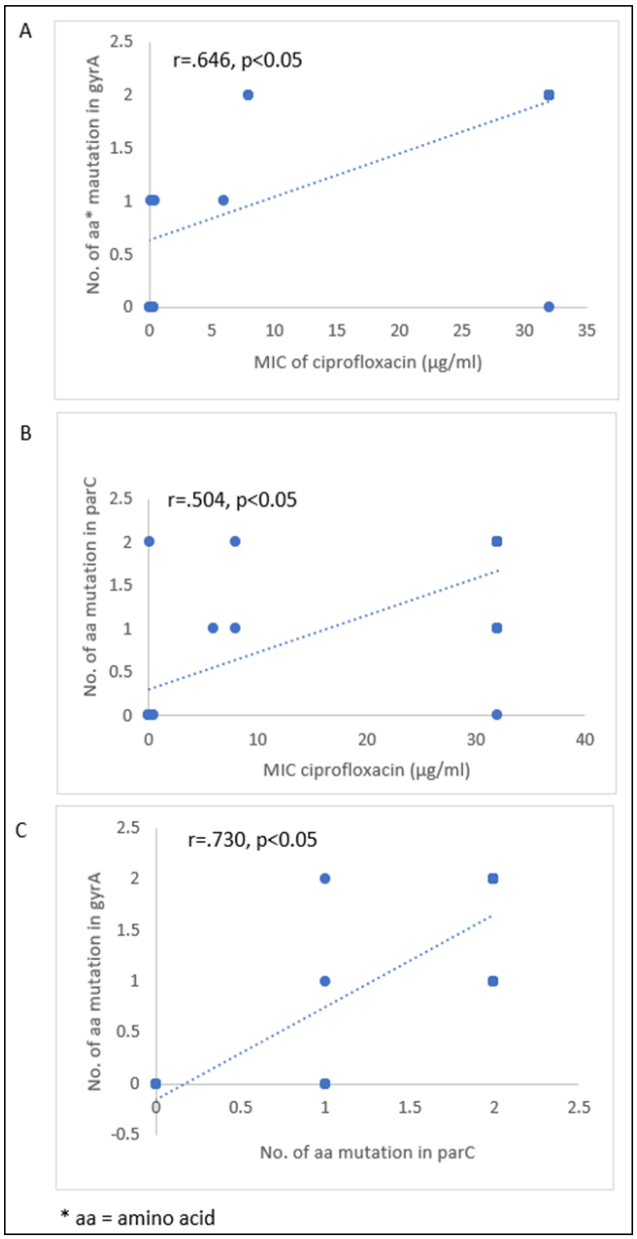

Fig 3: Correlation between mutations in QRDR of the A) *gyrA* gene and ciprofloxacin MIC, B) *parC* genes and ciprofloxacin MIC and C) *gyrA* gene and parC gene.

Table 2. Summary of amino acid substitutions in the quinolone-resistant determining region (QRDRs) of *gyrA* and *parC* genes in the quinolone-resistant (QR) ESBL-*E. coli* isolates with the corresponding minimum inhibitory concentrations (MICs) of ciprofloxacin (CIP).

MIC CIP[a] (ug/ml)	*Genes*		Isolate ID
	gyrA	*parC*	
32	-	-	4
0.125	Ser83Leu	Glu84Gln/ Val144Met	34
0.25	Ser83Leu		1,35,36
0.38	Ser83Leu		33
0.5	Ser83Leu		37
6	Ser83Leu	Ser80Ile	9
8	Ser83Leu/ Asp87Asn	Ser80Ile/ Asn167Thr	41
8	Ser83Leu/ Asp87Asn	Ser80Ile	2
≥32	Ser83Leu/ Asp87Asn	Ser80Ile	3,14,16,17,21
≥32	Ser83Leu/ Asp87Tyr	Ser80Ile	43
≥32	Ser83Leu/ Asp87Asn	Ser80Ile/ Glu84Gln	10,11,15
≥32	Ser83Leu/ Asp87Asn	Ser80Ile/ Glu84Gln	5,6,13,19,22,23,24 ,27,28,29,30,40,42

[a] CIP resistance value, ≥ 4 µg/ml

DISCUSSION

We focused on the genetic mechanisms of quinolone resistance in ESBL-producing *E. coli* isolates from our local community which was obtained from a tertiary hospital in Kuantan. The findings were analysed alongside the antibiotic susceptibility profile to commonly used antibiotics. Of note, during the period of the study, nalidixic acid (NA) was used as a surrogate antibiotic for AST, however, it has no longer a recommended treatment option due to high resistance rates in *E. coli*. As observed in this study, all 32 QR isolates showed 100% resistance to NA (Table 1).

As expected, most QR ESBL-*E. coli* isolates possessed mutations in the QRDR of the *gyrA* gene followed by *parC*. This implies that mutations in the *gyrA* are the most common mechanism of quinolones and fluoroquinolones (FQs) resistance followed by the *parC* mutations. Most of the mutations lead to the alteration of the amino acid sequence of the gyrA and parC which may attribute to phenotypic quinolone resistance. However, the reasons for the distribution of these mutation frequencies and locations are not yet well

known. Furthermore, double mutations in *gyrA* were more common than those of *parC*. This finding has been supported by a similar study reported from our neighbouring country, Thailand, which showed a higher occurrence of Ser83Leu mutation in *gyrA* in their isolates (89.1%) when compared to the *parC* gene (82.8%).[19] Another similar study in India by Bansal et al, reported that 98.1% of their QR-*E. coli* isolates possessed mutation in *gyrA* and 83.3% in *parC* gene.[20] Mutations in these two chromosomal genes would highlight one of the intrinsic pathways of quinolone and FQs resistance among *E. coli*. As these mutations are so common, thus, developing a kit to detect their presence is worthy, for rapid detection and evaluation of quinolone resistance.

The number and pattern of mutations particularly in *gyrA*, either single or double, are important sequential events in stepping up the quinolone resistance. Our study showed that a single Ser83Leu mutation of *gyrA* was associated significantly with high-level resistance to NA (MIC ≥256μg/ml) but may not to ciprofloxacin (MIC ranged from 0.25 to 0.5 μg/ml). However, double mutations in *gyrA* (Ser83Leu/Asp87Asn) were significantly associated with higher ciprofloxacin MIC levels (≥32 μg/ml) [Pearson correlation (r=.646, p= 0.000)] and this change was considered to play the initial step towards higher FQs resistance. Furthermore, combinations of point mutations in *gyrA* and *parC* were also required to generate a high level of resistance to FQs. The third most observed pattern of mutations was the combination of double mutations in *gyrA* and double mutations in *parC* (Ser83Leu/Asp87Asn and Ser80Ile/Glu84Val). So far, these point mutations in the QRDR of *gyrA* and *parC* genes have shown that resistance to FQs increased stepwise with the accumulation of these mutations.[21] In the E-test, the highest concentration for ciprofloxacin on the test strip was 32 μg/ml. Therefore, the combination of one or double mutations in *parC* with *gyrA* mutations may have demonstrated different ciprofloxacin MIC levels if performed with higher ciprofloxacin concentrations.

We found one isolate (QR-4) was highly resistant to ciprofloxacin (MIC, ≥32μg/ml) but did not have any amino acid alteration either in *gyrA* or in *parC*. The bacteria might undergo different quinolone resistance mechanisms such as plasmid-mediated which targeting protein protecting *qnr* genes, efflux pump overexpression *OqxAB* genes, or drug modifying encoding enzymes. This finding would suggest the other mode of mechanisms may also confer a high resistance but at a very low rate.[8]

In terms of antibiotic susceptibility profile, all the QR ESBL-*E. coli* isolates were also MDR bacteria. These pathogens, however, were susceptible to carbapenems despite the low level of resistance (0.7%) to ertapenem (a member of carbapenems). Thus, carbapenems are one of the last resorts for treating QR ESBL-*E. coli* infections but cautious and restricted use of these drugs is recommended. We found that QR ESBL-*E. coli* showed high resistance rates to most penicillin, except piperacillin-tazobactam (PTZ). Evidence from clinical trials in adults has shown that PTZ is an effective treatment for patients with urinary tract infections and other systemic infections such as respiratory tract infections, intra-abdominal infections, and febrile neutropenia.[22,23]

Furthermore, QR ESBL-*E. coli* also showed 100% susceptibility to amikacin and colistin and thus they also could be used as the last option drug against QR ESBL-*E. coli* infection. Interestingly, resistance to gentamicin and trimethoprim-sulfamethoxazole were much higher in QR ESBL-*E. coli* (25% and 75%, respectively) than QS ESBL-*E. coli* (0% and 33.3%) isolates. This finding indicates that isolates showing resistance to quinolones and FQs may carry resistance genes to aminoglycosides and other antimicrobials as well.

In this study, we did not investigate the further presence of *ß*-lactamase genes among the studied isolates. However, a similar study had been conducted before on *Enterobacteriaceae* bacteria isolated from the same population (HTAA).[24] In the study, ESBL-*K. pneumoniae* and *E. coli* isolates showed evidence of ESBL genes, namely bla_{CTM-M}, bla_{TEM}, bla_{SHV}, which 28% of 50 ESBL-positive isolates carrying the three ESBL genes.

In conclusion, mutations in *gyrA* and *parC* genes occurred in most of the QR ESBL- producing *E. coli* isolates, which may contribute to the development of multi-drug resistant bacteria. The resistance to ciprofloxacin revealed a high correlation with the accumulation of mutations in the QRDR of *gyrA* and

parC genes. Several antimicrobial agents commonly recommended for the treatment of *E. coli* infections were still effective against them *in-vitro*. This study highlighted the importance of DNA sequencing analysis in determining and understanding the mechanism of actions of quinolone resistance.

Authors' Contributions

Rahmatullah S. performed laboratory tests, compiled the results, and drafted the manuscript. Mahmud M.I.A.M. and Hamzah A.H designed the study, made the critical revision of the manuscript. Hamzah A.H. helped in bioinformatics and data analysis. Mahmud M.I.A.M. Roesnita B. gave the clinical inputs and logistic supports.

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Disclosure Statement

No competing financial interests exist.

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