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Original Article

In Silico Analysis of Amino acid Substitutions in DNA gyrase subunit A of Fluoroquinolone Resistant *P. aeruginosa* TOHO Strains, A Glance on Antibiotic Development

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Abstract

The broad consumption of antibiotics such as fluoroquinolones and the genetic adaption of opportunistic pathogenic bacteria including *Pseudomonas aeruginosa*, has led to develop of floroquinolone-resistant strains of *Pseudomonas aeruginosa*. Thus, the aim of the present study is to reveal the types of hot spot mutations occurring in selected DNA gyrase subunit A gene in fluoroquinolone-resistant *Pseudomonas aeruginosa* strains. For this purpose, 70 fluoroquinolone-resistant partial cds genes of *Pseudomonas aeruginosa* TOHO strains (P12-P377) for DNA gyrase subunit A have been aligned with wild genes for DNA gyrase subunit A by BLAST program and multiple alignment, to detect probable *gyrA* mutations. Based on analyses, hot spot mutations including Thr-83→Ile (92.44%), Ile-83→Thr (1.33%), Asn-87→Asp (0.44%) existing as single and Thr-83→Ile & Asn-87→Asp (5.78%) as double substitutions were detected. According to this survey, the point mutations in DNA gyrase subunit A gene need more consideration about fluroquinolone-resistant *Pseudomonas aeruginosa* isolates. The strains with hot spot SNP mutations may rise a huge concern from epidemiologic aspect for antibiotic therapy treatment.

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Introduction

Pseudomonas aeruginosa is an important opportunistic pathogen responsible for 10–15 % of the nosocomial infections in the world, this bacterium continues to be a serious cause of hospital infections, associated with a high rate of mortality ranging from 18% to 61%. Most of these infections are hard to treat due to the natural resistance of the species or ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents [1-3]. Generally, major factors in *P. aeruginosa* which enhance bacteria pathogenesis and drug resistancy are mutational mechanisms, a broad-spectrum Beta-Lactamase (ESBLs) of class A-B, D and efflux pumps [4-8]. So, there are different mechanisms for causing multidrug resistant (MDR) strains and limiting antibiotic therapeutic treatments, therefore unfortunately, the number of reports regarding to multidrug resistant (MDR) isolates of *P. aeruginosa* increases day to day [9,10]. In general lactams, aminoglycosides and quinolones are commonly antibiotic agents that are used for treatment of pseudomonas infection with reliable antibacterial activity [11]. Among these anibiotic classes, one of the most important antibiotics with broad-spectrum activity aginst both Gram-negative and Gram-positive bacteria are Quinolones that play an important role in treatment of serious bacterial infections, especially hospital-acquired infections. These antibiotics first time discovered in 1962 and nalidixic acid as first quinolone antibiotic was introduced in 1967 for treatment of urinary tract infections [12, 13]. Subsequent works led to the development of fluoroquinolones (e.g. ciprofloxacin), a large and effective family of compounds that possess bactericidal activity against Gram-negative bacteria such as *P. aeruginosa* by targeting and inactivation of two paralogous enzyme complexes including DNA topoisomerase IV and DNA gyras. These enzymes play important roles in DNA supercoiling in the closed circular DNA molecules and together remove the positive supercoils that accumulate ahead of a translocating DNA polymerase in DNA replication process and allowing to continue unhindered by topological strain [14,15]. Fluoroquinolones have the high oral bioavailability, convenient dosing, and excellent safety profile so makes them as a particular attractive treatment option for the treatment of *P. aeruginosa*. Therefore, in recent years paralleling the widespread use of the fluoroquinolones some MDR strains of *P. aeruginosa* are simultaneously resistant to this antibiotic. For example, in US intensive care units, the rate of *P. aeruginosa* resistance to ciprofloxacin tripled from 11% during 1990–93 to 32% in 2000, based on reports the increase in resistance is significantly associated with increased national use of FQs but for ciprofloxacin is especially

associated with cross-resistance to other antimicrobial agents [16].

As noted above the DNA topoisomerase IV and DNA gyras are as target for fluoroquinolones, these enzymes are made up of two subunits: *ParC* and *ParE* in the topoisomerase IV and *GyrA* and *GyrB* in the gyrase. Generally bacteria have developed resistance to FQs by two mechanisms consist of making targeted mutations (*GyrA*/*GyrB* for DNA gyrase and *ParC/ParE* for topoisomerase IV) or reduced access to the target by either decreased permeability or augmented expression of efflux pumps [14, 17, 18] . Among these mechanisms, resistace to FQs by mutagenesis in the gene encoding the DNA gyrase A subunit is as a major mechanism in Gram-negative bacteria including *P. aeruginosa* which occure within the Quinolone-Resistance-Determining Regions (QRDRs) as the single and double point mutations [19-22]. The main goal of this in silico investigation is to identify the types of substitutions in QRDR of DNA gyrase subunit A belonging to some strains from fluoroquinolone-resistant (FQR) gene in clinically resistant isolated *P. aeruginosa.*

Materials and Methods

Data collection

The search was done on NCBI nucleotide database to find complete or partial coding sequences of DNA gyrase subunit A belonging to fluoroquinolone-resistant strains of *Pseudomonas aeruginosa*. In this analysis 70 sequences of clinical isolates was selected which have been deposited in the GenBank databases under accession numbers AB753299 to AB753368. These sequences were reported by Kobayashi and his colleagues [23]. The selected sequences are belonging to DNA gyrase subunit A partial cds in TOHO strains. The selected sequences are partial cds and include some discontinuities, because they have been undergone several deletions and insertions in sequencing process.

Sequences screening

The selected sequences were aligned together by MEGA5.05 software [24] and compared with *Pseudomonas aeruginosa* DNA gyrase subunit A reference sequence (NC_002516.2, PAO1) via a multiple alignment. Based on alignment analysis the phylogenic tree of sequences was traced and redundant or similar sequences were omitted.

Sequence alignment

After primary screening of sequences, the remained mutant sequences and the RefSeq were aligned by MEGA5.05 software and the types and frequencies of hot spot single and double mutations in nucleotide sequences (codons) and amino acid substitutions were determined. For determination of amino acid substitutions, first the DNA sequences were translated into amino acid sequences in the correct reading frame and were aligned together via MEGA5.05 software.

Results

According to analyses, 3 types of hot spot single mutation and a double mutation were determined in the QRDR of *gyrA* gene by multiple alignment. In accordance with other investigations the predominant mutations were detected in codon 83 (Thr83→Ile), 83 (Ile83→Thr) and 87 (Asn87→Asp) within QRDR of gyrA gene as single mutations [19, 20, 22, 25-29]. Other hot spot mutations were double mutation in 83 and 87 codons with substitution of Thr83→Ile and Asn87→Asp (Table 1). Besides, in the present partial cds sequences the majority of alternations was detected as single mutation; while only one type of double mutation was identified.

Table 1. The details of mutations were observed in TOHO strains

Codon	Mutations	Amino acid substitution	Percentage
83	ACC→ATC	Thr→Ile	92.44%
83	ATC→ACC	Ile→Thr	1.33%
87	AAC→GAC	Asn→Asp	0.44%
83 87	ACC→ATC AAC→GAC	Thr→Ile Asn→Asp	5.78%

Discussion

Pseudomonas aeruginosa is known as an important pathogenic bacterium in nosocomial infections. The prevalence of *P. aeruginosa* FQR strains is increasing around the world and limiting the antibiotic therapeutic choices more and more [30].

FQs inhibit cell replication, transcription, and DNA repair in bacteria through disabling their essential enzymes of DNA gyrase and topoisomerase IV. Hence, mutations in these enzymes lead to appear FQR strains as the main mechanism of FQ resistance in bacteria. As the enzyme of DNA gyrase is the first target of FQs in Gram-negative bacteria including *P. aeruginosa*, so the mutations in *gyrA* gene appear early [31, 32].

In this study we tried to find out the probable hot spot SNP mutations in 70 samples which were reported as FQR *P. aeruginosa* strains by Kobayashi et al. in GenBank database. The results of the present survey confirm the same target site mutations including three singular substitutions in codons 83 (Thr83→Ile), 83 (Ile83→Thr), Asn87→Asp, and a double mutations in codons 83, 87 (Thr83→Ile & Asn87→Asp) within QRDR of *gyrA* gene as reported previously by other investigators [19, 25, 27, 28, 32-34].

According to previous investigation among the determined substitutions in this study, the *gyrA* gene mutants carrying the Thr83→Ile mutation have the key role in FQR strains and are significantly higher resistance to FQs than other alternations detected in the present survey. Furthermore, SNPs located in the Fluoroquinolone-Resistance-Determining Region (FRDR) of *gyrA* gene support FQR property in *P. aeruginosa* strains [3, 20, 29, 34].

It should be noted that according to this study more than 90% of FQR *P. aeruginosa* strains showed single or double mutations in the codon 83 which leads to Threonine → Isoleucine substitution at this position. Based on The hydrophobicity index (is a measure of the relative hydrophobicity, or how soluble an amino acid is in water, this index is zero for Glycine as standard) [35], Threonine is a polar – uncharged amino acid with low hydrophobicity (hydrophobicity index: +13) while Isoleucine is non-polar

amino acid with high hydrophobic property (hydrophobicity index: +99). Also in the codon 87, Asparagine as a polar-negative charged amino acid with low hydrophilic property (hydrophobicity index: -28) is replaced with Aspartic asid, however this amino acid is also a polar-negative charged amino acid but is more hydrophilic (hydrophobicity index: -55) in comparison with Asparagin. Similar to our previous study that has been done about *gyrA* gene mutations in fluoroquinolones resistant *Klebsiella pneumonia*, the most mutations in 83 and 87 codons lead to the substitution of amino acids with higher hydrophobic and hydrophilic property, respectively, compared to the first amino acids [36].

It is noteworthy that based on studies mutant substitutions in the codon 83 with hydrophobic amino acids generally confers more resistance rather than mutation in position 87. On the other hand, double mutations (83 and 87 codons) can increase levels of antibiotic resistance about two- to three-fold compared to single mutations [37].

Similar studies in other quinolone-resistance bacteria have been indicated spontaneous mutations in QRDR of *gyrA* gene at the 83 and 87 codons, however in these bacteria Serine is as main amino acid in the 83 position. Some studies showed that amino acid residues in these positions are crucial for binding of quinolone to *gyrA* subunit; however the molecular interactions are not known but some ligand docking studies on *gyrA* mutations in *Escherichia coli* and *Salmonella enterica* predicted the role of these mutant positions in altering of antibiotic-enzyme interactions. Based on these studies, change of amino acid, hydrophobicity and hydrogen bonds between enzyme and antibiotic are effective parameters in affinity level and interaction between enzyme and antibiotic so that change in amino acid hydrophobicity and reduction the hydrogen bonds between enzyme and antibiotic can leads to alteration of enzyme active site configuration. This alteration decreases the enzyme binding affinity to quinolones and therefore declines the drugs effectiveness [38, 39].

Conclusion

In conclusion, a vast range of epidemiologic studies and the present data in this survey confirm that, the misuse and inappropriate applications of FQs have led to continuous increase in the incidence of FQR strains of different bacteria including *Pseudomonas aeruginosa*. For this important reason, there is a serious need to substitute other therapeutic methods such as phototherapy rather than chemical therapy to control the rapid dissemination of antimicrobial resistance feature worldwide. We also suggest the detection of the 3-dimensional protein structures produced via mutant nucleotide sequences in future investigations.

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