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Study of Amino Acid Alteration in *graA* & *parC* Genes in Quinolone Resistant *Klebsiella pnemoniae*

Leila Asadpour*

Abstract

Objective: Fluoroquinolones are broad spectrum antibiotics which targets DNA gyrase and topoisomerase IV and prevents bacterial DNA replication and transcription. We aimed to determine amino acid alterations in *garA* and *parC* genes in quinolone resistant *Klebsiella pnemoniae* isolated from urinary tract infections in Rasht, Iran.

Materials and Methods: A total of 68 *K. pneumoniae* strains were isolated from urinary tract infections in Rasht, Iran, Resistance of *K. pneumoniae* to ciprofloxacin and the MIC of ciprofloxacin were determined according to the Clinical and Laboratory Standard Institute (CLSI) guideline. Quinolone resistance determining regions (QRDRs) of *gyrA* and *parC* were amplified in polymerase chain reaction (PCR) and subsequently sequenced. The changes in base and amino acid sequences were compared to the standard strain of *K. pneumoniae* in the GeneBank.

Results: Out of 68 *K. pneumoniae* clinical isolates, 16 isolates (23.5%) were phenotypically resistant to ciprofloxacin antibiotic, and 10 isolates (14.7%) had high levels of resistance. Investigation of the sequence of *gyrA* showed that in 7 out of 10 isolates, the mutation results in the substitution of an amino acid. Double mutation of Ser83Phe+Asp87Ala and Ser83Phe+Asp87Asn were the most common. In 4 out of 10 strains, the mutation in *parC* led to substitution of serine to isoleucine at codon 80.

Conclusion: Obtained results showed the high distribution of mutation hotspots in *gyrA* and *parC* in local isolates of *K. pneumoniae*.

Keywords: Amino acid alterations, Fluoroquinolone resistance, *gyrA*, *parC*, *Klebsiella pneumoniae*

Introduction

Quinolones are a class of broad-spectrum antibiotics that are effective against *Enterobacteriaceae*. Ciprofloxacin is a second-generation antibiotic of fluoroquinolones which is effective against Gram-negative and -positive bacteria (1,2). DNA gyrase and topoisomerase IV are the targets of this drug. DNA gyrase, a type II topoisomerase that is required for DNA replication and transcription, is the primary target of fluoroquinolones in Gram-negative bacteria. Ciprofloxacin prevents bacterial DNA from reconstruction, translation, and restoration through constraining DNA gyrase enzymes in Gram-negative bacteria (3,4). Topoisomerase IV is also as a secondary target of fluoroquinolones in Gram-negative bacteria (5). The resistance of Gram-negative bacteria against fluoroquinolones occurs due to chromosomal mutations in the quinolone resistance determining region (QRDR) of *gyrA* and *gyrB* genes, which code for DNA gyrase, and *parC* and *parE* genes, which code for topoisomerase IV (6,7). Mutations in the regions of the QRDR lead to changes in the enzyme structure and reduction of their binding to fluoroquinolones. In the present study, we aimed to determine amino acid alterations in *gyrA* and *parC* genes in quinolone resistant *Klebsiella pnemoniae* isolated from urinary tract infections in Rasht, Iran.

Materials and Methods

Test Bacteria

A total of 68 *K. pneumoniae* strains were isolated from urinary tract infections in Rasht, Iran, using conventional microbiological methods and confirmed by 16S rRNA gene amplification, as described previously (8, 9).

Identification of Ciprofloxacin Resistant Strains

Resistance of *K. pneumoniae* to ciprofloxacin and the MIC of ciprofloxacin were determined according to the Clinical and Laboratory Standard Institute (CLSI) guideline. *K. pneumoniae* ATCC 10031, a standard strain, was used as a control.

Detection of Mutations in *gyrA* and *parC*

Genomic DNA was extracted from each strain using a DNA isolation kit (CinnaGen, Iran). The extraction of nucleic acid was confirmed by agarose gel electrophoresis and subsequently used as the template DNA for polymerase chain reaction (PCR). PCR was performed in a total volume of 25 µL containing 0.5 µL dNTPS (10 mM), 5 µL enzyme buffer (10×), 3 µL forward and reverse primers

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Department of Veterinary Science, Rasht Branch, Islamic Azad University, Rasht, Iran.
***Corresponding Author:** Leila Asadpour, Tel: +989113383860; Email: L.asadpour@yahoo.com

(10 pmole), 2 µL template DNA (2 µg), 0.5 µL *Pfu* enzyme (2.5 U), and 14 µL deionized water. The *gyrA* and *parC* genes were amplified using specific primers (10). The primers used in this study are shown in Table 1.

The thermocycler program consisted of initial denaturation at 94°C for 4 minutes, 30 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and elongation at 72°C for 45 seconds. A final extension step was included for 10 minutes and the PCR products were detected by electrophoresis using a 1% agarose gel.

The PCR product was sent to Bioneer Company (South Korea) to determine the nucleotide sequence. After determining the nucleotide sequence of *gyrA* and *parC*, the change in base and amino acid sequences were compared to the standard strain of *K. pneumoniae* ATCC 13883 in the GeneBank using online software such as BLAST, Chromas v 1.45, and CLC main workbench v3.5.

Results

Out of 68 *K. pneumoniae* clinical isolates, 16 isolates (23.5%) were phenotypically resistant to ciprofloxacin antibiotic, and 10 isolates (14.7%) had high levels of resistance (completely resistant in antibiogram test and MIC > 64 µg/mL).

The amplifications of *gyrA* from ciprofloxacin resistant strains in the PCR reaction produced bands with an approximate length of 366 bp and from the amplification of *parC*, 312 bp bands were generated in all 10 tested isolates and subsequently confirmed by sequencing. Six different types of mutation patterns were found amongst the collection analyzed (Table 2). Investigation of the sequence of *gyrA* showed that in 7 out of 10 isolates, the mutation results in the substitution of an amino acid. In 3 strains, a double mutation of Ser83Phe + Asp87Ala was observed. In addition, 3 other different strains had a double mutation of Ser83Phe + Asp87Asn. In 1 strain, a single mutation of Ser83Ile was seen. In 6 out of 10 strains, the mutation in *parC* led to an amino acid substitution and such a substitution of serine to isoleucine occurred in 4 isolates at codon 80 and substitution of glutamic acid to lysine occurred in 2 isolates at codon 84.

Discussion

In this study, of 68 *K. pneumoniae* strains isolated from urinary tract infections, 10 isolates completely resistant to ciprofloxacin (MIC value between 64 and 1024 µg/mL) were investigated. Comparing nucleotide sequence of *gyrA* of the test strains with standard strain showed that the most common mutation occurred in codon 83 (70%). In 6 isolates, the mutation was a conversion of ATC to TTC, which led to the substitution of serine to phenylalanine. The second common mutation site (6 isolates) was in codon 87. Amongst them, 3 strains had a GAC to GCC conversion and the other 3 strains had a GAC to AAC conversion. This respectively led to the replacement of alanine and asparagine instead of aspartic acid, which have a high hydrophobicity in comparison with normal amino acids. In addition, one isolate had a conversion of ACC to ATC, which lead to a substitution of threonine instead of isoleucine in codon 161. Similarly, in *parC*, 4 isolates had a TCC to ATC conversion, which led to a substitution of threonine instead of isoleucine and 2 isolates had a GAA to AAA conversion, which led to a substitution of lysine instead of glutamic acid. The majority of ciprofloxacin resistant *K. pneumoniae* isolates in this study (60%) showed a QRDR region with double mutations in codons 83 and 87 of *gyrA*. This pattern was associated with high resistance to ciprofloxacin (MIC >256). This result is similar to Vila et al where they demonstrated that a mutation in codon 87, in addition to codon 83, was associated with increased fluoroquinolone resistance (11). In addition, Ardebili et al found that double mutations in *gyrA* and *parC* showed a higher level of ciprofloxacin resistance compared to the isolates with single mutations in *gyrA* or *parC* (12).

Mutations in codons 83 and 87 of *gyrA* and codon 80 of *parC* are among the most common mutations of *K. pneumoniae* strains resistant to fluoroquinolones. The change

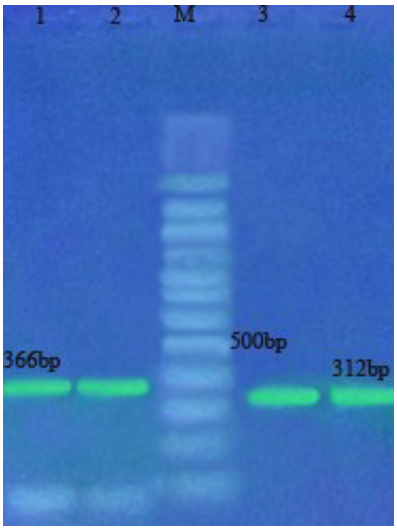

Figure 1. Agarose Gel Electrophoresis of *gyrA* and *parC* Genes PCR Amplicons. Lane M: 100bp DNA marker; lanes 1 and 2: 366bp amplicon of *gyrA* gene; lanes 3 and 4: 312bp amplicon of *parC*.

Table 1. Oligonucleotide Primers Used for Amplification of Particular Sequences of *Klebsiella pneumoniae gyrA* and *parC* Genes

Gene	Primers	Amplicon Size (bp)	Ref.
gyrA	F: 5′ CGCGTACTATACGCCATGAACGTA 3′	366	10
	R: 5′ ACCGTTGATCACTTCGGTCAGG 3′		
parC	R:5' CTGAATGCCAGCGCCAAATT3'	321	10
	F: 5' TGCGGTGGAATATCGGTCGC 3'		

Table 2. MIC of Ciprofloxacin and Amino Acid Substitution Profile in *gyrA* and *parC* in *Klebsiella pnemoniae* Isolates

Bacterial Isolate	MIC of Ciprofloxacin (µg/mL)	*gyrA* Amino Acid Substitution	*parC* Amino Acid Substitution
1	128	-	Ser80Ile
2	256	Ser83Ile	-
3	1024	Ser83Phe Asp87Asn	Ser80Ile
4	256	Ser83Phe Asp87Ala	-
5	64	-	Ser80Ile
6	1024	Ser83Phe Asp87Asn Thr161Ile	Glu84Lys
7	1024	Ser83Phe Asp87Asn	Glu84Lys
8	1024	Ser83Phe Asp87Ala	-
9	512	Ser83Phe Asp87Ala	-
10	256	-	Ser80Ile

in amino acids of 83 and 87 in the *gyrA* subunit of DNA gyrase enzyme is of special importance in the diagnosis of quinolone resistance such that it is reported in various studies (13,14). According to Heiat et al, based on GenBank data and reported studies, two codons (serine 83 and aspartic acid 87) in *gyrA* have the most frequent mutations including Ser83Phe (33%), Ser 83Tyr (20%), Asp87 Asn (25%), and Asp87Ala (8%) (15).

Norouzi et al (16) reported other different amino acid substitutions including Lys154→Arg and Ser171→Ala in *gyrA* and Ser129→Ala and Ala141→Val in *parC* in clinical isolates of *K. pneumoniae* from Kerman, Iran. Mutations in *gyrA*, as the primary target of fluoroquinolones, and complementary mutations in *parC* lead to the development of higher resistance levels in bacteria (17). In the present study, we had 3 ciprofloxacin resistant isolates that lacked any amino acid substitutions in *gyrA*. However, they had aSer80Ile conversion in *parC*. This result suggests the importance of mutations in *parC* in ciprofloxacin-resistant *K. pneumoniae*.

Conclusion

Our results showed the high distribution of mutation hotspots in *gyrA* and *parC* in local isolates of *K. pneumoniae*.

Ethical Issues

None to be declared.

Conflict of Interests

The authors declare no conflict of interests.

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References

1. Bonkat G, Muller G, Braissant O, et al. Increasing prevalence of ciprofloxacin resistance in extended-spectrum-β-lactamase-producing Escherichia coli urinary isolates. World J Urol. 2013;31(6):1427-32. doi: 10.1007/s00345-013-1031-5.
2. Ghasemian R, Shokri M, Makhlough A, Fallah M. Study of the resistance of Escherichia coli which creates community-acquired urinary tract infections to ciprofloxacin and co-trimoxazole antibiotics in Sari hospitals, Iran. Crescent J Med Biol Sci 2015;2(2):48-52.
3. Shin JH, Jung HJ, Lee JY, Kim HR, Lee JN, Chang CL. High rates of plasmid-mediated quinolone resistance QnrB variants among ciprofloxacin-resistant Escherichia coli and Klebsiella pneumoniae from urinary tract infections in Korea. Microb Drug Resist. 2008;14(3):221-6. doi: 10.1089/mdr.2008.0834.
4. Bansal S, Tandon V. Contribution of mutations in DNA gyrase and topoisomerase IV genes to ciprofloxacin resistance in Escherichia coli clinical isolates. Int J Antimicrob Agents. 2011;37(3):253-5. doi: 10.1016/j.ijantimicag.2010.11.022.
5. Fu Y, Zhang W, Wang H, et al. Specific patterns of gyr A mutations determine the resistance difference to ciprofloxacin and levofloxacin in Klebsiella pneumoniae and Escherichia coli. BMC Infect Dis. 2013;13:8. doi: 10.1186/1471-2334-13-8.
6. Koo SH, Kwon KC, Cho HH, Sung JY. Genetic basis of multidrug-resistant Acinetobacter baumannii clinical isolates from three university hospitals in Chungcheong province, Korea. Korean J Lab Med. 2010;30(5):498-506. doi: 10.3343/kjlm.2010.30.5.498.
7. Al-Marzooq F, Mohd Yusof MY, Tay ST. Molecular analysis of ciprofloxacin resistance mechanisms in Malaysian ESBL-producing Klebsiella pneumoniae isolates and development of mismatch amplification mutation assays (MAMA) for rapid detection of gyrA and parC mutations. Biomed Res Int. 2014;2014:601630. doi: 10.1155/2014/601630.
8. Gupta V, Yadav A, Joshi R. Antibiotic resistance pattern in uropathogens. Indian J Med Microbiol. 2002;20(2):96-8.
9. Wiskur BJ, Hunt JJ, Callegan MC. Hypermucoviscosity as a virulence factor in experimental Klebsiella

pneumoniae endophthalmitis. Invest Ophthalmol Vis Sci. 2008;49(11):4931-8. doi: 10.1167/iovs.08-2276.
10. Brisse S, Verhoef J. Phylogenetic diversity of Klebsiella pneumoniae and Klebsiella oxytoca clinical isolates revealed by randomly amplified polymorphic DNA, gyrA and parC genes sequencing and automated ribotyping. Int J Syst Evol Microbiol. 2001;51(3):91524.
11. Vila J, Ruiz J, Navia M. Molecular bases of quinolone resistance acquisition in gram-negative bacteria. In: Pandaly SG, ed. Recent Res Devel Antimicrob Agents Chemother. India: Reseach Signpost; 1999:32344.
12. Ardebili A, Lari AR, Beheshti M, Lari ER. Association between mutations in gyrA and parC genes of Acinetobacter baumannii clinical isolates and ciprofloxacin resistance. Iran J Basic Med Sci. 2015;18(6):6236.
13. Erac B, Gill A, Amyes S, Gulay Z. Mutations of gyrA in ciprofloxacin resistant Escherichia coli strains. Mikrobiyol Bul. 2002;37(2-3):125-30.
14. Minarini LA, Darini AL. Mutations in the quinolone resistance-determining regions of gyrA and parC in Enterobacteriaceae isolates from Brazil. Braz J Microbiol. 2012;43(4):1309-14. doi: 10.1590/S1517-838220120004000010.
15. Heiat M, Rezaeimehr M, Moghaddam M, Ranjbar R, Najafi A. Molecular genetic analysis of quinolone resistance-determining region of DNA Gyrase-A in fluoroquinolones resistant Klebsiella pneumoniae based on GenBank data and reported studies. Molecular Genetics, Microbiology and Virology. 2014;29(4):211-215.
16. Norouzi A, Azizi O, Hosseini H, Shakibaie S. Amino acid substitution mutations analysis of gyrA and parC genes in clonal lineage of Klebsiella pneumoniae conferring high-level quinolone resistance. J Med Microbiol Infect Dis. 2015;2(3):109-17.
17. Nawaz M, Sung K, Kweon O, Khan S, Nawaz S, Steele R. Characterisation of novel mutations involved in quinolone resistance in Escherichia coli isolated from imported shrimp. Int J Antimicrob Agents. 2015;45(5):471-476. doi: 10.1016/j.ijantimicag.2014.11.010.