Point Mutations in *gyrA* and *parC* Genes of Quinolone-Resistant Chlamydia Trachomatis in Iranian Women

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ABSTRACT

Background: Antimicrobial resistance is an increasingly common global issue, resulting in millions of death each year. Due to the emergence of bacterial resistance, it is not possible to fully treat some infections. According to a report by the World Health Organization (WHO) in April 2014, antimicrobial resistance is not a future prediction, but in fact a phenomenon happening right now, with the potential to affect people from different countries and age groups. Antibiotic resistance is characterized by bacterial changes, leading to the inefficacy of antibiotics in infection treatment. Today, antibiotic resistance is recognized as a major public health issue. Many studies have reported antibiotic resistance in different bacteria. Although, there are many reports about the prevalence of STD agents, there are only few documented reports of antibiotic resistance in chlamydia trachomatis. tetracyclines, quinolones, and azalides are common agents used in the treatment of infections. The present study aimed to evaluate resistance to quinolones among Iranian women with *chlamydial* infection. Material and Methods: This study included 300 women, who were referred to the obstetrics and gynecology clinics of teaching hospitals, affiliated to Shahid Beheshti University of Medical Sciences (Tehran, Iran) for abnormal vaginal discharge and recurrent infection (October 2012-2014). DNA sequencing, as well as polymerase chain reaction (PCR) assay, was performed to evaluate genetic resistance to quinolones. Results: The findings indicated point mutations in gyrase subunit A (*gyrA*) and topoisomerase IV subunit C (*parC*) genes of *Chlamydia trachomatis* strains from Iranian women. As the analysis of mutations indicated, they were in the quinolone resistance-determining regions (QRDRs) at positions 86, 93, 130, 131, 132, 134, and 135, of *gyrA* gene and positions 94 and 80, of *parC* gene. Conclusion: Based on the results, there have been some point mutations inside and outside QRDRs; this finding confirmed the resistance of chlamydial strains in Iranian women, treated by different classes of antibiotics.

Key Words: *Chlamydia trachomatis*, *gyrA*, *parC*, quinolone resistance, Antimicrobial resistance.

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INTRODUCTION

Chlamydia trachomatis is a Gram-negative bacterium, associated with four categories of diseases: 1) occular trachoma leading to blindness; 2) occulogenital diseases causing infertility, pelvic inflammatory disease, and reactive arthritis in adults; 3) lymphogranuloma venereum; and 4) perinatal infections [1-3]. Tetracycline, macrolides, and quinolones are among antibiotics used to treat chlamydial infections. On the other hand, the overuse of antimicrobial drugs has become a growing public health issue, leading to the increased antibiotic resistance of bacteria.

Despite proper drug treatment, chlamydial infections have shown a 5% to 20% recurrence rate [4-6]. The use of quinolones is quite common in many countries considering

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the low price and high accessibility of these drugs. Generally, quinolones are known as broad-spectrum antibacterial drugs in veterinary and clinical medicine. Considering the extensive use of these agents, different bacteria have shown increasing resistance. Quinolones function through exerting inhibitory effects on gyrase and topoisomerase IV in bacteria.

Fluoroquinolone resistance is associated with point mutations in the quinolone resistance-determining regions (QRDRs) of DNA gyrase (*gyrA*) and topoisomerase IV (*parC*) genes .Moreover, studies on *Escherichia coli* have indicated that mutations in codons 67, 82, 83, 84, and 106 of *gyrA* gene are responsible for resistance to quinolones [7, 8]. On the other hand, mutations at codons 80 and 84 in *parC* gene are responsible for resistance [9-11].

With this background in mind, the present study aimed to examine the drug resistance of *C. trachomatis* in Iranian women owing to the widespread use of quinolones and identify mutation-induced resistance for the prevention of inappropriate drug use and infection relapse.

MATERIALS AND METHODS

Statement of Ethics

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (SBMU.REC.1392.416). The Infectious Diseases and Tropical Medicine Research Center of the university funded the study (grant No, 416).

Vaginal discharge sampling was performed according to World Health Organization guidelines (using a speculum and dacron swabs).

94 *C. trachomatis* isolates were collected from 300 samples, which consisted of women with middle age of 29 and having abnormal vaginal discharge and a history of recurrent infection with using different classes of antibiotics such as Penicillins, Cephalosporins, quinolones, and were admitted to Shahid Beheshti teaching hospitals Tehran, Iran, from October 2012 to December 2014.

The confirmed *C. trachomatis* samples were stored at -70°C on 2SP medium (containing 20% glycerol) and subjected to molecular identification.

DNA extraction and PCR assay

For DNA extraction, the 2SP medium was used according to the DNA extraction kit (Bioneer, Korea). For identification of mutation regions in *gyrA* and *parC* genes, CTA3, CTA4, CTC3, and CTC4 primers were used from the literature [12]. CTA3 and CTA4 primers with nucleotide sequences of *gyrA*, as well as CTC3 and CTC4 primers with nucleotide sequences of *parC* gene, have been presented in the following table:

Table 1. CTA3 and CTA4 primers with nucleotide sequences of *gyrA*, and CTC3 and CTC4 primers with nucleotide sequences of *parC* gene

Genes	Primers	Nucleotide sequence	Fragment length (bp)
gyrA	CTA3	5'TTAAAACCTTCTCAGCGACG3'	362
gyrA	CTA4	5'GAAGGAAAAACTACAGGTTC3'	362
parC	CTC3	5'ATGGCCTCAAGCCTGTTCA3'	201
parC	CTC3	5'CAGTGGATTGCCAAAGTTCCC3'	201

To amplify 362-bp fragments from *gyrA* gene, PCR assay was performed in a thermocycler (AG22331; Eppendorf, Germany) under the following conditions: initial denaturation at 95ºC for 5 minutes; 35 cycles at 95ºC for 1 minute, at 41ºC for 1 minute, and at 72ºC for 2 minutes; and final extension at 72ºC for 10 minutes. Following the amplification process, the PCR products were added to 2% Agarose gel. In addition, for amplification of 201-bp fragments of *parC* gene, PCR assay was conducted in a thermocycler (AG 22331; Eppendorf, Germany) under the following conditions: denaturation at 95ºC for 5 minutes; 35 cycles at 95ºC for 1 minute, at 60ºC for 1 minute, and at 72ºC for 2 minutes; and extension at 72ºC for 10 minutes. The final products were then added to 2% Agarose gel.

For sequencing the genes, the samples were sent to Takapouzist Company (Iran).

Mutation was identified by comparing translated amino acid sequences to reference to *gyrA* and *parC* gene NCBI,GENE BANK , T-BLAST X tool.

RESULT

Fig. 1. PCR amplification of *gyrA* gene from chlamydia-positive patients: lane 1, DNA ladder (1000 bp); lanes 2, 3, 6, 7, 14, 15, positive sample; lane 12, positive control; lane 13, negative control.

Amino acid mutations in *C. trachomatis* samples

According to the analysis of amino acid changes in gyrA proteins, mutations were as follows:

- conversion of amino acid from serine to lucien at position 86
- deletion of amino acid at position 93 (proline)
- conversion from threonine to proline at position 130
- conversion from threonine to valine at position 130
- conversion from serine to valine at position 131
- conversion from serine to valine at position 132
- conversion from threonine to proline at position 134
- conversion from cysteine to serine at position 135

Analysis of *parC* gene

PCR and electrophoresis were performed using a pair of primers to detect mutations in *parC* gene. In 22% of the samples, 201-bp bands were formed (Figure 2).

Fig. 2. PCR amplification of *parC* gene from chlamydia-positive patients: lane 1, DNA ladder (1000 bp); lanes 2, 3, 7, positive sample; lane 11, negative control; lane 12, positive control.

According to the analysis of amino acid changes in *parC* proteins, mutations were as follows:

In parC proteins, mutations were as follows:

- conversion of amino acid from threonine to alanine at position 94
- conversion from serine to arginine at position 80

DISCUSSION

In the present study, 300 samples were examined for *C. trachomatis,* using nested PCR. After detecting the positive samples, PCR assay was used to amplify *parC* and *gyrA* genes. Following that, by sequencing the genes and converting them into proteins, mutations and changes in amino acids, leading to quinolone resistance, were examined.

Although there have been various studies on the prevalence of STDs related to *Neisseria gonorrhoeae* and *C. trachomatis* in Iran [13-16], as well as other countries, there have been fewer reports about drug resistance of bacteria, especially *C. trachomatis*. Nonetheless, there have been reports of recurrent *C. trachomatis* infection, which can be associated with the lack of proper treatment and drug resistance to different antibiotics in *C. trachomatis* [17]. For decades, tetracycline and erythromycin have been used to treat *C. trachomatis* infections. Moreover, macrolides, such as erythromycin or azalides (e.g., azithromycin) have been used owing to their high concentration in the cells. The first case of *C. trachomatis* resistance was reported in 1990 by Jones et al. [18]. Subsequently, a *C. trachomatis* strain, resistant to 64 µg/mL of tetracycline, was reported in France. In addition, three *C. trachomatis* strains were isolated in the United States, which showed multidrug resistance to azithromycin, ofloxacin, and doxycycline.

In the past, it was assumed that bacteria with an intracellular life cannot easily acquire drug resistance. However, according to the literature, *C. trachomatis* can acquire adenosine diphosphate/adenosine triphosphate (ADP/ATP) translocase enzymes from plants; therefore, there has been a possibility of horizontal transfer of drug-resistant and alien genes [19, 20].

A study by Dessus-Babus et al. showed that if *C. trachomatis* L2 strains were exposed to subinhibitory concentrations of fluoroquinolones, they underwent mutations in *gyrA* gene, and amino acid conversion occurred at position 83 (conversion of serine to leucine). Nevertheless, no changes were observed in *gyrB*, *parC*, or *parE* genes [12].

In 2002, Morrissey et al. showed that if *C. trachomatis* and *Pneumococcus* were exposed to subinhibitory concentrations of fluoroquinolones, the minimum inhibitory concentration increased after 30 passages in the cell culture. Based on the molecular methods, amino acid 83 converted from serine to isoleucine in *gyrA* gene [21].

In addition, studies by Rachel Binet et al. revealed that mutations in chlamydial rRNA genes, similar to *Mycobacterium* and *Helicobacter*, resulted in resistance to drugs, such as tetracycline and macrolides [22-24]. Moreover, in a study by Shigeaki Yokoi et al. from Japan (2004) on 23 *C. trachomatis* isolates, collected from men with nongonococcal urinary tract infection, 12 isolates showed an amino acid conversion in *parC* gene. Arginine 83 was converted to glycine, and in one case, arginine was converted to cysteine 66 [25].

In the present study, the analysis and comparison of the collected strains showed *gyrA* and *parC* gene mutations.

Mutations in *gyrA* gene were characterized by amino acid changes at positions 86, 94, 131, 132, 134, and 135, including QRDRs (amino acids 67-106). Additionally, in *parC* gene, mutations occurred in amino acids 80 and 94, which were also located in the QRDR. The obtained results were in agreement with the other similar studies around the world. However, differences in the position of amino acids might be associated with the endemicity of Iranian strains.

CONCLUSION

Regarding the presence of mutations in *parC* and *gyrA* genes from the isolated samples of Iranian women, the use of precise methods, including culture and molecular assays such as PCR, has been suggested to detect bacteria. According to the results, appropriate antibiotics should be selected in order to prevent the recurrence and consequences of infection, including pelvic inflammatory disorder, which are both costly and disturbing for patients.

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