MOLECULAR DETECTION OF QUINOLON RESISTANCE GENES IN CHLAMYDIA TRACHOMATIS ISOLATED FROM WOMEN WITH GENITAL INFECTIONS

Roya Torabizadeh¹, Gita Eslami², Latif Ghachkar¹ and Mehdi Goudarzi²

¹Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
²Department of Medical Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IRAN.

*Corresponding Author: Roya Torabizadeh
Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

ABSTRACT

Introduction and aims: Chlamydia trachomatis infection spread worldwide and causes different diseases. In many countries the most genital infection is chlamydia trachomatis. Tetracycline, macrolides and quinolones are common therapeutic options for treatment of infection. Resistance to quinolones in C. trachomatis clinical isolates has become an emerging challenge especially in women with genital infection. The aim of this study was to investigate the antimicrobial susceptibility patterns of C. trachomatis isolates resistance to quinolone agents and the mutation of gyr A and parC genes. With molecular methods in women with genital infection. Materials and methods: During a 15 months study, 200 specimens were collected from patients with genital infection. Nested PCR was used for identification of C. trachomatis. Presence of gyrase (gyrA) and topoisomerase IV (parC) and mutation was identified using PCR assay. for identification of C. trachomatis. Presence of gyrase (gyrA) and topoisomerase IV (parC) and mutation was identified using PCR assay. we understand that there are 54 cases from 80 cases which are positive for having mutation in gyr A gene (67.5%) and 18 cases are positive for having mutation in parC gene (22.5%). Result: Out of 200 specimens which had taken from women with vaginal discharge, D&C or abortion, 80 cases were positive for chlamydia trachomatis by Nested PCR. we understand that there are 54 cases from 80 cases which are positive for having mutation in gyr A gene (67.5%) and 18 cases are positive for having mutation in parC gene (22.5%). We understand that there are 54 cases from 80 cases which are positive for having mutation in gyr A gene (67.5%) and 18 cases are positive for having mutation in parC gene (22.5%). Our result revealed high resistance to quinolone among clinical chlamydia trachomatis isolated from patients, the percentage of mutation for both gyrA gene (67.5%) and parC gene (22.5%) were high, especially for gyr A gene, it seems that the second generation of quinolones are not effective drugs for treatment of chlamydial infections any more. Discussion: Our result revealed high resistance to quinolone among clinical chlamydia trachomatis isolated from patients, the percentage of mutation for both gyrA gene (67.5%) and parC gene (22.5%) were high, especially for gyr A gene, it seems that the second generation of quinolones are not effective drugs for treatment of chlamydial infections any more.

KEYWORDS: Chlamydia trachomatis, quinolone resistance, gyr A gene, par C gene.
permeability of bacterial membrane to drug. These two mechanisms are chromosomally mediated; also mobile elements have been described for carrying the qnr gene which confers resistance to quinolones.

Mechanism of action: Quinolones act by inhibiting the action of type II topoisomerase (DNA gyrase) and type IV topoisomerase, both enzymes have A₂B₂ subunits. Gyrase encoded by gyrA, gyrB genes. Topoisomerase encoded by parC, parE genes. Information about antimicrobial resistance profiles of C. trachomatis isolates in Iran is very sparse. The aim of this study was to investigate the antimicrobial susceptibility patterns of C. trachomatis clinical isolates against quinolones commonly used for treatment genital chlamydial infection and the mutation of QRDR gene region.

MATERIAL AND METHODS
A total of 80 clinical isolates of C. trachomatis were recovered from the 200 specimens of patients with genital infection who referred to the shahid Beheshti's hospital during October 2012 to February 2014 were included in this study. Identification of specimens was performed by Nested PCR. The quinolone resistance profile for all isolates was determined by PCR method. The confirmed samples as C. trachomatis were stored at -70°C on 2SP medium containing 20% glycerol and were subjected to molecular identification.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Nucleotidesquence</th>
<th>Fragment (bp)</th>
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<tbody>
<tr>
<td>GyrA</td>
<td>CTA3</td>
<td>TTAAAACCTTTCAGCAGCG</td>
<td>362 bp</td>
</tr>
<tr>
<td>GyrA</td>
<td>CTA4</td>
<td>GAAGAAAAACTACAGGTTC</td>
<td>362 bP</td>
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PCR conditions for amplification of 362bp fragment of the gyr A gene was done by thermocycler (AG 22331; Eppendorf, Hamburg, Germany) as follows: initial denaturation at 5 min at 95°C, followed by 35 cycles of 1 min at 95 °C, 1 min at 41°C, and 2 min at 72 °C; and final extension at 72 °C for 10 min to end amplification process. The PCR products add to a 2% gel agarose.

DNA extraction and PCR
DNA was extracted from bacteria on 2sp medium by using DNA extraction, Bioneer kit, korea.

For identification of presence of mutation region in gyrase (gyrA) and topoisomerase IV (parC), We needed to design specific primers to capable of attachment and amplification the AA 60-110 In QRDR region, that most frequent of mutations take place in this region so we used described primers CTA3, CTA4 and CTC3 and CTC4 adopted by the article (12).

The primers CTA3 and CTA4 with nucleotid sequence of gyrA were.
TTAAAACCTTTCAGCAGCG.
GAAGAAAAACTACAGGTTC.

The primers CTC3 and CTC4 with nucleotide sequence of parC were.
ATGGCCCTCAAGCCTGTCA.
CAGTGGATTGCAAAGGTCCC.

Figure1: Detection of gyr A gene by PCR. lane 1: ladder, lane 2 and 3 positive result.
PCR conditions for amplification of 201bp fragment of the parc gene was done by thermocycler (AG 22331; Eppendorf, Hamburg, Germany) as follows: initial denaturation at 5 min at 95°C, followed by 35 cycles of 1 min at 95 °C, 1 min at 600°C, and 2 min at 72°C; and final extension at 72 °C for 10 min to end amplification process. The PCR products add to a 2%gel agarose.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Nucleotidesequence</th>
<th>Fragment (bp)</th>
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<tbody>
<tr>
<td>parc</td>
<td>CTC3</td>
<td>ATGGCCTCAAGCCCTTCA</td>
<td>201bp</td>
</tr>
<tr>
<td>parc</td>
<td>CTC4</td>
<td>CAGTGATTGCCAAGTTCCC</td>
<td>201bp</td>
</tr>
</tbody>
</table>

CONCLUSION
In this study we enrolled 200 specimens of women with vaginal discharge, D&C, spontaneous abortion 80 specimens had positive result for Chlamydia trachomatis with molecular methods. determination of quinolone resistance with amplification of gyrA and parc genes cleared that 67.5 % of gyr A and 22.5% of parc genes had mutation in QRDR region, it means that in Iranian strains of Chlamydia trachomatis mutation of gyr A and parc genes could took place which refer to resistance strain of chlamydia trachomatis, so using of new generation of quinolones instead of old generation for treatment of genital infection of C.Trachomatis is recomended.

REFERENCES