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

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ABSTRACT

Introduction and aims: Chlamydia trachomatis infection spread worldwide and causes differrent 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.trahomatis 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 gyrA 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 gyrA 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 gyrA 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.

INTRODUCTION

Chlamydia trchomatis, a gram negative bacteria ,with two biphasic life cycle Elementry body(EB)and Reticulate body(RB)causes different diseases consist of four clinical categories: 1-occular trachoma 2-LGV 3-other oculogenital diseases in adults 4- perinatal infections.[1,2] In many countries the most genital infection is C.trachomatis. Tetracycline ,macrolides and quinolones are common therapeutic options for treatment of infection.[3] On 1990 resistance to antibiotic has been reported from C. trachomatis then resistance strain of C. trachomatis were described from France, USA and other countries.[4,5,6,7] It seems that antibiotic resistance to b rare in chlamydial infections. Intracellular nature of bacteria and also 2 forms of life cycle are good reason for elimination the rapid acquirement of resistance to antibacterial agents.[3,8]

For treatment of many infections consist of: gonococcal infections, osteomyelitis, enteric and respiratory tract infections, Fluroquinolones have been used. Two mechanism were described for resistance to quinolones 1- Alteration in target site of quinolonese 2-Over expression of efflux pump system or decreasing of

permeability of bacterial membrane to drug. These two mechanism 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 typeII topoisomerase (DNA gyrase) and type IV topoisomerase, both enzymes have A_2 B_2subunit.Gyrase encoded by gyrA, gyrB genes, Topoisomerase encoded by par C,pare E genes.(9,10,11) 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.

DNA extraction and PCR

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

For identification of presence of mutation region in gyrase (gyrA) and topoisomerase IV(parC), We neededde 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.
TTAAAACCTTCTCAGCGACG.
GAAGGAAAAACTACAGGTTC.

Gene	Primer	Nucleotidesequence	Fragment (bp)
Gyr A	CTA3	TTAAAACCTTCTCAGCGACG	362bP
Gyr A	CTA4	GAAGGAAAAACTACAGGTTC	362 bP

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 95ºC, followed by 35 cycles of 1 min at 95 ºC, 1 min at 41c, and 2 min at 72 ºC; and final extension at 72 ºC for 10 min to end amplification process. The PCR products add to a 2%gel agarose.

The primers CTC3 and CTC4 with nucleotide sequence of parC were.
ATGGCCTCAAGCCTGTTCA.
CAGTGGATTGCCAAAGTTCCC.

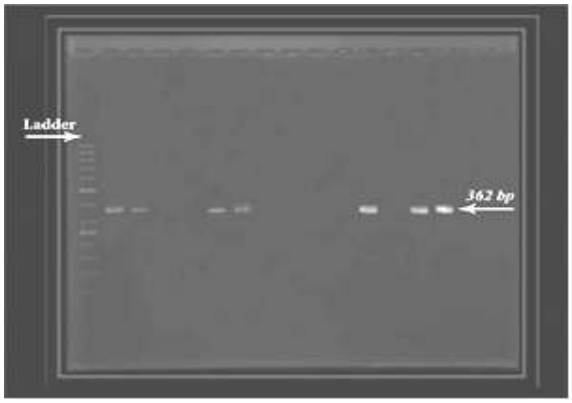

Figure1: Detection of gyr A gene by PCR.lane 1: ladder, lane2 and 3 positive result.

Gene	Primer	Nucleotidesequence	Fragment (bp)
parc	CTC3	ATGGCCTCAAGCCTGTTCA	201bp
parc	CTC4	CAGTGGATTGCCAAAGTTCCC	201bp

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 95ºC, followed by 35 cycles of 1min at 95 ºC, 1 min at 600C, and 2 min at 72ºC; and final extension at 72 ºC for 10 min to end amplification process. The PCR products add to a 2%gel agarose.

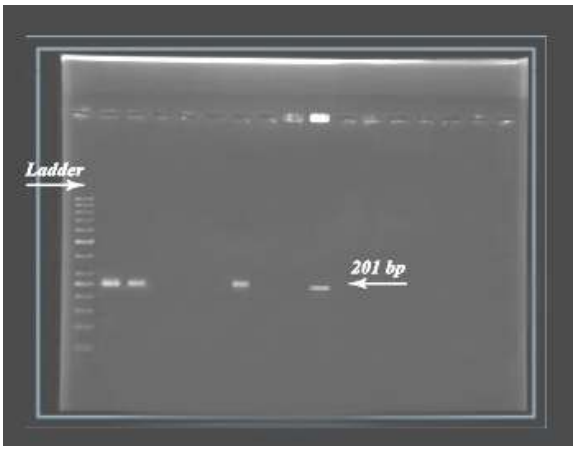

Figure2. Detection of par C gene by PCR.lane 1: ladder, lane2 and 3 positive result.

RESULTS

In this study we use described primers(12)which specificly designed for detection of mutations in the gyrA and parC region genes ,we understand that there are 54 cases from 80 cases are positive for having mutation in gyr A gene (67.5%).and 18 cases are positive for having mutation in par C gene. (22.5%).

DISCUSSION

Antibiotic resistance in chlamydia trachomatis due to it,s unique biphasic life cycle especially having elementry body is rare.[11,12,13] but there are studies that confirm antibacterial resistance in chlamydia on invitro or invivo situation .in 1990 jonnes et al reported antibiotic resistance for Chlamydia trachomatis.[5] In 1998, the first strain of chlamydia trachomatis which is resistance to Tetracyclin was isolated and reported from Toulose, France.[6] In 2000, Somani et al described 3 strains of chlamydia trachomatis which exhibited multidrug resistance to azithromycin,tetracyclin and doxycycline.[7] Most of researches have prepared in vitro condition which confirm resistance of chlamydiatrchomatis to antibacterial agent, also there are studies which work on pateints.Yokoi et al studied 23 men with nongonococcal urethritis and understand all 23 cases have mutation in ParC gene and also 12 cases from 23 ,have mutation in GyrA gene. Which refers to quinolone resistance in Chlamydia strains.

Our study revealed that there are antibactrial resistance in strain of chlamydia trachomatis in patients which we enrolled in the study, the percentage of mutation and resistance to quinolone was high for gyrA gene (67.5%) and parC gene (22.5%) especially for gyr A gene, so it seems that usage of quinolone like ciprofloxacine or ofloxacine for treatment of chlamydial infection is no more antibiotics. And instead of it, usage of new generation of quinolones may be have effective results.

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.

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