Original Article

Prevalence of *Mycoplasma genitalium* and *Mycoplasma hominis* isolates among Women with Cervicitis Referred to Karaj Health Care Centers

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Abstract

Background: *Mycoplasma* is a genus of bacteria often found in the normal flora of the mouth, respiratory system and urogenital tract; but potentially pathogenic species also exist which can cause serious respiratory and genital diseases in human including postpartum fever, pelvic inflammatory infections, and pyelonephritis. The aim of this study was to evaluate the prevalence of *Mycoplasma genitalium* and *Mycoplasma hominis* in women who referred to the health centers in Karaj and investigate the susceptibility of M. *genitalium* strains against fluoroquinolone antibiotics. **Materials and Methods:** Endocervical swabs were taken from 200 women with cervicitis. Nucleic Acid Amplification Tests (NAATs) were performed for detecting Mgpa gene in M. *genitalium* and RNH gene in *M. hominis*. Mutations in parC and gyrA genes, as well as antibiotic resistance, were studied in positive samples of M. *genitalium*. **Results:** M. *genitalium*(n=9) and *M. hominis*(n=11) positive samples were found among samples obtained from women with cervicitis. Positive samples of M.*genitalium* were examined for isolating the parC and gyrA genes. Six sequences of these genes were analyzed by MEGA5 software. Mutation in parC gene was observed in one sequence which %16 shows resistance. **Conclusion**: *M. hominis* and *M. genitalium* were detected in 5.5% and 4.5% of samples, respectively. Our findings showed a relatively medium prevalence of *M. hominis* and *M. genitalium* in women with cervicitis in Alborz province. The sequencing results of gyrA and parC genes in this study represent the occurrence of mutations which drive fluoroquinolones resistance. Therefore, further studies in this field are needed in Iran and other countries, and to address the problems of increasing antibiotic-resistant strains, irregular administration and antibiotic susceptibility testing, more attention should be paid.

Keywords: *Mycoplasma genitalium*, *Mycoplasma hominis*, Cervicitis, fluoroquinolone, PCR

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Introduction

Sexually Transmitted Infections (STIs) are a common major public health problem. In some developing countries, STI is one of the five common diseases. The World Health Organization (WHO) appraises that within 340 million new cases of STIs occur yearly. (1, 2) Mollicutes are found in both

STIs and healthy individuals (1). This study is designed to expound the role of these bacteria in women with cervicitis. Since the polymerase chain reaction (PCR)-based assays more simplified detection of M. *genitalium* and M. *hominis* in clinical specimens; it has been shown that M. *hominis* is related with non-gonococcal urethritis (NGU), bacterial vaginosis (BV), post-birth fever, and rarely with arthritis, peritonitis, meningitis, and bacteremia. M. *genitalium* is highly associated with NGU in men and salpingitis, cervicitis, pelvic inflammatory and endometritis diseases in women (3- 5). Although limited clinical data are available regarding the treatment of *M. genitalium* infections, eradiation rate of *M. genitalium* with a single 1g dose of azithromycin was reported to be 72–87% in most therapies. In the clinical range of *M. genitalium*, determining the antimicrobial resistance is critical for considering the treatment guidelines. The use of *Mycoplasma* culture method to evaluate antibiotic resistance is still a laborious and time-consuming process. Hence, in this study, we attempted to use a molecular procedure that not includes the isolation of *M. genitalium* and antimicrobial susceptibility testing in a laboratory to determine fluoroquinolone- resistance in bacterial clinical specimens. Fluoroquinolones act by binding to topoisomerase IV and DNA gyrase, which are target enzymes, and therefore interpose with DNA replication. DNA gyrase is composed of GyrA and GyrB each one two subunits, and topoisomerase IV consists of ParC and ParE each one two subunits. Alterations in GyrA subunit of DNA gyrase and/or the ParC subunit of topoisomerase IV in bacterial species are major mechanisms of fluoroquinolone resistance, including mycoplasmas and ureaplasmas. Alterations in GyrB and ParE play a complementary role in the increasing rates of fluoroquinolone resistance. Accordingly, in this study, we amplified the quinolone-resistance-determining region (QRDR) of the gyrA gene and the analogous region of the parC gene from the DNA of *M. genitalium* extracted from endocervical swab samples of women with cervicitis and determined their sequences. Amino acid changes in GyrA and ParC caused by mutations in the QRDRs of the gyrA and parC genes were compared with those previously reported in fluoroquinolone resistant mutants of other *Mycoplasma* and Ureaplasma spp. (6-8).

Methods

Inclusion criteria. This analytical cross-sectional study analyzed clinical samples collected from Karaj, capital of Alborz Province, Iran. The study group consisted of 201 women, aged 17 to 60 years, and the average age of 35 years. Clinical samples were collected (Endocervical swab). Questions were asked from each of the women with cervicitis and the relevant questionnaire was completed. Pregnant women were not studied in our study. Clinical symptoms, such as inflammation, itch, discharge and stench were considered as well as the case background including addiction, antimicrobial therapy over the last month, having abortions, number of abortions and infertility.

Clinical sample. The samples were collected from Sep 2015 to Aug of 2016. The swabs were rubbed on the endocervical surface. The samples were stored at 4°C in 7 mL of 2Sp medium transport media. DNA extraction was performed on cervical samples and stored at −20°C for qPCR.

DNA extraction and PCR amplification. Genomic DNA samples of cervical swabs were obtained in accordance with QIAamp DNA Mini Kit. Quantitative-PCR was used for detection of genital Mycoplasmas. The primers used for the detection of *M. hominis* were as follows: RNAH forward primer sequence: RNH1: 5´-CAATGGCTAATGCCGGATACGC-3' and reverse primer sequence: RNH2: 5´-GGTACCGTCAGTCTGCAAT-3' (6). In addition, primers for the detection of *M. genitalium* were: Mgpa forward primer sequence: Mgpa1: 5´-AGTTGATGAAA CCTTAACCCCTTG-3 and reverse primer sequence: Mgpa3: Reverse5´-CATTACCAGTTA AACCAAAGCCT -3´ (7). PCR reaction was performed using Master Mix (0.3 µl dNTPs +2.5 µl buffer PCR + 0.5 µl MgCl2 + 16 µl DW + 0.5 µl primer forward + 0.5 µl primer reverse + 0.2 µl Taq polymerase) +5 µl DNA template in a

total volume of 25µl. The PCR reaction was performed according to the following program: pre-denaturation for 5 minutes at 95ºC followed by 30 cycles of denaturation at 94ºC for 30 seconds, annealing for 30 seconds (at 57ºC for *M. hominis* and 56ºC for M. *genitalium*) and extension at 72ºC for 60 seconds. The last cycle was followed by an extra extension step at 72ºC for 5 minutes. The *M. genitalium* (ATCC: 33530) and *M. hominis* (ATCC: 15056) extracted DNA, as well as sterile double-distilled water, were used as positive and negative controls for PCR reaction, respectively. PCR products were electrophoresed on 1% agarose gel, and stained with Gel red (CinaGen Co, Tehran, and Iran) and visualized under a UV Tran illuminator.

Determination of the quinolone resistance determining regions of the gyrA and parC genes in Mycoplasma genitalium. From these DNA samples, segment of the parC gene and analogous region of the gyrA gene in *M. genitalium* were amplified by PCR (9). For the gyrA gene, primers MG-GYRA-A (5′-CGTCGTGTTCTTTATGGTGC-3′) and MG-GYRA-B (5′-ATAACGYYGTG CAGCAGGTC-3′) were used, and PCR primers for the parC gene were MG-PARC-A (5′-TGGGCTTA AAACCCACCACT-3′) and MG-PARC-B (5′-CGGGTTTCTGTGTAACGCAT-3′).PCR products were re-amplified using the same primer pair and the purified PCR products were sequenced (4).

Comparison of amino acid alterations in gyrA and parC with those associated with Fluoroquinolone resistance. In this study mutations in the QRDRs of the gyrA and parC genes were compared with other reported previously in fluoroquinolone-resistant mutants of others *Mycoplasma* and Ureaplasma spp.

Statistical analysis. Frequencies of infections were described with numbers and percent within 95% confidence interval. Collected clinical and epidemiological information were analyzed using the SPSS 24.0 software. Chi-square and Fisher's exact test were conducted to determine the relationship between variables. P-value less than 0.05 were considered as statistically significant.

Results

M. hominis and *M. genitalium* were detected in 5.5% and 4.5% of samples, respectively. Co-infection was detected with *M. genitalium* and *M. hominis* in two cases (0.9%). The *M. hominis* and *M genitalium* detected by qPCR, showed that women aged 36 to 45 years have more contamination with *M. hominis* and women aged 26 to 35 years have more contamination to *M. genitalium* but there is no significant difference between different age in tow groups (P=0.59, P=0.92) (see table2). Other parameters such as educational level, occupation, contraceptive method, and abortion history and infertility status were studied and there are no significant difference among different groups (see table2). G→T transition at nucleotide position 259 (Asp-87→Tyr) was detected in parC gene in one sample. No mutations were found in gyrA genes, thus there was no amino acid change in GyrA and ParC in the residual 5 specimens. To our training, the Asp-87→Tyr mutation in ParC, was comparable to changes at amino acid situation 84 in E. coli ParC, was generally apperceive in fluoroquinolone-resistant mutants of other *Mycoplasma* and Ureaplasma spp.

Table1: PCR method for the detection of Mycoplasma genitalium and Mycoplasma hominis.

PCR Results	Positive	Percent(95% CI)
M. genitalium	9	4.5 (4.21- 4.79)
M. hominis	11	5.5 (5.2-5.82)
M. genitalium + M. hominis	2	0.9 (0.77-1.03)
Total positive patients	18	8.99 (8.49-9.31)
Total	201	100

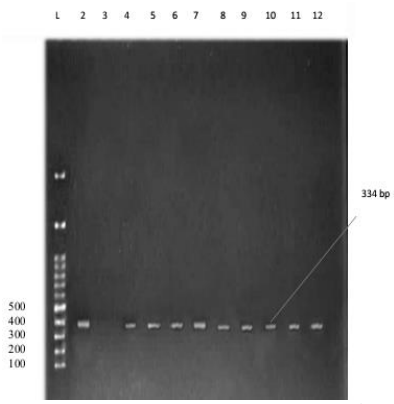

Figure 1. Agarose gel electrophoresis of Mycoplasma hominis. L: DNA size marker (100 bp DNA ladder) 2: positive control for M. genitalium 3: negative control, 4-12: positive samples.

Figure 2. Agarose gel electrophoresis of Mycoplasma genitalium. L: DNA size marker (100 bp DNA ladder) 2: negative control 3: positive control for M. genitalium, 4-7: positive samples.

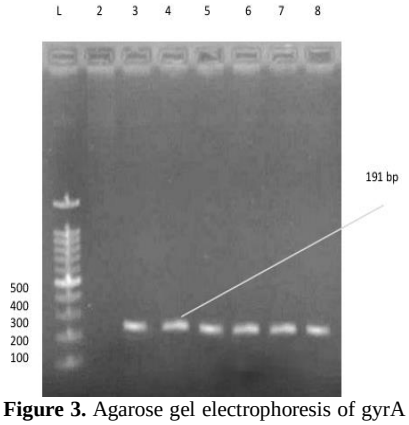

Figure 3. Agarose gel electrophoresis of gyrA gene. L: DNA size marker (100 bp DNA ladder) 2: negative control 8: positive control. (Escherichia coli), 3-7: positive samples.

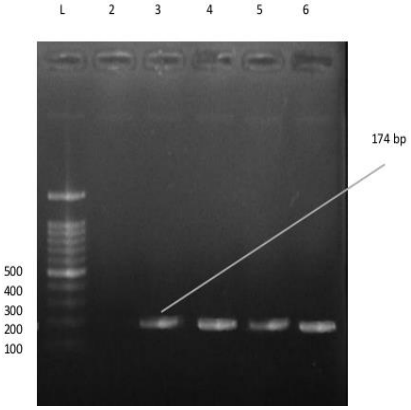

Figure 4. Agarose gel electrophoresis of parC gene. L:DNA size marker (100 bp DNA ladder) 2: negative control 3: positive control (Escherichia coli), 3-7: positive samples.

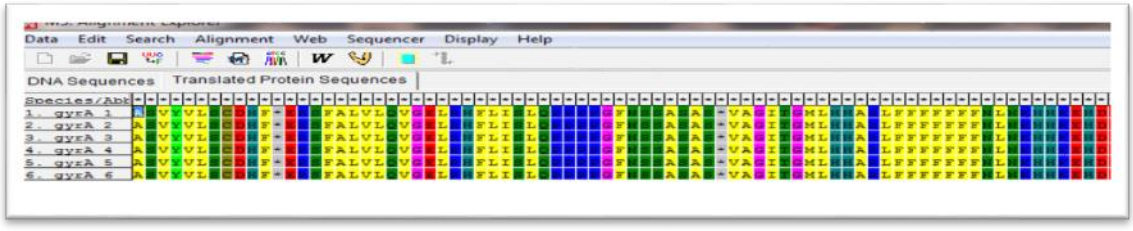

Figure 5. study of amino acid mutations in gyrA by MEGA5 software

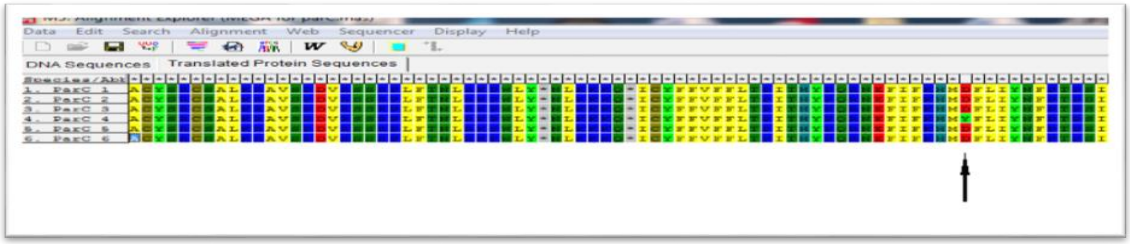

Figure 6. study of amino acid mutations in parC by MEGA5 software mutation in the parC gene at nucleotide position 259 (Asp-87→Tyr) was observed.

Table2: The characteristics of patients with M. genitalium and M. hominis.

		Total	***Positive for Mycoplasma Hominis***	***Positive for Mycoplasma genitalium***	***P value Mycoplasma Hominis***	***P value Mycoplasma genitalium***
		N(%)	**N(%)**	**N(%)**		
Ages (years)	<25 years old	30(14.9%)	2(18.1%)	1(11.1%)	0.59	0.92
	26-35 years old	74(36.8%)	2(18.1%)	4(44.4%)		
	36-45 years old	64(31.8%)	5(45.4%)	3(33.3%)		
	>45 years old	33(16.4%)	2(18.1%)	1(11.1%)		
Level of education	Illiterate	30(14.9%)	1(9%)	2(22.2%)	0.56	0.84
	low – literate	70(34.8%)	6(54.5%)	2(22.2%)		
	Diploma	79(39.3%)	3(27.2%)	4(44.4%)		
	Higher education	22(10.9%)	1(9%)	1(11.1%)		
job	Housewife	176(87.6%)	11(100%)	9(100%)	0.22	0.29
	Employee	25(12.4%)	0(0%)	0(0%)		
History of Abortion	Abortion	53(26.4%)	3(27.2%)	1(11.1%)	0.5	0.2
	No Abortion	148(73.6%)	8(72.7%)	8(88.8%)		
History of infertility	Infertility	26(12.9%)	1(9%)	2(22.2%)	0.5	0.3
	No infertility	175(87.1)	10(90.9%)	7(77.7%)		
Contraception method	No method	15(7.4%)	1(9%)	1(11.1%)	0.76	0.94
	Condom	35(17.4%)	1(9%)	1(11.1%)		
	Birth control pills	130(64.6%)	7(63.6%)	6(66.6%)		
	IUD	21(10.4%)	2(18.1%)	1(11.1%)		

Discussion

Mycoplasmas are causative agents of sexually transmitted diseases. Many studies have been conducted to realize the distribution and epidemiology of *mycoplasma* in Iran. Several studies have reported different outbreaks of *mycoplasma* in different parts of the world. Studies on women in different countries show a high prevalence of *Mycoplasma hominis*; Turkey 5%, Brazil 38.5%, Denmark 6.6%, and Italy 3.6% (8, 10-12). Worldwide studies have also been conducted to evaluate the frequency of *Mycoplasma genitalium*, including 98% in Denmark, 12.6% in Japan, 3.3% in the United Kingdom and 38% in France (11, 13- 15). In the present study, the prevalence of *Mycoplasma genitalium* and *mycoplasma hominis* was 4.5% and 5.5%, respectively. Several factors including the study population (receiving antibiotics, suffering from a genital infection, having multiple sexual partners, etc. number of samples, sampling method, age of the patients, culture, geographical area and the test method can justify the difference in prevalence. Is there a connection between symptoms of cervicitis and presence of *M. genitalium*? A link has been found between signs of cervicitis and *M. genitalium* in the United States, Japan, Sweden, and West Africa. Some causes of urethritis such as C. trachomatis and N. gonorrhoeae can cause cervical infection in women, but some women with cervicitis were not infected with these pathogens. Unlike our study, a study conducted in France on women with a vaginal discharge there was no relation between cervicitis and *M. genitalium*. Our findings suggest that, perhaps in women who have cervicitis as a consequence of its modest pathogenicity, the signs of cervicitis induced by *M. genitalium* are difficult to elicit (9). In the study ahead, change in amino acid sequence was found in ParC in *M. genitalium* isolated from the endocervical swab samples of women with cervicitis. This change of amino acid (Asp-87 → Tyr) in ParC was consistent with the change of amino acid position of 84 in ParC in E. coli, which it is suggested to be associated with resistance to other bacterial species, covering other mycoplasmas and ureaplasmas, such as fluoroquinolone resistance in *M. genitalium*. Nonetheless, the findings of this study showed that 1 (16%) of 6 *M. genitalium* present in the endocervical swab samples of women with cervicitis underwent a mutation leading to the decreased susceptibility to fluoroquinolones. For the treatment of urogenital infections, fluoroquinolones were most often used. Mutants with reduced susceptibility to fluoroquinolones of *M. genitalium* could have been selected in clinical practice is not known how the emergence of clinical polymorphisms of *M. genitalium* Containing changes such as amino acid changes in ParC in the present study can threaten the effectiveness of treatment of *M. genitalium* infections by fluoroquinolones. However, attainment of an amino acid change only in GyrA or ParC may be the first step in creating significant resistance to fluoroquinolones. Eventually, Aggregation of amino acid changes in GyrA and ParC could be induced by serial exposure of strains with a single amino acid change in GyrA or ParC to fluoroquinolones and could bring about the increase in the level of fluoroquinolone resistance. Fluoroquinolone resistance due to the emergence of *M. genitalium* with ParC alterations could be a disturbing issue. To prevent such resistances, antimicrobial resistance monitoring of *M. genitalium* is necessary to detect antimicrobial resistance and appropriate prevention However, in the case of *M. genitalium* infections, a serious barrier to controlling drug resistance is the difficulty of routine cultivation of this bacterium from clinical specimens. Therefore, molecular assays for non-culture specimens will be needed to monitor molecular markers associated with antimicrobial resistance in clinical strains of *M. genitalium*, as shown in the present study. In conclusion, amino acid alterations in ParC of *M. genitalium* analogous to those observed commonly in fluoroquinolone resistant mutants of other *Mycoplasma* and Ureaplasma spp. were observed in 1 (16%) of 6 *M. genitalium* found in the endocervical swab samples of women with

cervicitis. We know that the limitations of this study include absence of analyze of other mechanisms of fluoroquinolone resistance, such as mutations in the GyrB and ParE genes and changes in drug penetration and drug efflux, as well as a small number of samples examined. Nevertheless, the findings show that clinical strains of *M. genitalium* with diminished susceptibility to fluoroquinolones may appear. In addition, the non-culture method used in this study is useful for evaluating the antimicrobial resistance of clinical isolates of *M. genitalium* when *mycoplasma* culture and antimicrobial susceptibility testing from clinical specimens in laboratory experiments is not performed (4, 16).

Conflicts of Interest

There is no conflict of interest among authors.

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