Isolation and Molecular Identification of Mycoplasma Hominis in Infertile Female and Male Reproductive System

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1. Background

Infertility means failure to conception after one year of intercourse. In addition, if a woman is unable to hold the baby in her uterine and an abortion occurs, she is considered infertile. Sometimes the term of sterility is used instead of infertility in women, which affects approximately 10% to 15% of couples in the reproductive age (1). Human infertility factors include bacterial infections (25%), hormonal, genetic, and environmental factors (50%), and unknown causes (25%) (2). Infectious agents can affect the performance and abilities of the reproductive system by direct involvement of different parts of urogenital system, preventing the fertility or successful continue of pregnancy, or reducing fertility rate through systemic effects. The risk of infertility after infection directly depends on the patient’s age, number of previous episodes of infection, disease severity at the beginning treatment, and the interval between the start of symptoms to initiation of treatment; the risk of infertility will increase with repeated infection (3). The importance and role of Mycoplasma in female urogenital infections and their complications have been demonstrated (4). Bacteria such as Mycoplasma hominis and Ureaplasma urealyticum are very common bacterial causes of urogenital tracts infections in males and females (5). They decrease the sperm count mobility and increase the percentage of abnormal sperms; moreover, they can cause abortion (6). Studies have shown that PCR is a sensitive, specific, easy, and fast method for detection of M. hominis in genital samples (7, 8). In addition, the relatively high proportion of infertile males has been infected with M. hominis and U. urealyticum (9, 10).
2. Objectives

The aim of this study was to isolate and identify the molecular identity of *M. hominis* in samples from urogenital tract of infertile males and females referred to the Infertility Center of Kerman, Iran, in 2013.

3. Materials and Methods

In this descriptive study, 200 samples were purposefully collected from males and females who attended the Infertility Center of Kerman during the first six months of 2013. According to WHO standards, 100 semen samples with abnormal spermiogram parameters were collected from infertile males and were kept at -20 °C until DNA extraction. In addition, 100 vaginal swab samples were collected from infertile females by midwives, were completely solved in a PBS solution, and were kept at -20 °C until DNA extraction. A volume of 100 mL of sample was poured into a microtube and then centrifuged for ten minutes at 13000 rpm. The supernatant was discarded and the precipitate was maintained for DNA extraction at the bottom of the microtube. For bacterial DNA extraction, the purification kit, Sinagen Company (CAT NO: PR-881613CinnaPure-DNA (Cell culture, Tissues, Gram negative Bacteria, and CSF), 50 Preps were used.

The samples’ DNA was extracted according to the Kit manufacturer guidelines and finally, the solution below the column containing the purified DNA was kept at -20 °C until performing PCR.

3.1. Polymerase Chain Reaction

To perform PCR, the Master mix (Sinagen Company, Iran) was used, which contains Taq polymerase enzyme (0.5 IU/L), MgCl$_2$ (4 mmol/L), and dNTPs (4 mM/L). Specific primers were used to identify the genus *Mycoplasma* and *M. hominis* (Tables 1 and 2) (11).

3.2. Sequencing

After purification, the PCR products were sent to the Bioneer Co, South Korea, to determine the nucleotide sequence. Bidirectional sequencing was performed with forward and reverse primers and finally, two sequences were obtained for each sample. Sequences multiple alignment was done using BioEdit software (available through http://www.ncbi.nlm.nih.gov/blast/). Clustal W method. MEGA 5 software was used to compare the samples nucleotide sequences and displaying similarities matrix in Excel. Using MEGA 5 software based on Neighbor-joining tree and with Bootstrap 1000, the phylogenetic tree of the samples was analyzed and drawn. Then the obtained sequence was compared with other sequences of *M. hominis* in NCBI gene bank site to ensure its specificity. Using the above software, the isolate similarity matrix of this study with isolates from rest of the world, recorded in the gene bank, was plotted. The phylogenetic tree of the samples was then drawn.

The PCR temperature program for amplification of target gene was performed using the specific primers based on the research protocol of Vosooghi et al. (10).

4. Results

Initially, the samples with positive results for *Mycoplasma* genus were separated and PCR was performed on them for identification of *M. hominis*. Formation of 344 bp band on an agarose gel indicated the positive strains of *M. hominis*. Of 100 obtained samples from infertile males, the presence of *Mycoplasma* genus was confirmed in 45 cases among which 33% were infected with *M. hominis* (Tables 3 and 4). From 100 obtained specimens from infertile females, the presence of *Mycoplasma* genus was confirmed in 43 cases of which 41.8% were infected with *M. hominis* (Figures 1 and 2).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Target Gene</th>
<th>Sequence</th>
<th>Length, bp</th>
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<td>GSO 16S rRNA</td>
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<td>163</td>
<td></td>
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<tr>
<td>MGSO 16S rRNA</td>
<td>R: 5'-TGCACTCATCGTCACTGATTAAACCTC -3'</td>
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<td>RNA H1 16S rRNA</td>
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<td>RNA H2 rRNA</td>
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<table>
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<th>Temperature</th>
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<tr>
<td>Prog.2:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Seg.1: Denaturation</td>
<td>94 °C</td>
<td>1</td>
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<tr>
<td>Seg.2: Annealing</td>
<td>55 °C</td>
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<td>Seg.3: Extension</td>
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<td>Prog.3: Full Extension</td>
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<td>7</td>
<td>1</td>
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The phylogenetic tree was developed based on Neighbor-joining method using the MEGA5 software, and the corresponding file was saved as images, as can be seen in Figure 3.

**5. Discussion**

*Mycoplasma hominis* was isolated and identified as one of the infectious agents in urogenital secretions. Many studies have been done on *Mycoplasma* in Iran and around the world; however, such a research has not been done so far in Iran. In general, identification of *Mycoplasma* helps to understand the distribution and epidemiology of this infectious agent. Urogenital infections have a key role in male infertility. Bacteria or its toxins bind to the sperm, stimulate immune system, and damage sperms in all anatomic areas of urogenital tract and reproductive system. Most of infertile males have a history of sexually transmitted diseases (STDs) such as gonococcal or nongonococcal urethritis. Moreover, the asymptomatic infected males will finally infect their partners and cause secondary couple infertility problems. Most of infertile males’ wives have asymptomatic STDs, which might be related to their infected husbands (12). The first priority of this study was to determine the molecular identity of *M. hominis* as the causing factor of urogenital infections.
Figure 3. Phylogenetic Tree of *Mycoplasma hominis* Isolated and Comparing Them With Other Isolates Recorded in Gene Bank

by using PCR. As PCR is a highly specific and rapid method for isolation of the factor, the obtained samples were not enriched in this study. Ahmadi et al. mentioned that the PCR is usually faster than conventional tests and can be used as a reliable method for isolation of *Mycoplasma* from urogenital specimens (9). Bacterial infection is one of the major causes of male and female infertility. Several bacteria genus are involved in infertility, the most common and most important of which are *Mycoplasma* species such as *M. hominis* that play an important role in developing infertility by creating infections without clinical symptoms that lead to not referring to the physician and the disease progression (13). In our study, the rate of infection with *Mycoplasma* genus (44%) were higher than the results of Ghazi Saeedi et al. study on first drops of urine (12%) and the prostatic secretions (14.6%) of males for isolation of *Mycoplasma* species (13). The contradictory results could be due to differences in the types of samples used for the experiments. Moreover, the samples used in this study were obtained from
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PCR is much more accurate and sensitive than culture. In Ahmadi et al. research on semen samples of infertile males by PCR, the infection rate with M. hominis was 15.5% (9). The prevalence of urogenital infection with Mycoplasma in genitourinary tracts of people not suffering from genital infections and infertility should be measured and their molecular identity in normal flora should be determined as well as analyzed.

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Authors’ Contributions

Study concept and design: Dr. Kheirkhah. Acquisition of data: Mohseni and Jamalizadeh. Analysis and interpretation of data: Jamalizadeh and Farsinejad. Drafting the manuscript: Mohseni and Habibzadeh. Critical revision of the manuscript for important intellectual content: Kheirkhah and Mohseni. Statistical analysis: Mohseni and Jamalizadeh. Technical and material support: Jamalizadeh and Habibzadeh. Study supervision: Kheirkhah and Mohseni.

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References


