Original Article

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# A Comparison between the Molecular Identity of Mycoplasma Hominis in Urine Samples of Patients with Urinary Tract Infections and Similar Strains Available in GenBank

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## ABSTRACT

**BACKGROUND AND OBJECTIVE:** Mycoplasmal infections are one of the most important urinary infections. Various studies have applied culture studies and biochemistry to separate the bacteria from the urinary tract. However, it should be noted that molecular and phylogenetic analyses are based on epidemiologic evaluations. The purpose of this study was to determine the molecular identity of Mycoplasma hominis, separated from the urine samples of patients with urinary tract infections in Kerman, Iran and compare the sequences with other strains in GenBank.

**METHODS:** In this cross-sectional study, 5 ml mid-stream urine samples of 50 patients with urinary tract infections were collected. After the specialists confirmed the diagnosis of urinary tract infections in patients via paraclinical tests, segments of 16S rRNA gene were amplified, using specific primers of Mycoplasma hominis via polymerase chain reaction (PCR) technique. After purifying the PCR product, the sequences of bacterial strains were determined. Then, the sequences were aligned and the strains were compared with each other and other strains available in GenBank, using BioEdit software.

**FINDINGS:** Three Mycoplasma hominis strains were separated in this study. The alignment of sequences and comparison with strains available in GenBank did not indicate a significant difference between the strains. Based on phylogenetic analyses in this study and the phylogenetic tree, one of the strains (H6) was highly similar to the strains of GenBank and belonged to the same family. On the other hand, two strains (H11 and H15) were of a different lineage and were completely different from other strains in the present study and those recorded in GenBank.

**CONCLUSION:** In this study, after applying the PCR technique and bacterial separation, the sequences were compared with those in GenBank. All three strains were Mycoplasma hominis, based on 16S rRNA gene sequence.

**KEY WORDS:** Mycoplasma hominis, Urinary tract infection, Polymerase chain reaction, Nucleotide sequence, 16S rRNA gene

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## Introduction

Urinary tract infections (UTIs) are the second most common infections in the body, accounting for 8.3 million physician visits per year. According to previous research, women are more prone to these infections (1). Chlamydias, mycoplasmas and ureaplasmas are the primary pathogens responsible for UTI, followed by gonococci, Enterobacter, Streptococcus, Enterococcus and Gardnerella vaginalis; the last four are generally less common than others (2).Mycoplasma is a member of Mycoplasmataceae family. Since this genus lacks any cellular wall, it has a limited capacity to grow and synthesize among other freely-living bacteria. This genus also requires a complex and rich environment for growth and can cause infections in different hosts such as humans, animals, plants and insects. Even though Mycoplasmas are naturally found in male urinary tract, they are potentially pathogenic. Out of 17 species obtained from humans, Mycoplasma pneumoniae, Mycoplasma hominis, Mycoplasma genitalium and Ureaplasma urealyticum are of grave importance among Mycoplasma spp. in the human body (3).Mycoplasma hominis can be separated from the urinary tracts of approximately 35% of men and women, who lack any signs of UTI. However, it has been reported that these organisms contribute to the formation of bacterial vaginosis, pelvic inflammatory diseases, septicemia following childbirth, spontaneous abortion, endometritis, pyelonephritis and urethritis (4). The acquisition of Mycoplasma hominis while passing through the labor canal can lead to meningitis, blood and eye infections and brain abscess in newborns (5, 6). This species can be also separated from brain abscess (7).According to previous research, the prevalence of Mycoplasma hominis is higher in individuals with UTI symptoms, compared to those with no signs of disease (8). However, considering the challenges in culture studies and bacterial separation in laboratory settings, the role of this species in human infections, especially in subsequent undesirable complications, has been less discussed in Iran (9). According to previous studies, molecular methods with their significant features are more sensitive than culture studies for Mycoplasma separation (10). So far, Mycoplasma hominis separation and phylogenetic analyses have not been performed in patients with UTI in Iran. It should be mentioned that this species was separated from infertile men's semen at Royan Institute via polymerase chain reaction (PCR) and the results were under phylogenetic analysis (11-13). In other countries, culture studies are performed to separate these bacteria (8). Separation and identification of Mycoplasma hominis in patients with UTIs are of pivotal importance and can be considered as an important part of control programs for sexually transmitted diseases. The purpose of this study was to evaluate Mycoplasma hominis separation from the urinary tracts of male and female patients, diagnosed with UTI and compare the sequences of obtained strains with those available in GenBank.

## Methods

This cross-sectional study was conducted during April-June 2013 after obtaining permission from the ethics committee of Kerman University of Medical Sciences. In this study, we evaluated the kidneys of patients, referring to the laboratory of Shafa Hospital with a clinical diagnosis of UTI, confirmed by physicians. Moreover, paraclinical tests confirmed the diagnosis of UTI. Clinical symptoms of the patients included frequent urination, dysuria, pelvic pain and changes in urine color. Since PCR is an efficient and rapid technique for the separation of responsible agents, the collected samples were not enriched and were directly evaluated by PCR. After sedimentation of the original samples, DNA extraction was performed using Qiagen PCR Template Purification Kit 50. In this study, a segment of 16S rRNA gene was used as the goal gene in order to detect Mycoplasma. The standard strain of Mycoplasma

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hominis (PG21) was regarded as the positive control for PCR reactions and distilled water was used as the negative control. Nucleotide sequences, properties of the primers used in the diagnosis of Mycoplasmas and Mycoplasma hominis species are demonstrated in table 1. Moreover, the PCR temperature protocol for amplification of desired genes is shown in table 2. The PCR product was electrophoresed on Agarose gel. The used gel (1%) was stained with ethidium bromide solution with a ratio of 0:5 µg/ml and a 100 bp marker was applied. After the end of electrophoresis, the gel was removed from the buffer and placed in a UV transilluminator device.

**Table 1. Nucleotide sequences and the used primers for the detection of Mycoplasma hominis by PCR**

| Primers | Target gene | Sequence | Length |
|---|---|---|---|
| GSO<br>MGSO | 16S rRNA | F: 5′-GGGAGCAAACA GGATTAGATACCCT -3′<br>R: 5′-TGCACCATCTGTC ACTCTGTTAACCTC -3′ | 163 |
| RNA H1<br>RNA H2 | 16S rRNA | F: 5′-CAATGGCTAATG CCGGATACGC-3′<br>R: 5′-GGTACCGTC AGTCTGCAAT-3′ | 344 |

**Table 2. The temperature protocol of PCR test**

| Step | Temperature | Time (min) | Cycle |
|---|---|---|---|
| Prog. 1: Predenature | C°94 | 6 | 1 |
| Prog. 2: | | | |
| Seg.1:Denature | C°94 | 1 | |
| Seg.2:Annealing | C°55 | 1 | |
| Seg.3:Extension | C°70 | 1 | 33 |
| Prog. 3: Full extension | C°72 | 7 | 1 |

Images were captured using a special camera and then printed.The positive samples were purified, using High Performance Purification Kit and sent to Bioneer Company (South Korea) to determine the sequences. The obtained sequences were first edited and prepared. Afterwards, those sequences, which could not be examined, were modified and the files were converted to FASTA format so that the sequences could be useful in DNA sequence analysis. By using the BLAST program available on the National Center for Biotechnology Information (NCBI) website, we were able to search for sequences similar to each obtained sequence. After performing primary evaluations, the studied sequences and those from NCBI GenBank were evaluated in terms of alignment using Mega5 software.

For this purpose, initially, all the sequences were matched in terms of size in similar areas, using the mentioned software. Afterwards, the alignment results were prepared for display, using BioEdit software. The alignment results were organized based on the sequence of ATCC23114 strain, and the position of the segment was evaluated from position 197 to position 491 of 16S rRNA gene. The phylogenetic tree was plotted based on UPGMA method, using Mega5 software and the files were saved as images.

## Results

After PCR test on 50 urine samples of patients with UTI, the positive samples of the genus were confirmed after observing a 163 bp band on Agarose gel (fig 1). The positive samples were under PCR reaction to determine the Mycoplasma hominis species. Formation of a 344 bp band on Agarose gel indicated the existence of positive Mycoplasma hominis species (fig 2). The PCR results showed that in 30 out of 50 urine samples, Mycoplasma species could be found. Also, three Mycoplasma hominis strains were turned positive, using PCR test, as indicated in table 3.

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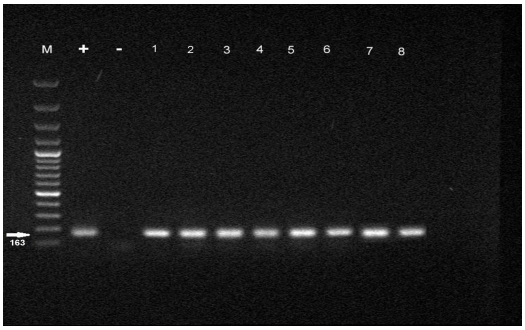



**Figure 1. Electrophoresis of PCR product using specific primers of Mycoplasma (163 bands were observed in eight positive genera), M: 100bp marker, +:Positive control, -:Negative control, 1-8: Positive Mycoplasma samples**

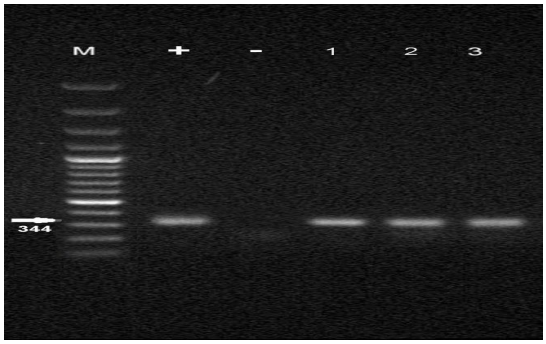



**Figure 2. The electrophoresis of PCR product using specific primers of Mycoplasma (344bp bands were observed in three positive Mycoplasma hominis species), M: 100bp marker, +:Positive control, -: Negative control, 1-3: positive Mycoplasma hominis samples**

The alignment of obtained sequences and comparison with GenBank sequences showed that the strains were Mycoplasma hominis, based on 16S rRNA gene sequence. Nucleotide substitution from T to C was observed at position 5 in five sequences from GenBank, with access numbers EU443618 to EU443622. In the present study, at position 7, a gap was seen in sequence 6, which is quite rare in 16S rRNA gene. In position 246, in GU419506 sequence, a substitution from A to G was observed, and in position 279, a gap was observed. In sequences 11 and 15, a gap was seen at position 273 and a substitution from A to T was seen at position 276. In total, we can conclude that the studied sequences were not significantly different from the sequences in GenBank. The small observed differences require repeated sequencing to approve their originality. The phylogenetic tree can be divided into two main branches. The first branch includes two studied strains, i.e., H15 and H11, which are different from other branches. In another part of the tree, three branches can be seen: one branch with a GU 419506 sample, indicating the difference between this sample and other samples and two branches covering other samples. H6 was included in the majority of sequences (fig 3).

**Table 3. The frequency of samples based on the PCR of Mycoplasma genus and Mycoplasma hominis species**

| Sample | PCR of the strain | PCR of the genus | | |
|---|---|---|---|---|
| | N(%) | Number | Total percentage | Percentage of positive genus |
| Positive | 30(60) | 3 | 6% | 10% |
| Negative | 20(40) | 27 | 54% | 90% |
| Total | 50(100) | 30 | 60% | 100% |

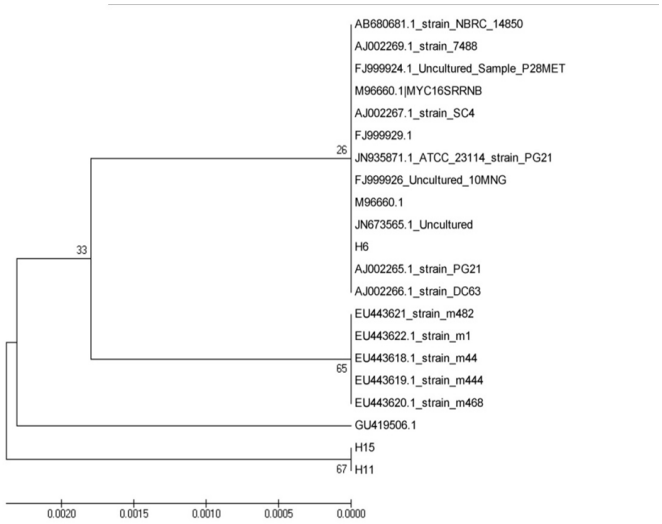



**Figure 3. The phylogenetic tree for comparison between the strains in this study and those in GenBank (H6, H11 and H15 are the strains in the present study)**

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## Discussion

In this study, it was determined that Mycoplasmas are highly sensitive to environmental conditions such as pH, temperature and available compounds in the culture medium, due to the absence of a cellular wall. During sampling or transfer to the lab, these bacteria may be weakened or destroyed and cannot be restored in the culture medium. Various studies have been conducted to compare culture studies and PCR technique for the clinical diagnosis of UTI. All conducted studies have confirmed the priority of PCR to culture studies in diagnosis (14, 15).Moreover, living bacteria are not required in molecular methods, and the results are less affected by sampling and transfer conditions. On the other hand, culture studies, which are generally time-consuming, require specific culture media and impose heavy costs on patients (16). In this study, separation of the infective agent was carried out via PCR. This technique is a fast and specific test for separating Mycoplasma agents in UTIs. In similar studies conducted around the world, this method is recommended for a more accurate diagnosis and is regarded as a suitable technique with high sensitivity (14-17). Ahmadi et al. indicated that PCR is faster than the usual tests and can be used as a reliable method for separating genital Mycoplasmas (12). Vosooghi et al. performed a study to separate Mycoplasma hominis from genital secretions of infertile men, referring to the Infertility Center of Kerman. In their study, PCR method showed high sensitivity and specificity, compared to culture studies. Considering the high speed and accuracy of this method, it could be used as a suitable diagnostic test at the Infertility Center of Kerman for testing infertile men (18). In the present study, segments of 16S rRNA gene were used in the PCR test for amplification, using specific primers for separating Mycoplasma genus and Mycoplasma hominis species. Many scholars have applied the PCR method for diagnosing different types of Mycoplasmas, based on the amplification of 16S rRNA sequences, which could identify the genus and species of Mycoplasmas (19). Since in previous research, especially a study by Shahhosseini et al., the used primers had extremely high sensitivity and specificity, the sensitivity and specificity of the primers were not estimated in the present study. The mentioned study indicated the high sensitivity (100%) and high specificity (94%) of these primers for separating Mycoplasma genus and Mycoplasma hominis species (20). In the present study, among 50 Mycoplasma samples responsible for UTIs in men and women, in 60% of cases, positive Mycoplasma genus was confirmed, indicating the significant role of Mycoplasma infections in causing UTIs in men and women. Moreover, Ghazisaeedi and colleagues in their study showed that the prevalence of Mycoplasma infections in patients with UTIs was 53%, which was in accordance with the results of the present study (21). The results of this study showed that the severity of Mycoplasma infection was higher in women than men in Kerman, Iran. Generally, the overall rate of UTIs is higher in women than men, which is related to the anatomical location of female genitourinary tract and the short length of the urinary tract in women. The results of the present study were similar to the findings of previous research (22). Mosavyan et al. performed a study to determine the prevalence of Mycoplasma responsible for infection in patients, referring to Imam Khomeini Hospital of Ahvaz, Iran. Among patients with genitourinary Mycoplasma infections, 31.4% and 68.6% were male and female, respectively. The results of this study also showed that Mycoplasma infections are more common among women than men (23). In the present study, among 30 patients with urinary Mycoplasma infections, three patients (10%) had Mycoplasma hominis species and 27 patients (90%) were infected with other strains or probably had Ureaplasma urealyticum. In multiple studies, other Mycoplasma strains have been separated from UTIs (12, 24). The results indicated that Mycoplasma

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hominis is not the most important agent in patients with UTI in Kerman. Despite the conducted research in Iran and other countries, genitourinary Mycoplasma infections, nucleotide sequence determination and phylogenetic analysis of Mycoplasmas (as agents of genitourinary infections) have not been discussed in Iran, so far. Therefore, considering the difference between the strains in terms of pathogenicity and variation in the tendency of bacteria to different tissues, molecular evaluations and study of heterogeneity seem necessary. In the present study, after performing PCR and bacterial separation, the positive strains were selected to perform molecular studies, and the acidic nucleotide sequence was determined by DNA purification. By comparing the obtained sequences with those in GenBank, it was determined that all three strains were Mycoplasma hominis, based on 16S rRNA gene sequence. According to phylogenetic studies and the phylogenetic tree, one of the strains (H6) was highly similar to other strains in GenBank and belonged to the same family. However, the other two strains (H11 and H15) were of a different lineage and were completely isolated from other strains and those recorded in GenBank. Therefore, we can consider these strains to be endemic to Kerman. The results of this study can pave the way for UTI treatment. Based on these findings, we can identify the infective agents of UTIs in future studies. Moreover, Mycoplasma hominis strains with a clear molecular identity can be used in future studies. Inthis study, for the first time, Mycoplasma hominis separation and identification in urine samples of patients with UTIs were performed in Kerman province. It is suggested that the obtained sequences be compared with those in GenBank in order to determine the similarities and differences.

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