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# Investigating the Prevalence of *Mycoplasma genitalium* and *Mycoplasma hominis* Among Women with Vaginal Infection in Zabol in 2017

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**ABSTRACT**

**Background & Objective:** *Mycoplasma hominis*, which belongs to the Mycoplasmataceae family, is an opportunistic pathogen of the genitourinary system. *Mycoplasma genitalium*, causing urethritis-endometritis-cervicitis, plays a role in prostatitis This study aimed to investigate the prevalence of *M. genitalium* and *M. hominis* among women with vaginal infection in Zabol, Iran.

**Materials & Methods:** In this cross-sectional study, 69 endocervical samples were taken from women aged 18 to 60 years who suffered from vaginal infections. DNAs extracted from the samples were applied as a template for 16SrDNA coding gene amplification using specific primers in two separate PCR reactions.

**Results:** The highest infection rate was in the age group of 25 to 35 years, with a prevalence of 75%. The highest rate of negative PCR results (54%) was in the age group of 25 to 35 years, followed by the age groups of 36 to 45 years (28%), 18 to 24 years (4%), and older than 45 years (3%). The lowest rate was in the age group younger than 18 years (2%). Considering their levels of education, the highest rate of infection was seen in the subjects with bachelor's degrees. The rate of *Mycoplasma genitalium* infection was equal in the subjects who had and did not have a miscarriage (50%). Only 5.7% of the subjects with negative PCR samples had a miscarriage and the rest (94.3%) did not experience a miscarriage.

**Conclusion:** Overall, the present study showed that the rate of *Mycoplasma* vaginal infections was very low Also there is no significance deference for infection rate between pregnant women with or without miscarriage history. However, those with *Mycoplasma*-negative PCR samples had a low miscarriage rate.

**Keywords:** *Mycoplasma genitalium*, *Mycoplasma hominis*, Vaginitis



## Introduction

*Mycoplasma hominis*, which belongs to the *Mycoplasmataceae* family, is an opportunistic pathogen of the genitourinary system. The presence of these bacteria as a member of microbial flora in healthy people and their role in the pathogenesis of the genitourinary system have always been controversial. Among the major causes of the pathogenicity of these bacteria, the change in vaginal conditions and their replacement with normal flora, including *Lactobacillus*, can be mentioned. Rapid diagnosis of genital mycoplasmas in patients with genital infections, due to their role in miscarriage, postpartum fever, preterm labor, and chorioamnionitis, is very important **(1)**. Mycoplasmas are the smallest single-celled organisms that are pathogenically isolated from plants, animals, and humans. Some of these bacteria are normal flora of the respiratory and genital systems **(2)**. *M. hominis* also plays a role in prostatitis, postpartum fever, recurrent spontaneous miscarriage, pyelonephritis, stillbirth, low birth weight, neonatal

pneumonia, and neonatal meningitis **(3)**. *Mycoplasma genitalium*, in addition to causing urethritis-endometritis-cervicitis, plays a role in prostatitis **(4)**. Among the mycoplasmas isolated from humans, the role of *M. hominis*, as an opportunistic organism causing infection in the genital tract, was proved. Genital mycoplasmas, especially *M. hominis* and *M. genitalium*, reside naturally in the genitourinary systems of men and women who have sexual intercourses **(5)**. Using the conventional Polymerase chain reaction (PCR), the isolation rate of *M. genitalium* ranged from 0-6% in the United Kingdom and was 34.4% in New Zealand **(6)**. *M. hominis* is the first bacterium of human origin isolated in 1973. This bacterium is found in the vagina of 2.3% of women with bacterial vaginosis and 10% of healthy women. In pregnant women with bacterial vaginosis, the risk of premature rupture of the fetal membranes, preterm labor, and postpartum endometritis (after cesarean section) is increased. *M. hominis* also plays a role in prostatitis, postpartum fever, recurrent spontaneous miscarriage, pyelonephritis, stillbirth, low birth weight, neonatal pneumonia, and neonatal meningitis **(3)**. Mycoplasmas are hard to grow and require special conditions to proliferate in culture. Bacterial culture is a standard method for the detection of mycoplasmas; however, this method is very difficult and requires specific culture media **(7)**.

Studies have shown that PCR is a more efficient molecular diagnostic method compared to culture in the detection of these bacteria **(8)**. Nowadays, this technique is widely used to identify pathogenic microbes in patient samples. Using this technique, the pathogenic factors are detectable in more patients. Proving a pathogen is essential for considering the optimal treatment that patients require. Culture methods lack sufficient sensitivity and specificity, which may be due to prior treatment with antibiotics and/or pathogens that are inherently difficult to cultivate. Among these pathogens, species of *Bordetella*, *Legionella*, *Coxiella*, and *Mycoplasma* can be noted. This study aimed to investigate the use of PCR technique for differential diagnosis of *Mycoplasma hominis* and *Mycoplasma genitalium* in samples taken from women with vaginal infection referred to Imam Khomeini Hospital and Amir al-Momenan Hospital in Zabol, Iran. Given the potential impact of mycoplasmas on the complications of maternal infection and mortality, timely diagnosis and treatment are needed more than ever.

## Materials and Methods

In this descriptive cross-sectional study, under the supervision of a gynecologist, 69 endocervical samples were taken from women aged 18 to 60 years old who suffered from vaginal infections and referred to Imam Khomeini Hospital and Amir-al-Momen Hospital in Zabol, Iran, in 2017. The inclusion criteria of this study were having one or more symptoms of genital infection, such as burning and itching in the genital tract and increased discharge and discoloration, and not taking antibiotics and vaginal cream two days before the referral. A questionnaire was designed to collect the subjects' data, including their age, marital status, history of urinary tract infection, miscarriage, history of Pap smear, and use of vaginal cream, antibiotics, and oral contraceptives, as well as the data related to the diagnosis made by their physicians and the subjects' clinical symptoms. Sampling was performed by obtaining the subjects' full consent and without imposing any additional costs on the subjects. The samples were collected by two sterile swabs and speculums from the endocervical and vaginal regions, placed in 500 µL PBS, and transferred to the laboratory for carrying out the diagnostic test in the shortest time possible.

Chromosomal DNAs were extracted from all the samples of transient buffers prepared using the Gram-negative DNA Extraction Kit and applied as a template for 16 SrDNA coding gene amplification using specific primers. For amplification, the PCR technique was used (Table 1). The PCR reaction was performed in a final volume of 25 µL in 35 cycles at an initial temperature of -95°C and at temperatures of 62°C, for primer binding in *M. genitalium*, 59.5°C, for *M. hominis*, and 72°C, for continuing the reaction, each one lasted for a minute. At the end of the study, the data obtained from the laboratory studies were described via SPSS 16 (version 16; SPSS Inc., Chicago, IL US), using statistical methods, i.e., mean, frequency, standard deviation, tables, and figures.

**Table 1.** The primer sequences used

| **Used to identify *Mycoplasma genus*** |
|---|
| My-ins(5'-GTAATACATAGGTCGCAAGCGTTATC-3'),520bp<br>MGSO-2-Bi (5'-CACCATCTGTCACTCTGTTAACCTC-3') |
| Used to identify *Mycoplasma hominis* |
| RNAH1 5' (CAATGGCTAATGCTGGATACGC)344bp<br>RNAH2 5' GGTACCGTCAGTCTGCAAT |
| Used to identify *Mycoplasma genitalium* |
| MGF: 5/-TACATGCAAGTCGATCGGAAGTAGC-3/427bp<br>MGR: 5/-AAACTCCAGCCATTGCCTGCTAG-3 |

## Results

Out of the 69 available samples examined, 11 samples (14.5%) were unacceptable due to poor storage and transportation conditions. Out of the other 58 samples, the *Mycoplasma genus*-PCR results were positive for 5 samples (Figure 1). Among these five positive PCR results, the *Mycoplasma genitalium*-PCR results were positive for 4 samples (Figure 2). No samples were positive for *Mycoplasma hominis*.

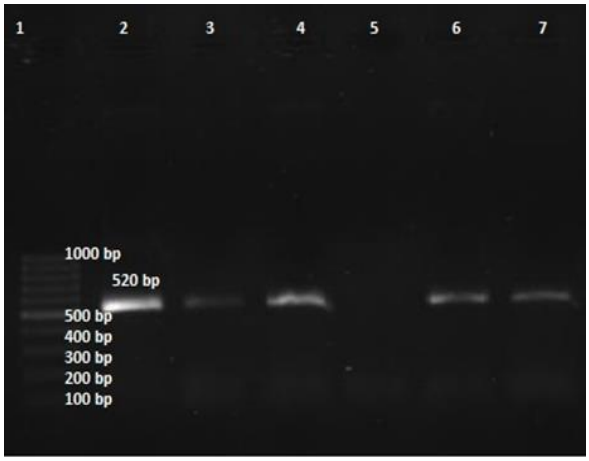


**Figure 1.** The *Mycoplasma genus*-PCR results

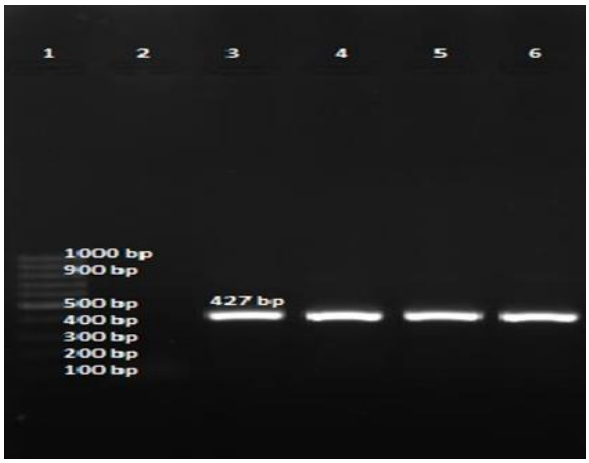


**Figure 2.** The *Mycoplasma genitalium*-PCR result

In the present study, the subjects' age ranged from 18 to 60 years old, with a mean age of 33 years. The highest rate of infection (75%) was in the age group of 25 to 35 years. The highest rate of negative PCR results (54%) was in the age group of 25 to 35 years, followed by the age groups of 36 to 45 years (28%), 18 to 24 years (4%), and older than 45 years (3%). The lowest rate (2%) was for the age group of younger than 18 years old. These differences were statistically significant (*P*=0.000).

In this study 91.4% of the subjects were married and 8.6% of them were single. All cases of *Mycoplasma* positive were among the married subjects. These differences were not statistically significant (*P*=0.773).

The rate of *M. genitalium* infection was 8.3% in the rural population and 6.5% in the urban population. The rate of *mycoplasma genus* was 91.7% in the rural population and 91.3% in the urban population. These differences were not statistically significant (*P*=0.858).

Considering their levels of education, the highest rate of infection was seen in the subjects with bachelor's degrees (50% of *M. genitalium* cases), followed by those with associate degrees (25% of *M. genitalium* cases) and diplomas (25% of *M. genitalium* cases). These differences were not statistically significant (*P*=0.739). The highest rate of *Mycoplasma* infection was seen in the subjects who belonged to a moderate economic level (50%), followed by those who had a good economic level (25%) and a poor economic level (25%). These differences were not statistically significant (*P*=0.708).

The rate of *M. genitalia* infection in those who had a miscarriage was equal to those who did not have a miscarriage (50%). However, 94.3% of those with negative PCR samples had no miscarriages and only 5.7% of these subjects had a miscarriage. These differences were statistically significant (*P*=0.009).

In terms of the subjects' gravidity, the subjects with 4 gravidae (50%), followed by those who had 3 and 2 gravidae (25%), had the highest *M. genitalia* infection. Of those who had negative PCR results, the highest gravidity was 3 (28.3%) followed by 5, 2, 4, 1, and 7 (20.8%, 15.1%, 11.3%, 9.4%, 5.7%, and 5.7%, respectively) and the lowest gravidity was 6 (3.8%). These differences were not statistically significant (*P*=0.857).

## Discussion

Genital infections can cause mortality and morbidity **(9-11)**. *Mycoplasma* bacteria, as a microbial flora in healthy people, are opportunistic pathogenic agents of the genitourinary system. Changes in vaginal conditions and the replacement of various pathogens, including *Lactobacillus* instead of normal flora, are major pathogens of mycoplasmas. Due to complications such as miscarriage, postpartum fever, preterm labor, and chorioamnionitis, rapid diagnosis and treatment of genital mycoplasmas are important **(1,12)**.

In the present study, 16% of the samples were unacceptable. This may be because mycoplasmas lack cell walls; hence, they are sensitive to environmental conditions and may become weak or die during sampling or transferring to the laboratory. In the current study, the PCR results showed that 8.6% of the samples had *Mycoplasma genus* and 6.8% of them had *Mycoplasma genitalium*. Several studies conducted in different countries all around the world have

demonstrated that the frequency of *M. genitalium* was 4.5% in Norway, 4.8% in New Zealand, 5% in Sudan, 7% in the USA, 7.6% in Venezuela, 17.2% in Kenya, and 38.2 % in France **(13-19)**.

The most positive cases were seen in 29-39 years old; these results were similar to studies conducted in India and Turkey, which is due to the closeness of the culture of these countries with Iran **(20)**. Some studies showed that these bacteria are directly related to the economic and social status of women, financial poverty, relationships with multiple sexual partners, the use of birth control pills, and the age of sexually active women **(21)** although in our study we did not see this conclusion.

In our study, women in the second to fourth pregnancies were prone to mycoplasma infection. Elemental studies have shown that people with more than 2 pregnancies have a higher incidence of mycoplasma infection.

Studies have shown that the prevalence of these bacteria in the female reproductive system is directly correlated with socioeconomic status, poverty, sex with multiple partners, contraceptive use, and the age of sexually active women **(22)**. However, in this study, the rate of vaginal infection was not associated with economic status, level of education, place of residence, and marital status.

## Conclusion

Overall, the present study showed that the rate of *Mycoplasma* vaginal infections is very low. Furthermore, this study indicated that the samples' marital status, place of residence, level of education, economic status, and gravidity were not correlated with *Mycoplasma* vaginal infections. The rates of *Mycoplasma genitalium* infection in the samples who had and did not have a miscarriage were equal. However, those with *Mycoplasma*-negative PCR samples had a low miscarriage rate.

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## Conflict of Interest

Authors declared no conflict of interests.

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