Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 10 (6): 260-269, 2015
ISSN 1816-4935 / DOI: 10.3923/jm.2015.260.269

Sequences of *Mycoplasma hominis* in Patients with Urinary Tract Infection in a Hospital in Kashan, Iran

[1]Mahmood Safari, [2]Ashraf Bakhshi, [2]Mahzad Erami, [3]Babak Kheirkhah, [4]Ali Pourbakhsh and [1]Homayun Pourbabei

[1]Department of Medical Microbiology, Kashan University of Medical Sciences, Kashan, Iran
[2]ShahidBeheshti Hospital, Kashan University of Medical Sciences, Kashan, Iran
[3]Department of Microbiology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Tehran, Iran
[4]Department of Large Animal Internal Medicine, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Tehran, Iran

Corresponding Author: Ashraf Bakhshi, Shahid Beheshti Hospital, Kashan University of Medical Sciences, Kashan, Iran Tel: 989132631958

ABSTRACT

Mycoplasma hominis is normally found in the urinary tract of human and its role in Urinary Tract Infection (UTI) has been proved. This bacterium causes inflammatory responses and accumulation of leucocytes in urethra. In spite of the presence of the bacteria, the urine culture might be negative. *Mycoplasma hominis* can be transferred sexually and causes human infertility. The present study was conducted to detect and identify *Mycoplasma hominis* by molecular methods in urine samples of the patients with UTI, who were referred to our hospital. A total number of 864 urine samples from the patients with UTI were subjected to this study. After routine culture, urine analysis were performed on the samples. The DNA was extracted from the sediments of the urine samples, using phenol and chloroform method. Polymerase Chain Reaction (PCR) was conducted on the extracted DNA to detect the 16S rRNA of *Mycoplasma hominis*, with the primers; RNAH1 and RNAH2. Based on the results of PCR tests, out of 100 pyuria positive samples, 9 and 1% were infected with *Mycoplasma* sp. and *Mycoplasma hominis*, respectively. The sequencing of amplified product of 16S-rRNA revealed a single nucleotide substitution (269 T A), compared with the reference gene of this species.

Key words: *Mycoplasma hominis*, pyuria, molecular identification, PCR

INTRODUCTION

Urinary Tract Infection (UTI) caused by bacteria is frequently encountered in the outpatient settings in the USA, accounting for 8.6 million visits in 2007 (Hooton *et al*., 2013). UTIs are among the most common infectious diseases of human and the most common nosocomial infections in the developed world (Ulett *et al*., 2013). It has been estimated that 40-50% of women and 5% of men will develop UTI in their lifetime and that UTI accounts for more than 1 million hospitalizations and $1.6 billion of medical expenses each year in the USA (Ulett *et al*., 2013). Acute uncomplicated lower UTI, also known as cystitis, is characterized by burning sensation on urination and frequent urination without fever or flank pain (Trestioreanu *et al*., 2010). Most UTIs in women are acute uncomplicated cystitis caused by *Escherichia coli* (86%), *Staphylococcus aprophyticus* (4%), *Klebsiella* spp. (3%), *Proteus* spp. (3%), *Enterobacter* spp. (1.4%), *Citrobacter* spp. (0.8%), or *Enterococcus* spp. (0.5%) (Colgan and Williams, 2011). It has been reported that 7.6% of the UTI

cases are caused by both *Mycoplasma* and *Ureaplasma* (Latthe *et al*., 2008). *Mycoplasma hominis* is generally a genitourinary (GU) pathogen (Henao-Martinez *et al*., 2012). *Mycoplasma* are the smallest-living, atypical bacteria that possess a very small genome and are characterized by their strict dependence on the host for their nutrients (Al-Daghistani and Abdel-Dayem, 2010). The genome of this bacterium is composed of 580,000 base pairs and contains 482 genes (Larsen and Hwang, 2010). The role of mycoplasmas in the etiopathogenesis of the urogenital system is still a subject of controversy (Bayraktar *et al*., 2010). The importance of mycoplasmas is obscured by the presence of *Mycoplasma hominis* and *Ureaplasmaurea lyticum* in many asymptomatic persons from whom these bacteria could be isolated. The prevalence of *Mycoplasma* infectious has been reported to vary from 10-40% in the male seminal fluid and has been shown to have a role in varicose infertile men with higher rate of as then ozoospermia (Al-Daghistani and Abdel-Dayem, 2010). Mycoplasmas have been associated with various pathological conditions and intrauterine infections, including pyelonephritis, pelvic inflammatory disease, chorioamnionitis, endometritis and postpartum fever, leading to significant complications, such as; preterm birth, low birth, spontaneous abortion, stillbirth, premature birth, infertility and perinatal mortality (Bayraktar *et al*., 2010). *Mycoplasma hominis*, a commensal bacterium of the GU tract, is a rare cause of mediastinitis, deep sterna wound infection and blood-culture-negative endocarditis (Myers *et al*., 2010). Genital mycoplasmas represent a group of microorganisms that are commonly found in the GU tract of pregnant and non-pregnant women. They are most commonly present in the genital tract of sexually active women (Bayraktar *et al*., 2010). However, generations of subclinical genital infection or nongonococcal urethritis have been detected in 25% of infertile men (Al-Daghistani and Abdel-Dayem, 2010). The *Mycoplasma hominis* colonization values range between 20 and 30% around the world (Bayraktar *et al*., 2010). *Mycoplasma hominis* is a fastidious slow-growing organism, which may not be readily identified through routine culture protocols and whose growth might further be inhibited by sodium polyanethole used in some culture media. These factors are the major limitations to the identification of *Mycoplasma hominis* using standard culture media and explain the delay in correct diagnosis and treatment in patients (Myers *et al*., 2010). Molecular methods have revolutionized our understanding of these bacteria and the simultaneous use of culture and molecular detection methods, such as PCR, offers great sensitivity in the detection of mycoplasmas (Larsen and Hwang, 2010).

Although, many studies on *Mycoplasma hominis* have been performed in Iran and around the world to determine the prevalence of this bacterium in clinical samples, no research has been conducted so far to study its molecular characteristics. Thus, the aim of the present study was to investigate and identify the characteristics of *M. hominis* in patients with UTI, who were referred to Shahid Beheshti Hospital in the second half of 2014 and reduce its hazardous staining prognoses.

MATERIALS AND METHODS

***Mycoplasma* isolation:** In this descriptive study, 864 patients with UTI symptoms, who were referred to the Shahid Beheshti laboratory during the latter half of 2014, were included. The patients were provided with necessary instructions for appropriate urine sample collection and the mid-stream urine was collected in sterile tubes and transported to the Department of Microbiology Laboratory. Urinary analysis test was performed routinely with the samples and the presence of pyuria was determined.

DNA extraction: The DNA was extracted from the samples according to the procedures of Kojima *et al*. (1997). Based on the European Pharmacopoeia 2005, the negative and positive

controls were PPLO broth medium and a standard strain of *Mycoplasma hominis* (PG21), respectively. A total of 500 µL of the samples were placed in a 1×5 µL Eppendorf tube and microcentrifuged at 13,000 rpm for 15 min. Then, 100 µL of the lysis buffer were added to 100 µL of the precipitate and the tube was placed in a 56°C bath for 4 h. Subsequently, 200 µL of saturated phenol were added to the tube and centrifuged at 13,000 rpm for 20 min. The supernatant was transferred to another tube and an equal volume of phenol/chloroform mixture (1:1) was added to it. After centrifuging at 13,000 rpm for 20 min, the aqueous phase was transferred to another tube and an equal volume of pure chloroform was added to it and again centrifuged at 13,000 rpm for 5 min. Subsequently, the supernatant was transferred to a new tube, mixed with 1/10 volume of sodium acetate (3 M), precipitated in a -20°C refrigerator with a twofold volume of cool and pure ethanol (20 min) and again centrifuged at 13,000 rpm for 15 min. Then, 200 µL of 70% ethanol were added and the tube was centrifuged at 13,000 rpm for 5 min. Finally, the DNA was dried and re-suspended in DDW at 4°C to be used for PCR (Kojima *et al*., 1997).

PCR: Based on the previous studies on *Mycoplasma* genus amplification, two primers (forward and reverse) (Stellrecht *et al*., 2004) were used and a 163-bp region of 16S rRNA gene was amplified. For *Mycoplasma hominis*, the primer sets RNAH1 and RNAH2 (Stellrecht *et al*., 2004), which amplify a 334 bp region of the lipoprotein gene were used. The primer sequences with their corresponding genes are shown in Table 1. The DNA amplification was performed in a total reaction volume of 25 µL containing 5 µL of DNA, 1 µL of each primer, 0.5 µL of dNTP mix (200 mM) (CinnaGen Inc.), 2 µL of $MgCl_2$ (50 mM) (Cinnagen Inc.), 2.5 µL of PCR buffer (10×) (CinnaGen Inc.) and 0.25 µL of Taq DNA polymerase (5 U μL^{-1}) (CinnaGen Inc.). The reaction mixture was thermocycled for 30 times beginning with an initial denaturation step at a minimum of 94°C. The temperature and time profiles of each cycle were as follows: The 94°C for 3 min (annealing) and 72°C for 1 min (extension). The PCRs were completed with a final extension step at 72°C for 10 min and the PCR products were stored at 4°C. The PCRs were performed using two programmable thermal cyclers (Primus and Master gradient). Positive and negative controls were included in all the tests. Each aliquot of every PCR product was mixed with 2 µL of loading buffer (6×). The PCR products and 100 bp DNA ladder were separated by electrophoresis on 1% agarose gel, stained with 0.5 µL mL^{-1} ethidium bromide (100 V for 1h) and viewed using a UV Transilluminator.

Bioinformatics studies: After purification, the best PCR products were sent to MWG Institute, Germany, to determine and verify their sequences. The nucleotide sequences of the samples were compared using MEGA software v. 5.04 and the similarity matrix was illustrated in Excel. The phylogenetic tree of the samples was analyzed and constructed by Bootstrap 1000 software via neighbor-joining tree method. Subsequently, the obtained sequences were compared with other sequences of *Mycoplasma hominis* available in GenBank to ensure their distinctiveness. The similarity matrix developed in the present study was compared with others constructed in Iran and other regions of the world, which have been recorded in GenBank. The phylogenetic

Table 1: Nucleotide sequences and primers used for identification of *Mycoplasma hominis* by PCR

Primer	Target gene	Sequence	Length (bp)	Reference
	16S rRNA	F: 5′-GCTGCGGTGAATACGTTCT-3′	163	Kojima *et al*. (1997)
		R: 5′-TCCCCACGTTCTCGTAGGG-3′		
RNAH1	16S rRNA	F: 5′-CAATGGCTAATGCCGGATACGC-3/	334	
RNAH2		R: 5′-GGTACCGTCAGTCTGCAAT-3′		

tree for all the samples was constructed and all the sequences were compared with each other in accordance with the shortest sequence.

RESULTS

The results obtained from the electrophoresis of the PCR products demonstrated the existence of 163 and 334 base pair bonds. These unique bonds belonged to *Mycoplasma* and *Mycoplasma hominis*, respectively (Fig. 1 and 2). Out of the 864 urine samples cultured in the laboratory, 100 were pyuria positive and among them, 10 samples (10%) were infected with mycoplasmas and 1 sample (1%) was infected with *Mycoplasma hominis*. All of the obtained sequences were similar (Fig. 3), except for m.hom-Kashan, which was different from the source sequence, exhibiting substitution of T with A. The lack of difference between the sequences of the samples studied in the present study and the source sequence could be owing to the absolute coincidence of the samples with the source sequence of *Mycoplasma hominis*, which proved the lineages of this species. The sequences of the sample (Table 2) that were related to

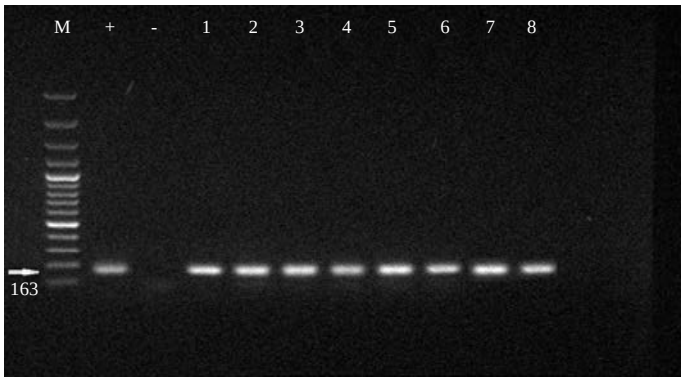

Fig. 1: Specificity of the PCR detection assay using the primers 163 bp. M: Marker 100 bp, +: Positive control, -: Negative control and 1-8: Suspected sample

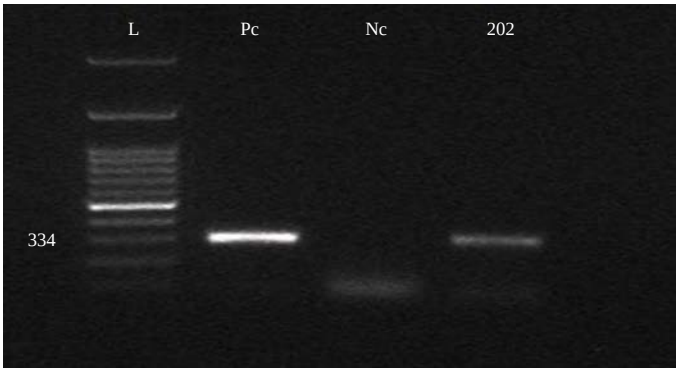

Fig. 2: Specificity of the PCR detection assay using the primers RNAH1 and RNAH2. M: marker 100 bp; PC: positive control [*Mycoplasma hominis* (PG21)], NC: Negative control [PPLO broth media], 1: Positive *Mycoplasma* genus samples. The formation of 334 bp in positive specie

Table 2: Similarities matrix of *Mycoplasma hominis* in this study

Seq->	JN935871 (%)	M. hom-kashan (%)	AB680681.1 (%)	EU443618 (%)	FJ999929.1 (%)	EU443619.1 (%)	EU443620.1 (%)	EU443621. (%)	EU443622.1 (%)	JN673565.1 (%)	*M. arginini* (%)
ATCC_23114	ID	100	100	100	100	100	100	100	100	100	94
M.hom-kashan	100	ID	100	100	100	100	100	100	100	100	94
AB680681.1	100	100	ID	100	100	100	100	100	100	100	94
EU443618.1	100	100	100	ID	100	100	100	100	100	100	94
FJ999929.1	100	100	100	100	ID	100	100	100	100	100	94
EU443619.1	100	100	100	100	100	ID	100	100	100	100	94
EU443620.1	100	100	100	100	100	100	ID	100	100	100	94
EU443621.1	100	100	100	100	100	100	100	ID	100	100	94
EU443622.1	100	100	100	100	100	100	100	100	ID	100	94
JN673565.1	100	100	100	100	100	100	100	100	100	ID	94
M. arginini	94	94	94	94	94	94	94	94	94	94	ID
M. arthritidis	93	93	93	93	93	93	93	93	93	93	95
M. buccale	94	94	94	94	94	94	94	94	94	94	94
M. canadense	94	94	94	94	94	94	94	94	94	94	99
M. genitalium	78	78	78	78	78	78	78	78	78	78	79
M. genitalium	78	78	78	78	78	78	78	78	78	78	79
M. orale	93	93	93	93	93	93	93	93	93	93	92
M. phocidae	95	95	95	95	95	95	95	95	95	95	94
M. pnsseumoniae	78	78	78	78	78	78	78	78	78	78	78
M. salivarium	92	92	92	92	92	92	92	92	92	92	91
M. spumans	93	93	93	93	93	93	93	93	93	93	95

Seq->	*M. arthritid* (%)	*M. buccale* (%)	*M. canadense* (%)	*M. genitalium* (%)	*M. genitali* (%)	*M. orale* (%)	*M. phocidae* (%)	*M. pneumoniae* (%)	*M. salivarium* (%)	*M. spumans* (%)
ATCC_23114	93	94	94	78	78	93	95	78	92	93
M.hom-kashan	93	94	94	78	78	93	95	78	92	93
AB680681.1	93	94	94	78	78	93	95	78	92	93
EU443618.1	93	94	94	78	78	93	95	78	92	93
FJ999929.1	93	94	94	78	78	93	95	78	92	93
EU443619.1	93	94	94	78	78	93	95	78	92	93
EU443620.1	93	94	94	78	78	93	95	78	92	93
EU443621.1	93	94	94	78	78	93	95	78	92	93
EU443622.1	93	94	94	78	78	93	95	78	92	93
JN673565.1	93	94	94	78	78	93	95	78	92	93
M. arginini	95	94	99	79	79	92	94	78	91	95
M. arthritidis	ID	97	95	77	77	94	95	78	94	93
M. buccale	97	ID	95	77	77	96	96	77	95	94
M. canadense	95	95	ID	78	78	93	96	77	92	96
M. genitalium	77	77	78	ID	100	77	76	97	78	78
M. genitalium	77	77	78	100	ID	77	76	97	78	78
M. orale	94	96	93	77	77	ID	94	77	96	92
M. phocidae	95	96	96	76	76	94	ID	76	93	97
M. pnsseumoniae	78	77	77	97	97	77	76	ID	78	78
M. salivarium	94	95	92	78	78	96	93	78	ID	91
M. spumans	93	94	96	78	78	92	97	78	91	ID

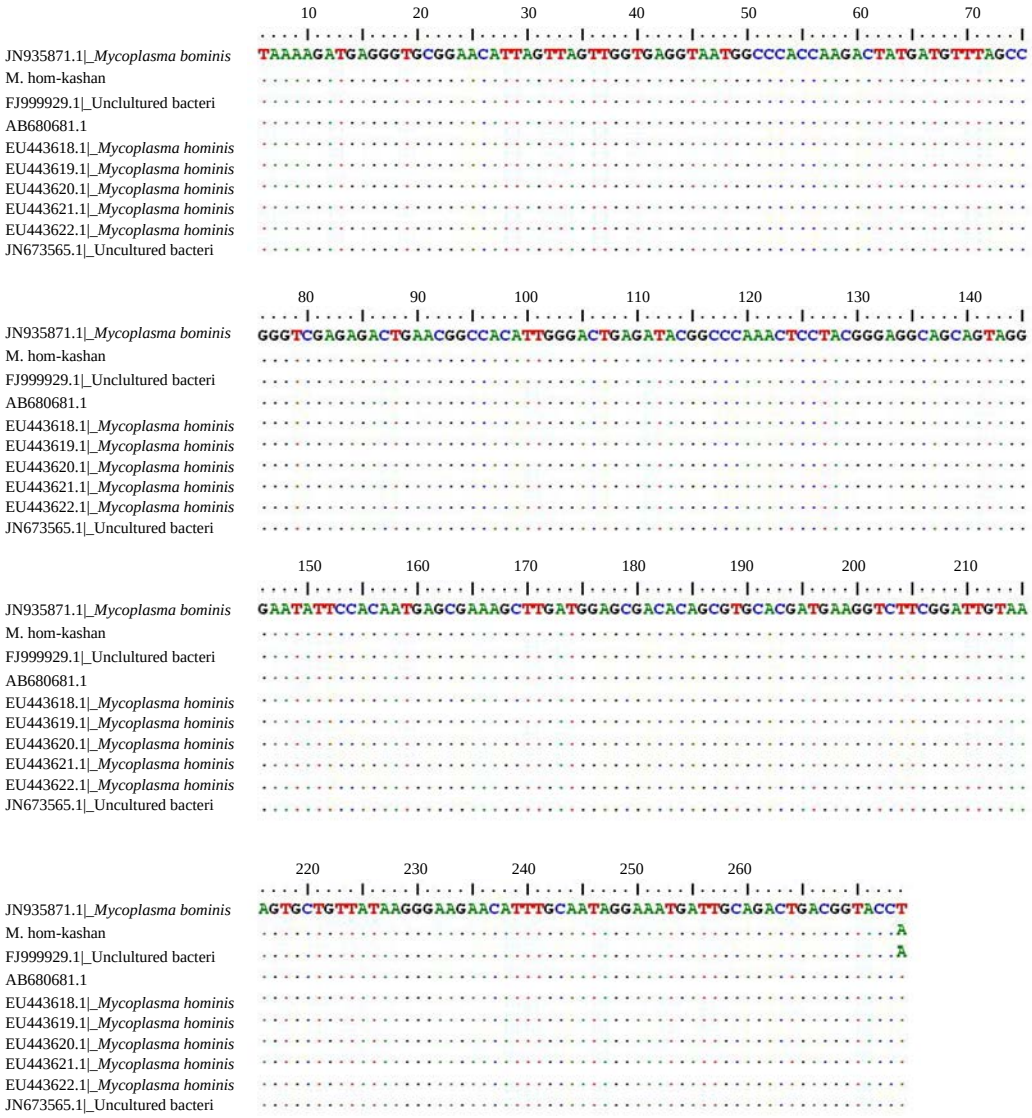

Fig. 3: Comparison between sequence of *Mycoplasma hominis* isolates in this study and others similar sequences in gen bank

Mycoplasma hominis sequences presented no deviation, whereas the genomic region of the 10 samples infected with mycoplasmas was correlated to that of the other species of *Mycoplasma* with relative similarity. These species were placed in different branches of the phylogenetic tree, which confirmed the resultant resolution of the arranged sequence. The similarity matrix (Fig. 3) revealed 100% similarity with *Mycoplasma hominis* and up to 95%similarity with the sequences of other mycoplasmas. The sequencing results were deposited in GenBank as m.hom-Kashan strains.

DISCUSSION

One of the pivotal outcomes of this study is the determination of the *Mycoplasma hominis* sequence. Furthermore, this study is the first to determine and analyze the sequence and phylogenetic characteristics of *Mycoplasma hominis* in Iran by using bioinformatics software.

In a previous study, Kenri *et al*. (2012) reported that *Mycoplasma pneumoniae* strain 309, a type 2a (subtype 2 variant) strain, has variations in the P1 protein, which is responsible for the attachment of this bacterium to the host cells. Furthermore, Musatovova and Baseman (2009) identified common and distinct sequences among the Texas clinical strains of *Mycoplasma genitalium* and obtained 18 DNA sequence variants, which were compared with all the other available clinical sequences. A detailed analysis revealed silent mutations of six amino acid residues within the encoded region of the MgPaadhesin in numerous clinical strains. In addition, missense mutations of limited numbers of amino acids were observed. The alignment of putative amino acid sequences revealed the simultaneous occurrence of several mutations and the existence of identical or similar protein variants in strains from different locations (Musatovova and Baseman, 2009). Pereyre *et al*. (2002) investigated the mechanisms of intrinsic resistance of *Mycoplasma hominis* to 14 and 15 membered macrolides and compared them with those of *M. pneumoniae*, which is naturally susceptible to macrolides. In addition, two *Mycoplasma hominis* clinical isolates with acquired resistance to 16-membered macrolides were also examined for mutations in domains II and V of 23S rRNA and in ribosomal proteins L4 and L22. Overall, their study achieved exhaustive characterization of the intrinsic resistance of *Mycoplasma hominis* to 14 and 15 membered macrolides and provided the first description of *Mycoplasma* clinical isolates resistant to macrolide, lincosamide and streptogramin antibiotics and harboring a mutation at position 2611 in the 23S rRNA (Pereyre *et al*., 2002).

Sasaki *et al*. (2002) determined the complete genomic sequence of an intracellular bacterial pathogen, *Mycoplasma penetrans* HF 2 strain. The genome was found to contain the two component system, but lacked the essential cellular gene, uridinekinase. The relatively large genome of *M. penetrans* HF 2 among the *Mycoplasma* species may be owing to its rich core proteome and the presence of a number of paralog families corresponding to 25.4% of all coding sequences (CDSs). The largest paralog family was found to be the p35 family, which encodes surface lipoproteins, including the major antigen, P35. A total of 44 genes for p35 and p35 homologs were identified and 30 of them were noted to form one large cluster in the chromosome. The genetic tree of p35 paralogs suggested the occurrence of dynamic chromosomal rearrangement in paralog formation during evolution. Thus, *M. penetrans* HF 2 may have acquired diverse repertoires of antigenic-variation related genes to allow its persistent infection in humans (Sasaki *et al*., 2002). Jamalizadeh (2013) studied *Mycoplasma hominis* in infertile men referred to the Infertility Clinic of Kerman and genetically classified mycoplasmas into five separate lineages. It was pointed out that there was a rare similarity between the five lineages. In addition, two lineages presented a significant genetic similarity with those available in the GenBank of Japan, Denmark, Russia and the United Kingdom and were arranged into one ancestry. The other three lineages were arranged into ancestry independent of the whole lineages and those available in GenBank. These lineages could be recorded in GenBank as native Iranian ones. Although, a significant genetic similarity had been indicated between seven other lineages, there was no similarity between those available in GenBank and the five individual lineages (Jamalizadeh, 2013). In another study, Mohseni examined *M. genitalium* in infertile men referred to the Infertility Clinic of Kerman and classified this bacterium into four different ancestries, with rare similarity between them. In addition, there was a significant similarity between the four lineages and those available in GenBank of Denmark and the USA and were thus classified into one ancestry. One lineage was arranged into an ancestry independent of the rest of the lineages and those available in GenBank. This lineage could be recorded in GenBank as the native Iranian one. The other two lineages were placed in two ancestries; however, there was a rare similarity between them and the rest of the lineages as well as those available in GenBank (Mohseni, 2013).

In the study by Lathle *et al*. (2008) no significant difference was observed between the *Mycoplasma hominis* substance (10%) and the findings obtained by examining 1032 samples (with 7.6% of the cases positive for *Mycoplasma* and *Ureaplasma*) (Latthe *et al*., 2008). In addition, no significant difference between the obtained results and the findings reported by Gonzalez Pedraza *et al*. (2003) was noted, who examined 1507 patients with GU infection (2% *Mycoplasma hominis*). Latthe *et al*. (2008) reported that the highest rate of staining for *E. coli* in the urinary samples was 35%, similar to that mentioned for this bacterium (44.34%) by Gonzalez-Pedraza *et al*. (2003) with no significant difference between the results obtained in both the studies.

Maeda *et al*. (2004) reported that the rate of infection with *Mycoplasma hominis* in 153 patients with non gonococcal urethritis in Japan was 2.6%. Furthermore, Nassar *et al*. (2008) indicated that the rate of infection with *Mycoplasma hominis* in 2400 urinary samples (with 200 samples being pyuria positive) was 3%, similar to that observed by Baka *et al*. (2009) in 153 women with chronic GU infection (3.3%) in Greece. In addition, in a study carried out in Japan with 100 urinary samples, Takahashi *et al*. (2006) reported that the rate of infection with *Mycoplasma hominis* was 4%. Thus, it can be observed that there are no significant differences in these above-mentioned results.

CONCLUSION

In the present study, infection with *Mycoplasma hominis* in patients who are pyuria positive, but show negative routine culture was pointed out. Occasionally, the existence of mycoplasmas in the GU tract could be detected with no clinical symptoms. Thus, microbial screening for pyuria-positive patients is necessary, especially for teenagers, because GU infections resulting from the colonization of these bacteria could cause serious diseases, such as Pelvic Inflammatory Disease (PID) and infertility, if left untreated. The results obtained in the current study not only open a new window to treatments for the above-mentioned diseases but also make it possible to access the lineages of *Mycoplasma hominis* through identification of its molecular characteristics for future research on infectious factors correlated to human GU system.

ACKNOWLEDGMENTS

This research was supported by the Kashan University of Medical Sciences, Shahid Beheshti Hospital, Kashan, Iran and the Faculty of Specialized Veterinary Sciences, the Reference *Mycoplasma* Laboratory, Razi Vaccine and Serum Research Institute, Karaj, Iran.

REFERENCES

Al-Daghistani, H.I. and M. Abdel-Dayem, 2010. Clinical significance of asymptomatic urogenital *Mycoplasma hominis* and *Ureaplasma* urealyticum in relation to seminal fluid parameters among infertile Jordanian males. Middle East Fertility Soc. J., 15: 29-34.

Baka, S., E. Kouskouni, S. Antonopoulou, D. Sioutis and M. Papakonstantinou *et al*., 2009. Prevalence of *Ureaplasma* urealyticum and *Mycoplasma hominis* in women with chronic urinary symptoms. Urology, 74: 62-66.

Bayraktar, M.R., I.H. Ozerol, N. Gucluer and O. Celik, 2010. Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma* urealyticum in pregnant women. Int. J. Infect. Dis., 14: e90-e95.

Colgan, R. and M. Williams, 2011. Diagnosis and treatment of acute uncomplicated cystitis. Am. Family Phys., 84: 771-776.

Gonzalez-Pedraza, A., C. Ortiz, R. Mota, R. Davila and E. Dickinson, 2003. Role of bacteria associated with sexually transmitted infections in the etiology of lower urinary tract infection in primary care. Enfermedades Infecciosas y Microbiol. Clin., 21: 89-92.

Henao-Martinez, A.F., H. Young and J.J.L. Nardi-Korver and W. Burman, 2012. *Mycoplasma hominis* brain abscess presenting after a head trauma: A case report. J. Med. Case Rep., Vol. 6. 10.1186/1752-1947-6-253

Hooton, T.M., P.L. Roberts, M.E. Cox and A.E. Stapleton, 2013. Voided midstream urine culture and acute cystitis in premenopausal women. New Engl. J. Med., 369: 1883-1891.

Jamalizadeh, S., 2013. Isolation and molecular identification *Mycoplasma hominis* reproductive use Infertilemen and women. M.Sc. Thesis, Islamic Azad University Science and Research Unit of Sirjan, Kerman, Iran.

Kenri, T., A. Horino, M. Matsui, Y. Sasaki and S. Suzuki *et al*., 2012. Complete genome sequence of *Mycoplasma pneumoniae* type 2a strain 309, isolated in Japan. J. Bacteriol., 194: 1253-1254.

Kojima, A., T. Takahashi, M. Kijima, Y. Ogikubo and M. Nishimura *et al*., 1997. Detection of *Mycoplasma* in avian live virus vaccines by polymerase chain reaction. Biologicals, 25: 365-371.

Larsen, B. and J. Hwang, 2010. *Mycoplasma*, *Ureaplasma* and adverse pregnancy outcomes: A fresh look. Infect. Dis. Obstet. Gynecol. 10.1155/2010/521921

Latthe, P.M., P. Toozs-Hobson and J. Gray, 2008. *Mycoplasma* and *Ureaplasma* colonisation in women with lower urinary tract symptoms. J. Obstetrics Gynecol., 28: 519-521.

Maeda, S., T. Deguchi, H. Ishiko, T. Matsumoto and S. Naito *et al*., 2004. Detection of *Mycoplasma* genitalium, *Mycoplasma hominis*, *Ureaplasma parvum* (biovar 1) and *Ureaplasma urealyticum* (biovar 2) in patients with non-gonococcal urethritis using polymerase chain reaction-microtiter plate hybridization. Int. J. Urol., 11: 750-754.

Mohseni, N., 2013. Isolation and molecular identification *Mycoplasma* genitalium reproductive use infertile men and women. M.Sc. Thesis, Islamic Azad University Science and Research Unit of Sirjan, Kerman, Iran.

Musatovova, O. and J.B. Baseman, 2009. Analysis identifying common and distinct sequences among Texas clinical strains of *Mycoplasma genitalium*. J. Clin. Microbiol., 47: 1469-1475.

Myers, P.O., E. Khabiri, G. Greub and A. Kalangos, 2010. *Mycoplasma hominis* mediastinitis after acute aortic dissection repair. Inter. Cardiovas. Thoracic Surg., 11: 857-858.

Nassar, F.A., F.H. Abu-Elamreen, M.E. Shubair and F.A. Sharif, 2008. Detection of *Chlamydia trachomatis* and *Mycoplasma hominis*, *Genitalium* and *Ureaplasma urealyticum* by polymerase chain reaction in patients with sterile pyuria. Adv. Med. Sci., 53: 80-86.

Pereyre, S., P. Gonzalez, B. de Barbeyrac, A. Darnige and H. Renaudin *et al*., 2002. Mutations in 23S rRNA account for intrinsic resistance to macrolides in *Mycoplasma hominis* and *Mycoplasma fermentans* and for acquired resistance to macrolides in *M. hominis*. Antimicrob. Agents Chemother., 46: 3142-3150.

Sasaki, Y., J. Ishikawa, A. Yamashita, K. Oshima and T. Kenri *et al*., 2002. The complete genomic sequence of *Mycoplasma penetrans*, an intracellular bacterial pathogen in humans. Nucl. Acids Res., 30: 5293-5300.

Stellrecht, K.A., A.M. Woron, N.G. Mishrik and R.A. Venezia, 2004. Comparison of multiplex PCR assay with culture for detection of genital Mycoplasmas. J. Clin. Microbiol., 42: 1528-1533.

Takahashi, S., K. Takeyama, S. Miyamoto, K. Ichihara and T. Maeda *et al*., 2006. Detection of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum* DNAs in urine from asymptomatic healthy young Japanese men. J. Infect. Chemother, 12: 269-271.

Trestioreanu, A.Z., H. Green, M. Paul, J. Yaphe and L. Leibovici, 2010. Antimicrobial agents for treating uncomplicated urinary tract infection in women. Cochrane Database Syst. Rev. 10.1002/14651858.CD007182.pub2

Ulett, G.C., M. Totsika, K. Schaale, A.J. Carey, M.J. Sweet and M.A. Schembri, 2013. Uropathogenic Escherichia coli virulence and innate immune responses during urinary tract infection. Curr. Opin. Microbiol., 16: 100-107.