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Frequency of *Mycoplasma hominis* and *Ureaplasma urealyticum* in Females With Urogenital Infections and Habitual Abortion History in Ahvaz, Iran; Using Multiplex PCR

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Background: Infections due to *Mycoplasmatales* members can cause infertility, preterm delivery and neonatal morbidity and mortality. Therefore, rapid diagnosis is of great importance to control these infections and diminish their outcomes.
Objectives: The current study aimed to develop the multiplex PCR assay to detect two genital mollicutes from a single amplification reaction and the study of their relation with habitual abortion and urogenital infection in infected females.
Materials and Methods: Urine and genital samples from symptomatic females (20-54 years old) in Imam Khomeini hospital, Ahvaz, Iran, were screened for the two common mollicutes, *Mycoplasma hominis* and *Ureaplasma urealyticum*; using multiplex PCR. The prevalence of these mollicutes in 200 healthy females was also evaluated.
Results: The results of the current study showed that the presence of *M. hominis* in urine (P = 0.006) and genital samples (P = 0.01) was associated with urogenital infections. Statistical analysis based on the age, revealed that the highest prevalence of *M. hominis* (71.4%) and *U. urealyticum* (60%) were in females within 30-34 and 35-39 years old, respectively. Similarly, in genital samples, the highest incidence of *M. hominis* (54.5%) and *U. urealyticum* (53.8%) was found in females within 28-33 and 34-39 years old. Interestingly, further analyses revealed direct strong relation between the mollicutes used in this study and habitual abortion as well as urogenital infections.
Conclusions: There was a strong relation between the presence of the studied *M. hominis* and *U. urealyticum* with urogenital infection in the females under study in comparison with those of control groups. The studied mollicutes were highly associated with habitual abortion in symptomatic females. The multiplex PCR was developed for simultaneous, early, and easy detection of these potential pathogens.

Keywords: Mycoplasma hominis; *Ureaplasma urealyticum*; Female Urogenital Disease; Abortion; Habitual

1. Background

Unlike other prokaryotes, the class mollicutes lack the cell wall and with unusually different genome are relatively small in size. The largest group is formed by the genus *Mycoplasma* of which more than 90 species have been described (1). *Mycoplasmatales* are associated with infection of the genitourinary tract, reproductive failure, and neonatal morbidity and mortality. Infection with genital mycoplasmas has been linked with infertility (2-4). *Ureaplasma* spp. are the main cause of non-chlamydial, non-gonococcal urethritis and acute prostatitis (2). In pregnancy, *Ureaplasma* can cause chorioamnionitis and preterm delivery (5).

Mycoplasma homonis has been associated with pyelonephritis, pelvic inflammatory disease and postpartum septicemia (2). Diagnosis of mycoplasmal infections is usually made by serological determination or by culture. However, serological procedures are often hampered by interspecies cross reactions, and cultivation is costly since it requires special media and expertise. It can take 2-5 days to culture *Ureaplasma* spp. and *M. hominis* (2, 6). The need for an improved detection method for *M. hominis* and *Ureaplasma urealyticum* is evident. Polymerase chain reaction (PCR) to amplify specific short segments of nucleic acid sequences is a promising rapid diagnostic test (7, 8).

2. Objectives

The current study aimed to develop the multiplex PCR assay to detect two genital mollicutes from a single am-

Implication for health policy/ practice/ research/ medical education:
The present study focused on determining the prevalence of mollicutes infection in reproductive system of females in Ahvaz. With regard to the outcomes of these infections that can lead to infertility, determining of their prevalence can help clinicians to prevent or treat these infections. Therefore infertilities due to bacterial infections can be diminished.

plification reaction and the study of their relation with habitual abortion and urogenital infection in infected females.

3. Materials and Methods

3.1. Bacterial Strains

The following microorganisms were purchased from the American Type Culture Collection (ATCC) and National collection of type cultures (NCTC): *U. urealyticum* (NCTC 10177T) and *M. hominis* (ATCC 23114). These microorganisms were used as positive controls.

3.2. Clinical Specimens

All specimens were received in the clinic of Imam Khomeini hospital. Specimens included 155 cervicovaginal swabs from females with vaginal discharges or genital infections and 110 urine samples from females with urine infections. Also control urine and swab samples (100 of each) were obtained from asymptomatic females. The symptomatic subjects completed standardized questionnaires about their age, educational status, any symptoms of urogenital infection and history of abortion. The swabs were transported to the laboratory in mycoplasma transport medium (PPLO broth, Difco, USA). Approximately 20 ml of clean-catch urine was then collected in a sterile plastic container. Urine samples concentration increased 10 fold by centrifugation for 30 min at 1600 ×g prior to testing. All of the specimens were stored at-70°C until DNA extraction (2).

3.3. DNA Extraction

DNA was extracted from standard strains and clinical samples as described previously by Cadieux et al. 1999 (9). Briefly, 1 ml of the sample was centrifuged at 12000 ×g for 10 min. The pellet was washed in PBS buffer and resuspended in 30 µl of dH2O. After boiling for 10 min, an aliquot of 7 µl was used directly in PCR experiments as DNA template (9).

3.4. Multiplex PCR Assay

The sequences of primers for each bacterium are listed in Table 1. Multiplex amplification reaction was prepared in 25 µl final volume containing: 10 ρM of each RNH primer and 5 ρM of each U primer (Tuba Negin, Tehran, Iran), 1.5 U Taq DNA polymerase, 1× PCR buffer, 0.2 mM dNTPs, 3 mM MgCl 2 (Cinagene, Iran), 0.1-0.5 µg DNA template and water up to 25µl. The PCR amplification was carried out using a thermal cycler (Biorad, USA) with thermal profile as follow: initial denaturation step at 95°C for 4 min, 35 cycles at 95°C for 50 sec, annealing temperature at 53.7°C for 50 sec, extension step at 72°C for 60 sec, followed by final extension at 72°C for 7 min. The PCR products were visualized and photographed under UV light after electrophoresis for 45 min at 100 V through 1% agarose gel containing ethidium bromide (1 µg/mL).

Table 1. Nucleotide Sequences of Primers

Analysis, organism, and primer	Target or DNA sequence (5'-3')	Length, bp	Reference
Multiplex PCR			
U. urealyticum	Urease gene	167	(10)
U9 primer	GAG ATA ATG ATT ATA TGT CAG GAT CA		
U8 primer	GAT CCA ACT TGG ATA GGA CGG		
M. hominis	16S rDNA	334	(11)
RNH1 primer	CAA TGG CTA ATG CCG GAT ACG C		
RNH2 primer	GGT ACC GTC AGT CTG CAA T		

Table 2. Frequency Distribution of *M. hominis* and *U. urealyticum* in Urogenital Samples by Multiplex PCR Method

Bacteria	Genital samples			Urine samples		
	Case group, n = 110, No. (%)	Control group, n = 100, No. (%)	P Value	Case group, n = 155, No. (%)	Control group, n = 100, No. (%)	P Value
M. hominis	7 (6.4)	0 (0)	0.01	11 (7.1)	0 (0)	0.006
U. urealyticum	15 (13.6)	8 (7.2)	0.191	13 (8.4)	5 (3.2)	0.303
Total	22 (20)	8 (7.2)	-	24 (15.5)	5 (3.2)	-

Table 3. Frequency Distribution of *M. hominis* and *U. urealyticum* in the Different Age Groups

Urine samples			Genital samples		
Age group, y	***M. hominis*, No. (%)**	***U. urealyticum*, No. (%)**	**Age group, y**	***M. hominis*, No. (%)**	***U. urealyticum*, No. (%)**
20-24	0 (0)	1 (6.7)	16-21	1 (9.1)	1 (7.7)
25-29	1(14.3)	2 (13.3)	22-27	2 (18.2)	3 (23.1)
30-34	5 (71.4)	2 (13.3)	28-33	6 (54.5)	1 (7.7)
35-39	1 (14.3)	9 (60)	34-39	1 (9.1)	7 (53.8)
40-44	0 (0)	1 (6.7)	40-45	0 (0)	1 (7.7)
45-49	0 (0)	0 (0)	46-51	0 (0)	0 (0)
50-54	0 (0)	0 (0)	52-57	0 (0)	0 (0)
-	-	-	58-63	1 (9.1)	0 (0)
Total	7 (100)	15 (100)	Total	11 (100)	13 (100)

3.5. Statistical Analysis

Statistical analysis was performed by SPSS statistical software package V.11.5 and Chi-Square test. Statistical significance was assumed at the $P < 0.05$ level.

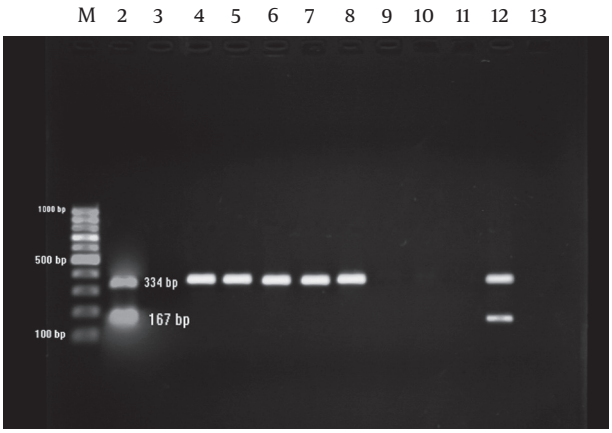
4. Results

M. hominis and *U. urealyticum* were detected by amplifying 334 and 167 bp amplicons, respectively; using multiplex PCR (Figure 1).

M. hominis was detected in 6.4% of urine samples collected from symptomatic but no asymptomatic women (P=0.01) and in 7.1% of genital samples from symptomatic but not from asymptomatic women (P = 0.006) (Table 2).

In urine samples, the highest frequencies of *M. hominis* and *U. urealyticum* were observed in females within 30-34 years old (71.4%) and 35-39 years old (60%), respectively (Table 3). On the other hand, in genital samples, the highest frequencies of *M. hominis* and *U. urealyticum* was observed in females within 28-33 years old (54.5%) and 34-39 (53.8%), respectively (Table 3).

Figure 1. PCR Amplification of *M. hominis* and *U. urealyticum* From Urogenital Samples.



Lane M: Marker; Lane 2: Positive control; Lane 4-8: samples positive for M. hominis; Lane 9-11: samples negative for both of the M. hominis and U. urealyticum; Lane 12: simultaneous detection of *M. hominis* and *U. urealyticum*.

Table 4. Frequency Distribution of *M. hominis* and *U. urealyticum* in Urogenital Samples in Females With History of Habitual Abortion

Samples	Habitual abortion, No. (%)	*M. hominis*	P Value	*U. urealyticum*	P Value
Genital	15 (9.7)	5 (33.3)	P < 0.001	10 (66.7)	P < 0.001
Urine	10 (9.1)	2 (20)	P = 0.014	7 (70)	P < 0.001

In addition, the relation of habitual abortion with the presence of the studied mollicutes in either urine or genital samples was also analyzed. Surprisingly, the incidences of both mollicutes were considerably higher in urogenital samples among symptomatic females with habitual abortion history in comparison with those with no habitual abortion history (Table 4). *M. hominis* and *U. urealyticum* were recovered in 33.3% and 66.7% of genital samples, respectively; from symptomatic females with habitual abortion history (P < 0.001). Similar results were also obtained in the case of urine samples (Table 4).

5. Discussion

The role of mycoplasmas in the genital and extragenital systems is speculative and depends on epidemiologic data (6). Clinical studies showed that mycoplasma incidence is raised in the presence of an anaerobic primer pathogen such as *Trichomonas vaginalis*, *Chlamydia trachomatis* or *Neisseria gonorrhea* (6). In current study, statistical analyses revealed the correlation between the incidence of *M. hominis* in symptomatic as well as asymptomatic females with urine (P = 0.01) and genital (P = 0.006) infections (Table 2), while no significant relation was observed between the incidence of *U. urealyticum* and urine (P = 0.191) and genital (P = 0.303) infections.

Mycoplasmas can grow in the stress environment created by primary pathogen. Notably, these microorganisms colonize numerously in sexually active women, but they cannot be detected due to less sensitivity of microbiological cultivation methods unless an infection occurs (11). Based on the literature, diseases such as pelvic inflammatory disease, infertility (12), habitual abortion (13), bacterial vaginosis (14), cervicitis (15), non-gonococcal urethritis (7) and chorioamnionitis (5) have been reported to be associated with *M. hominis* and *U. urealyticum* infection. Therefore, if these microorganisms are really pathogens, their early detection would be of high value. Hence, a sensitive, specific, fast, cheap and easy applicable diagnostic method is necessary.

The current study found, *M. hominis* and *U. urealyticum* in urogenital infected samples with the incidence of 6.4 to 13.6%. However, studies in other countries showed that the incidence of *M. hominis* and *U. urealyticum* was relatively higher (6, 16). Since *M. hominis* and *U. urealyticum* have been found significantly associated with low socioeconomic background, the lower incidence in the current study is not surprising, considering the low number of sexual partners among Iranians, limitations in sexual relationships for non-married people and public awareness on using contraceptive drugs (17, 18). The highest incidence of *M. hominis* and *U. urealyticum* was observed in females with urogenital infections between 30-39 years old all of whom were sexually active.

These results support previous reports on the presence of *M. hominis* and *U. urealyticum* in sexually active adults (10, 11). Apparently, the level of colonization of genital mycoplasmas is highly affected by fluctuation of estrogen and progesterone hormones (19). In addition, within this range; more sexual activity, desired condition of urogenital tract mucosa and utilization of contraceptive pills cause higher level of colonization of mycoplasmas in comparison with those of non-sexually active adults (11, 20). In the current study, 25 out of 265 females with urogenital infections had history of habitual abortion. Statistical analyses showed the direct relation between the presence of *M. hominis, U. urealyticum*, and habitual abortion (P < 0.001). This result was in agreement with previous report on habitual abortion in the presence of these mollicutes (13).

The current study found strong relation between the presence of the studied *M. hominis* and *U. urealyticum* with urogenital infection in females in comparison with those of the control groups. In addition, it was shown that the studied mollicutes were highly associated with habitual abortion in symptomatic females. Eventually, the multiplex PCR in the current study was developed for simultaneous, early and easy detection of these potential pathogens.

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Authors' Contribution

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A = Study Design; B = Data Collection; C = Statistic Analysis; D = Data interpretation; E = Manuscript preparation; F = Literature preparation; G = Funds collection

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References

1. van Kuppeveld FJ, van der Logt JT, Angulo AF, van Zoest MJ, Quint WG, Niesters HG, et al. Genus- and species-specific identification of mycoplasmas by 16S rRNA amplification. *Appl Environ Microbiol.* 1992;**58**(8):2606-15.
2. Stellrecht KA, Woron AM, Mishrik NG, Venezia RA. Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. *J Clin Microbiol.* 2004;**42**(4):1528-33.
3. Marais NF, Wessels PH, Smith MS, Gericke A, Richter A. Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum infections in women. Prevalence, risks and management at a South African infertility clinic. *J Reprod Med.* 1991;**36**(3):161-4.
4. Stacey CM, Munday PE, Taylor-Robinson D, Thomas BJ, Gilchrist C, Ruck F, et al. A longitudinal study of pelvic inflammatory disease. *Br J Obstet Gynaecol.* 1992;**99**(12):994-9.
5. Cassell GH, Waites KB, Crouse DT. Perinatal mycoplasmal infections. *Clin Perinatol.* 1991;**18**(2):241-62.
6. Serin MS, Evruke C, Kibar F, KÖKSAL F. Comparison of PCR and cultivation methods to determine the incidence of infections due to mycoplasma hominis and mycoplasma fermentans in women genitourinary tract. *Eastern J Med.* 2013;**6**(2):48-52.
7. Yoshida T, Maeda S, Deguchi T, Miyazawa T, Ishiko H. Rapid detection of Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum, and Ureaplasma urealyticum organisms in genitourinary samples by PCR-microtiter plate hybridization assay. *J Clin Microbiol.* 2003;**41**(5):1850-5.
8. Zariffard MR, Saifuddin M, Sha BE, Spear GT. Detection of bacterial vaginosis-related organisms by real-time PCR for Lactobacilli, Gardnerella vaginalis and Mycoplasma hominis. *FEMS Immunol*

Med Microbiol. 2002;**34**(4):277-81.
9. Cadieux N, Lebel P, Brousseau R. Use of a triplex polymerase chain reaction for the detection and differentiation of Mycoplasma pneumoniae and Mycoplasma genitalium in the presence of human DNA. *J Gen Microbiol.* 1993;**139**(10):2431-7.
10. Golshani M, Eslami G, Ghobadloo Sh Mohhammadzadeh, Fallah F, Goudarzi H, Rahbar AA Soleimani, et al. Detection of Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum by multiplex PCR in semen sample of infertile men. *Iranian J Public Health.* 2007;**36**(2).
11. Schlicht MJ, Lovrich SD, Sartin JS, Karpinsky P, Callister SM, Agger WA. High prevalence of genital mycoplasmas among sexually active young adults with urethritis or cervicitis symptoms in La Crosse, Wisconsin. *J Clin Microbiol.* 2004;**42**(10):4636-40.
12. Peerayeh Shahin Najar, Samimi Roghayeh. Detection of Ureaplasma Urealyticum in Clinical Samples from Infertile Women by Polymerase Chain Reaction. *Iranian J Pharmacol Ther.* 2007;**6**(1):23-26.
13. Bayoumi Faten Sayed, Hussein Ibtessam MR, Hind MG. The role of Mycoplasmal infection and anticardiolipin antibodies as autoimmune parameters in pregnancy loss. *J Med Sci.* 2006;**6**:585-90.
14. Sha BE, Zariffard MR, Wang QJ, Chen HY, Bremer J, Cohen MH, et al. Female genital-tract HIV load correlates inversely with Lactobacillus species but positively with bacterial vaginosis and Mycoplasma hominis. *J Infect Dis.* 2005;**191**(1):25-32.
15. Dessi D, Delogu G, Emonte E, Catania MR, Fiori PL, Rappelli P. Long-term survival and intracellular replication of Mycoplasma hominis in Trichomonas vaginalis cells: potential role of the protozoon in transmitting bacterial infection. *Infect Immun.* 2005;**73**(2):1180-6.
16. Mardh PA, Westrom L. Tubal and cervical cultures in acute salpingitis with special reference to Mycoplasma hominis and T-strain mycoplasmas. *Br J Vener Dis.* 1970;**46**(3):179-86.
17. Domingues D, Tavora Tavira L, Duarte A, Sanca A, Prieto E, Exposto F. Genital mycoplasmas in women attending a family planning clinic in Guine-Bissau and their susceptibility to antimicrobial agents. *Acta Trop.* 2003;**86**(1):19-24.
18. Keane FE, Thomas BJ, Gilroy CB, Renton A, Taylor-Robinson D. The association of Mycoplasma hominis, Ureaplasma urealyticum and Mycoplasma genitalium with bacterial vaginosis: observations on heterosexual women and their male partners. *Int J STD AIDS.* 2000;**11**(6):356-60.
19. Sonnex C. Influence of ovarian hormones on urogenital infection. *Sex Transm Infect.* 1998;**74**(1):11-9.
20. de Moreno NO, de Richard L, Leon X. [The presence of genital mycoplasmas in women of reproductive age]. *Rev Med Panama.* 1993;**18**(3):238-41.