

Frequency of *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma urealyticum* among Females Patients Attending Gynecology and Obstetrics Clinics at Ain Shams University Hospital

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(Received: April 07, 2020; accepted: May 13, 2020)

Citation: Abdel Salam SA, Khattab MA, Faisal MM. Frequency of *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma urealyticum* among Females Patients Attending Gynecology and Obstetrics Clinics at Ain Shams University Hospital. *J Pure Appl Microbiol.* 2020;14(2):1413-1421. doi: 10.22207/JPAM.14.2.39

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Abstract

Mycoplasmas and *Ureaplasmas* inhabit the genitourinary tract of sexually active males and females. *Mycoplasma genitalium* infection as well as *Ureaplasma urealyticum* are related to many reproductive health problems such as cervicitis, urethritis and pelvic inflammatory disease (PID). The aim of this study was detection of *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma urealyticum* cervical colonization among childbearing age females attending Gynecology and Obstetrics clinics at Ain Shams University Hospital by using polymerase chain reaction. This study was conducted on 145 women attending Gynecology and Obstetrics clinics at Ain Shams University Hospital. The patients were divided into three groups according to symptoms. Cervical samples were collected using a sterile swab and placed in a liquid-based transport medium. The detection of *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma urealyticum* was performed by using polymerase chain reaction. This study was conducted during the period from January 2018 till August 2018 on 145 women attending Gynecology and obstetrics clinic at Ain Shams University Hospital. Ninety females (62%) were positively colonized with *Mycoplasma* spp and *U. urealyticum*. *M. genitalium* was detected in 25 patients (28%), 10 patients (11%) were positive for *M. hominis* and 55 patients (61%) were positive for *U. urealyticum*. Group (1): 60 patients attending STD clinic. Twenty patients (33.3%) were positive for *U. urealyticum*, five (8.3%) were positive for *M. hominis* and seventeen (28.3%) were positive for *M. genitalium*. Group (2): 50 patients with premature rupture of membranes. Twenty eight patients (56%) were positive for *U. urealyticum*, two patients (4%) were positive for *M. hominis* and seven patients (14%) were positive for *M. genitalium*. Group (3): 35 patients with pelvic inflammatory disease. Seven patients (20%) were positive for *U. urealyticum*, three patients (8.6%) were positive for *M. hominis* and one patient (2.9%) was positive for *M. genitalium*. The highest prevalence of *U. urealyticum* was associated with premature rupture of membranes. The highest prevalence of *M. genitalium* was determined in STD clinic patients. This study shows a high prevalence of genitourinary infections due to *U. urealyticum* which was considerably higher when compared to *Mycoplasma* spp. and is significantly associated with premature rupture of membranes. *M. genitalium* was confirmed as an important cause of STD. The use of PCR for identification of *Mycoplasmas* and *U. urealyticum* on cervical samples should be recommended. Further studies are needed to definitely associate with Spontaneous rupture of membranes and *U. urealyticum* colonization.

Keywords: *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, childbearing age females

INTRODUCTION

Genus *Mycoplasmas* and Genus *Ureaplasmas* are included in class Mollicutes. Different *Mycoplasma* species (*Mycoplasma hominis*, *Mycoplasma genitalium*) and *Ureaplasma urealyticum* inhabit the genitourinary tract of many sexually active men and women. The percentage of women with vaginal colonization by *Mycoplasma* spp, and *Ureaplasma* spp increases after puberty¹.

By adulthood, approximately 80 percent of healthy females acquire *Ureaplasma* spp and up to 50 percent acquire *M. hominis* in their cervical or vaginal secretions².

M. hominis and *U. urealyticum* are found frequently in the urogenital tract in both healthy persons and symptomatic patients³. *M.*

genitalium is a 'true' pathogen and causes sexually transmitted infection (STI) as urethritis in males. Furthermore, there is increasing evidence that *M. genitalium* infection is related to adverse outcomes in reproductive health in women, including urethritis, cervicitis, endometritis, pelvic inflammatory disease (PID) and infertility^{4,5}.

There is no adequate evidence that *M. hominis*, *U. parvum* or *U. urealyticum*, can cause cervicitis, vulvovaginitis, urethritis, PID or infertility, but some studies showed an association between the colonization by one of these microorganisms and a disease condition^{6,7}.

However, there is limitation on availability of data regarding *Mycoplasmas* and *Ureaplasmas* infections, so studies are needed to understand the contribution of these organisms to unfavorable

reproductive health outcomes in women and to improve guidelines for both screening and treatment.

Aim of the study

The aim of the current study is to detect the frequency of *M. genitalium*, *M. hominis* and *U. urealyticum* in child bearing age females attending Gynecology and Obstetrics clinics at Ain Shams University Hospital by using polymerase chain reaction.

PATIENTS & METHODS

This study was conducted during the period from January 2018 till August 2018. 145 women attending Gynecology and Obstetrics clinics at Ain Shams University Hospital and fulfilling inclusion criteria were included.

The inclusion criteria were: females in childbearing period (from 20 to 47 years), and complaint of only one of the following: symptoms

of vaginitis/cervicitis, (vaginal discharge, pain, itching, burning sensation and dyspareunia), history of premature child delivering or premature rupture of membranes and diagnosis of PID.

Exclusion criteria was administration of antimicrobial therapy within the last 30 days before the evaluation.

Sample collection

Cervical samples were collected using a sterile swab and placed in a liquid-based transport medium. Informed consent was obtained from patients according to the regulations of Scientific research Ethical Committee Faculty of Medicine - Ain Shams University). The samples were stored in -20 until testing.

All cervical samples were subjected to DNA extraction using QIAGEN DNA extraction Kit® (QIAGEN, USA), for DNA purification according to manufacturer's instructions.

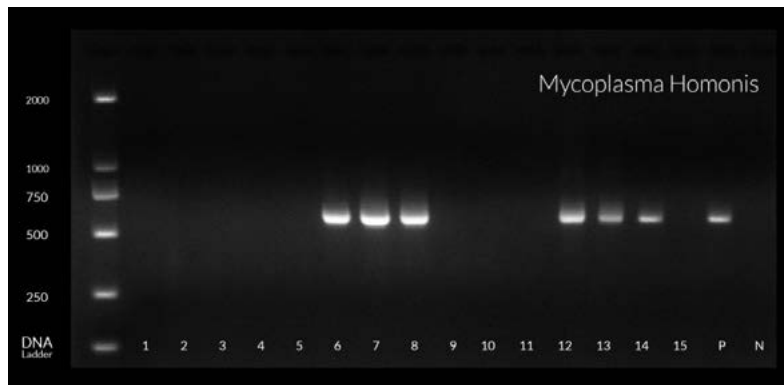


Fig. 1. Gel Electrophoresis of *Mycoplasma hominies* gene, product size (600) bp

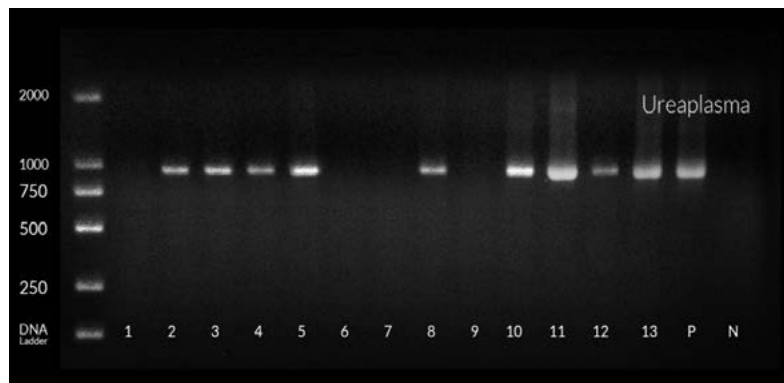


Fig. 2. Gel Electrophoresis of *Ureaplasma urealyticum* gene, product size (900) bp

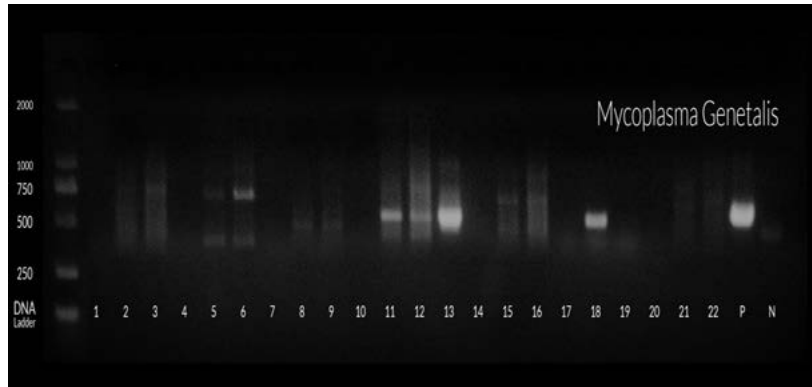


Fig. 3. Gel Electrophoresis of *Mycoplasma genitalis* gene, product size (500) bp

Amplification of *M. genitalium*, *M. hominis* and *U. urealyticum* genes was done using primers shown in Table (1).

PCR amplification conditions were 95°C for 10 min for denaturation, followed by 35 cycles at 65°C for *Mycoplasma genitalis*, 58°C for *Ureaplasma urealyticum* and *Mycoplasma hominis* for annealing and a final extension period of 15 min at 72°C.

Beta globin was used as internal control, amplification conditions were 95°C for 10 min for denaturation, followed by 35 cycles at 55°C for annealing and then final extension at 72°C for 15

min. Then the detection of amplified products were done using 2% agarose gel containing ethidium bromide. Then the products were visualized on a UV transilluminator (365 wavelength). Qiagen gel pilot 250 bp Plus was used as a ladder for molecular weight as shown in Figs (1, 2, and 3).

Data entry and statistical analyses were performed using SPSS (statistical package of social sciences) version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

This study was conducted on 145 women attending Gynecology and Obstetrics clinics at Ain

Table 1. Primer sequences used in the polymerase chain reaction

| organism | Direction | Primer sequence | Product size |
|--|------------|---|--------------|
| <i>Mycoplasma genitalis</i> ⁸ | MgPa1 | 5' AGT TGA TGA AAC CTT AAC CCC TTG G 3' | 500 bp |
| | MgPa3 | 5' CCG TTG AGG GGT TTT CCA TTT TTG C 3' | |
| <i>Ureaplasma urealyticum</i> | UU AS | 5' ACT ATA TTT CTA TAG CGT CGC AA 3' | 900 bp |
| | UU S | 5' TCA CCT TAA GTT GGG GAT AA 3' | |
| <i>Mycoplasma hominis</i> | Mh s | 5' ACC CAT TGG AAA CAA TGG CTA ATG CCG GAT ACG 3' | 600 bp |
| | MhAs | 5' ATA GAC CCA GTA AGC TGC CTT CGC CT 3' | |
| <i>Beta globin</i> | β-globin F | 5' TGA GTC TAT GGG ACG CTT GA 3' | 250 bp |
| | β-globin R | 5' AAA AAT TGC GGA GAA GAA AAA 3' | |

Table 2. Distribution of the patients in studied groups

| Groups | Patients N % |
|----------------------------|--------------|
| Total 145 | |
| STD Clinic | 60 (41.4%) |
| Premature rupture membrane | 50 (34.5%) |
| PID | 24.1%)(35 |

Table 3. Distribution of *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma urealyticum* among total positive colonized patients n = 90:

| Detected Pathogen | Total N Positive cases(90) |
|-------------------------------|----------------------------|
| <i>Mycoplasma genitalium</i> | 25 (28%) |
| <i>Mycoplasma hominis</i> | 10 (11%) |
| <i>Ureaplasma urealyticum</i> | 55 (61%) |

Table 4. Distribution of *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma urealyticum* among different groups

| | | Disease group | | | | | | | | |
|----------------------|----------|-----------------|-------|---------|---------------------------------|-------|---------|----------------------------------|-------|---------|
| | | STD Clinic (60) | | | Premature rupture membrane (50) | | | Pelvic inflammatory disease (35) | | |
| | | N | N % | P-value | N | N % | P-value | N | N % | P-value |
| <i>U. plasma</i> | Negative | 40 | 66.7% | > 0.05 | 22 | 44.0% | > 0.05 | 28 | 80.0% | ≤ 0.05 |
| | Positive | 20 | 33.3% | > 0.05 | 28 | 56.0% | ≤ 0.05 | 7 | 20.0% | > 0.05 |
| <i>M. hominis</i> | Negative | 55 | 91.7% | > 0.05 | 48 | 96.0% | > 0.05 | 32 | 91.4% | > 0.05 |
| | Positive | 5 | 8.3% | > 0.05 | 2 | 4.0% | > 0.05 | 3 | 8.6% | > 0.05 |
| <i>M. genitalium</i> | Negative | 43 | 71.7% | > 0.05 | 43 | 86.0% | > 0.05 | 34 | 97.1% | ≤ 0.05 |
| | Positive | 17 | 28.3% | ≤ 0.05 | 7 | 14.0% | > 0.05 | 1 | 2.9% | > 0.05 |

P> 0.05 Not significant
 P ≤ 0.05 Highly significant

Shams University Hospital. Their age ranged from 20 to 47.

These patients were divided into three groups according to the clinical presentations as shown in Table 2:

Group (1): Sixty patients(41.4%) were attending Sexually Transmitted Diseases(STD) clinic with symptoms of vaginitis/cervicitis (mean age 31± 4)

Group (2): Fifty patients (34.5%)with diagnosis of premature rupture of membranes (mean age 32± 4)

Group (3):Thirty-five patients (24.1%)with diagnosis of PID (mean age 33± 3)

The distribution of *M. genitalium*, *M. hominis* and *U. urealyticum* detection among total positive patients is shown in Table 3. *U.*

urealyticum was the most frequent microorganism detected.

Table 4 shows that in the group (1) there were 20 patients (33.3%) positive for *U. urealyticum*, five patients (8.3%) were positive for *M. hominis* and seventeen patients (28.3%) were positive for *M. genitalium*. A highly statistical association was observed between *M. genitalium* detection and patients attending STD clinic.

In the Group 2, there were 28 patients (56.0%) positive for *U. urealyticum*, two patients (4.0%) were positive for *M. hominis* and seven patients (14.0%) were positive for *M. genitalium*. A highly statistical association was observed between *U. urealyticum* detection and premature rupture of membranes.

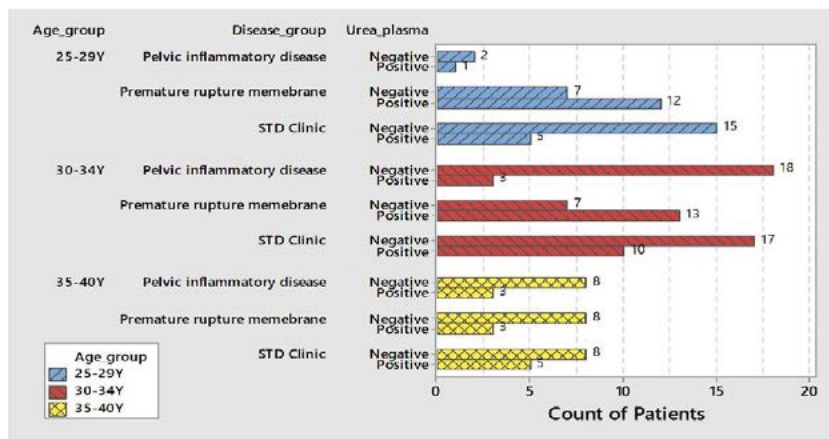


Fig. 4. Distribution of *Ureaplasma urealyticum* with different age groups and disease group

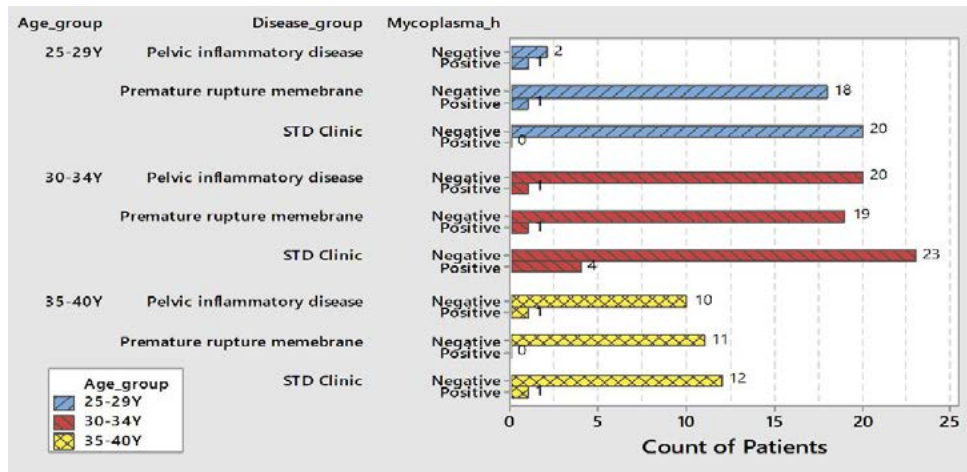


Fig. 5. Distribution of *Mycoplasma hominis* with different age groups and disease group

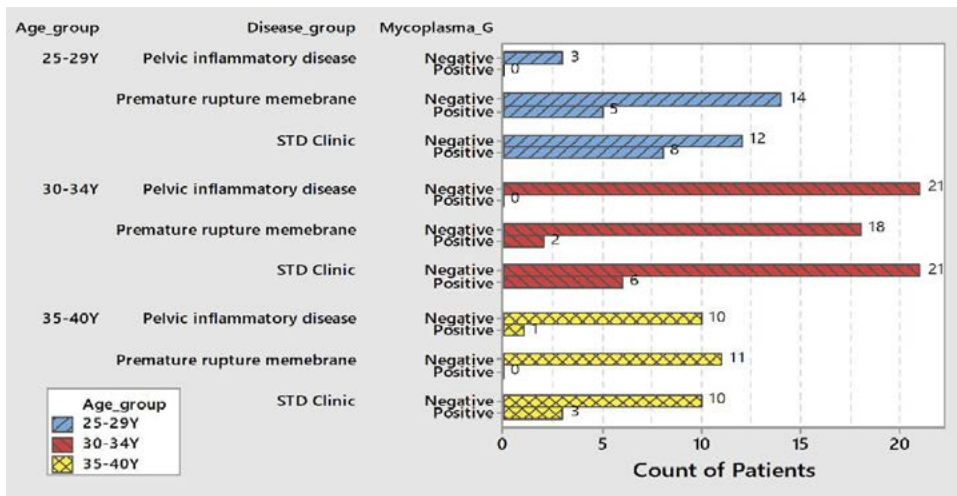


Fig. 6. Distribution of *Mycoplasma genitalis* with different age groups and disease group

In the Group 3, there were seven patients (20.0%) positive for *U. urealyticum*, three patients (8.6%) positive for *M. hominis* and one patient (2.9%) positive for *M. genitalium*. No statistical association between diagnosis of PID and *M. genitalium*, *M. hominis* and *U. urealyticum* was observed.

Fig. (4) shows that there was a high prevalence of *Ureaplasma urealyticum* among patients with premature rupture of membranes especially in the Age group from 25 to 29 years old and age group from 30 to 34 years old.

Fig. (5) shows that the *Mycoplasma hominis* was highly prevalent among female patients attending STD clinic especially among age group from 30-34 years.

Fig. (6) shows that the high prevalence of *Mycoplasma genitalis* was in patients attending STD clinic especially among age group 25-29 years.

DISCUSSION

M. hominis and *U. urealyticum* are found frequently in the urogenital tract of humans in both patients and healthy individuals³. However, *M. genitalium* is a ‘true’ sexually transmitted pathogen causing urethritis in males and there is increasing evidence that *M. genitalium* infection could be related to unfavorable reproductive health outcomes in women, including urethritis, cervicitis, PID, endometritis and infertility and adverse birth outcomes^{4,5}.

The aim of the current study was to detect the distribution of *M. genitalium*, *M. hominis* and *U. urealyticum* in child bearing age females attending Gynecology and Obstetrics clinics at Ain Shams University Hospital by using polymerase chain reaction.

The results of our study showed that out of 145 patients, 90 patients (62%) were positive for *Mycoplasma* spp and *U. urealyticum*. 28% were positive for *M. genitalium*, 11% were positive for *M. hominis* and 61% were positive for *U. urealyticum*. These results are similar to those described by Bayraktar *et al.*⁹, who reported *U. urealyticum* detection in 44% of patients and *M. hominis* in 4% of patients. Moreover, the prevalence of *M. genitalium* was similar to that reported by Balkus *et al.*⁴

In this study the prevalence of *M. genitalium* was 28% among studied group, most of cases were detected in age group ≤ 30 years. Lokken and coworkers reported lower prevalence of *Mycoplasma genitalium* in Uganda (14%) and Kenya (12.9%–16%) than the prevalence in this study¹⁰. Also, Getman *et al.*, reported prevalence of *M. genitalium* infections in all females (14 to 70 years of age) was 16.3%. *M. genitalium* infections were significantly more prevalent in subjects ≤ 30 years of age than in subjects > 30 years of age¹¹. Furthermore, Campos *et al.* who studied the prevalence of *Mycoplasma genitalium* and *Mycoplasma hominis* in urogenital tract of Brazilian women reported that the prevalence of *M. hominis* and *M. genitalium* were detected in 31.8% (96/302) and 28.1% (85/302), respectively and also reported that women under age 25 represent a higher risk for *M. hominis* infection¹². As for *Mycoplasma hominis* infections are found to be less common than *Ureaplasma* infections. In a study performed by Sarier *et al.* reported that *Mycoplasma hominis* infections occur mainly in severely immunosuppressed patients¹³.

Furthermore, patients were divided into three groups: Group (1): patients attending STD clinic, Group (2): patients with diagnosis of Premature rupture of membranes and Group (3): patients with diagnosis of PID.

There was a high prevalence of *U. urealyticum* in the three groups, where it represents 33% in patients attending STD clinic, 56.5% in females with premature rupture of

membrane and 20% in PID.

Furthermore, Bayraktar and coworkers reported that there was a high prevalence of *U. urealyticum* among pregnant women in age group 18–34 years. They concluded also that *U. urealyticum* was more frequently detected than *M. hominis* in both the controls and patients⁹.

There is an association between the presence of genital *mycoplasmas* and increased risk of obstetrical complications, such as, preterm labor, spontaneous abortion and delivering low birth weight¹⁴. Bayraktar and coworkers stated that there is an association between *U. urealyticum* and preterm labor⁹.

Luton *et al.* performed a study on 218 pregnant women of different gestational age and followed them. He reported that there is a significant increase in the prevalence of *Mycoplasmas* as a cervical colonizer during whole pregnancy¹⁵. Moreover, Lillis *et al.* reported that 70 women (17.5%) were positive for *M. genitalium* among 400 women attending a STD clinic. *M. genitalium* was significantly associated with age < 25 years¹⁶.

There are different methods that can be used for detection of *Mycoplasmas*, like culture, antigen detection, antibody detection and molecular methods. Moreover, the molecular methods allowing simultaneous detection of different species of genital *Mycoplasmas* is very useful in a clinical setting. Quantitative PCR analysis plays an important role in their diagnosis as it also can show microbial load^{17,18}. Furthermore, detection of *U. urealyticum* in culture is difficult so PCR is a gold standard method for its detection. Recent study performed by Sarier *et al.*, they stated that using quantitative PCR in diagnosis of *U. urealyticum* is a valuable test for demonstrating microbial load and avoiding false-positive results. Thus, quantitative PCR analysis plays an important role in the diagnosis since it also can show microbial load¹⁸.

CONCLUSIONS

This study shows a high prevalence of *U. urealyticum* and *M. genitalium* in comparison to *M. hominis* as genitourinary infections. There is a significant association between prevalence of *U. urealyticum* and age. *U. urealyticum* is more prevalent in patients with premature rupture of

membrane in the current study so there may be an association between *U. urealyticum* infection and obstetrical complications. So testing the presence of *Mycoplasma* species and *U. urealyticum* during pregnancy may be necessary for better pregnancy outcomes and can be included in the routine follow up of pregnancy. The use of molecular techniques like PCR for identification of *Mycoplasma* spp and *U. urealyticum* on cervical samples can be beneficial in comparison to culture methods. Further studies are recommended to prove the association between spontaneous abortion and premature rupture of membranes and *U. urealyticum* colonization.

ACKNOWLEDGMENTS

The authors are grateful to members of obstetrics and gynecology of Ain Shams University hospitals, for their support to carry out this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors designed the study. MMF collected samples from patients. SAAS and MAK performed the molecular work of the study performed PCR on the samples. All authors analyzed the data. All authors wrote the manuscript. All authors read and approved the manuscript.

FUNDING

None.

ETHICS STATEMENT

Informed consent was taken from all patients according to the Ethical Committee regulation of Faculty of Medicine Ain Shams University.

DATA AVAILABILITY

All Datasets generated or analyzed during this study is available.

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