Abdel Salam et al. | J Pure Appl Microbiol | 14(2):1413-1421 | June 2020
Article 6256| https://doi.org/10.22207/JPAM.14.2.39

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

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

Shimaa A. Abdel Salam[1], Mona A. Khattab[1]* and Malames M. Faisal[2]

[1]Department of Medical Microbiology & Immunology Faculty of Medicine Ain Shams University, Cairo, Egypt.
[2]Department of Gynecology & Obstetrics Faculty of Medicine Ain Shams University, Cairo, Egypt.

***Correspondence:** monaadelkhattab@yahoo.com

(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

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 on145 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)_:_ 50patientswith 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 associates 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 puberty[1].

By adulthood, approximately 80 percent of healthy females acquire *Ureaplasma* spp and up to 50 percent acquire *M. hominis* in their cervical or vaginal secretions[2].

M. hominis and *U. urealyticum* are found frequently in the urogenital tract in both healthy persons and symptomatic patients[3]. *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.

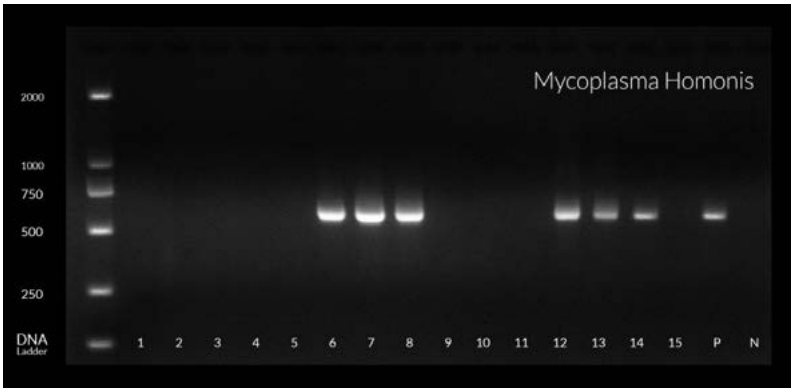

Fig. 1. Gel Electrophoresis of *Mycoplasma hominies* gene, product size (600) bp

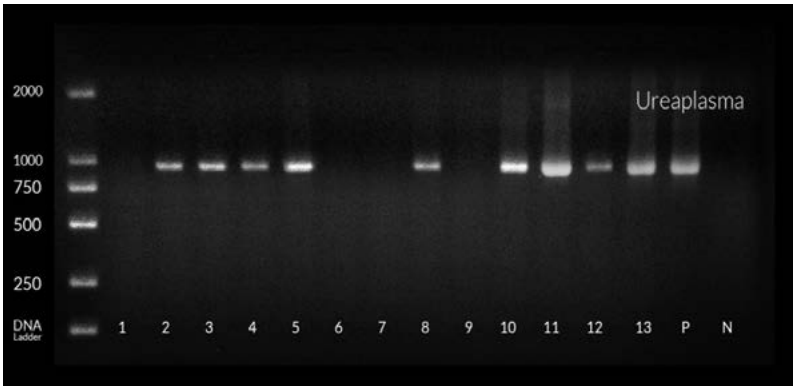

Fig. 2. Gel Electrophoresis of *Ureaplasma urealyticum* gene, product size (900) bp

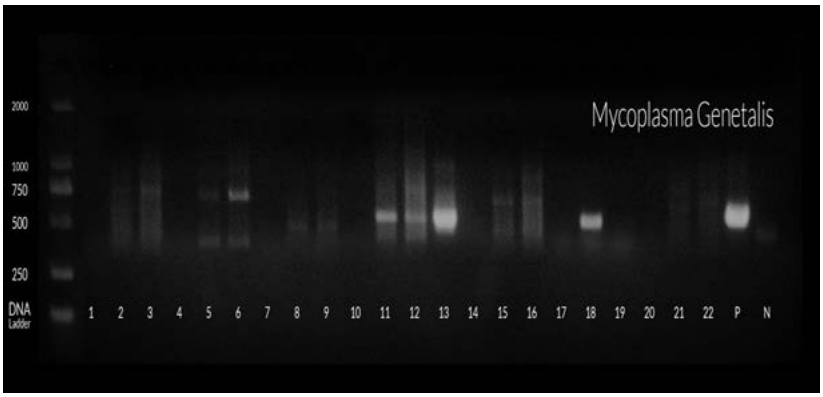

Fig. 3. Gel Electrophoresis of *Mycoplasma genitalis* gene**,** product size (500) bp

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

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)
Mycoplasma genitalium	25 (28%)
Mycoplasma hominis	10 (11%)
Ureaplasma urealyticum	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
U. plasma	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
M. hominis	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
M. genitalium	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.

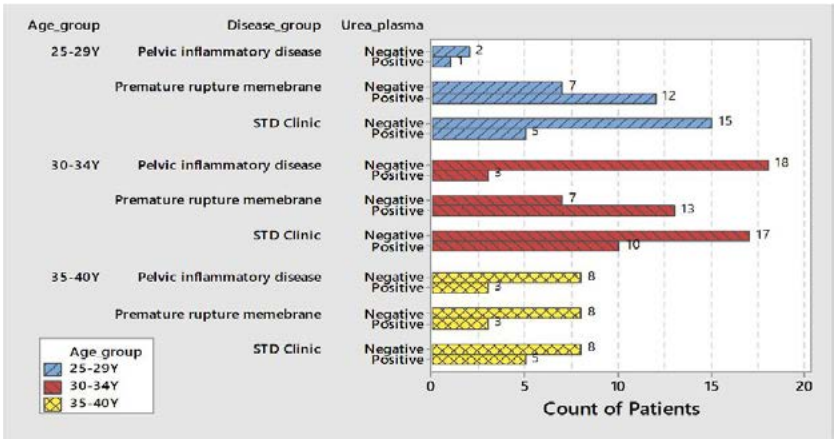

Fig. 4. Distribution of *Ureaplasma urealyticum* with different age groups and disease group

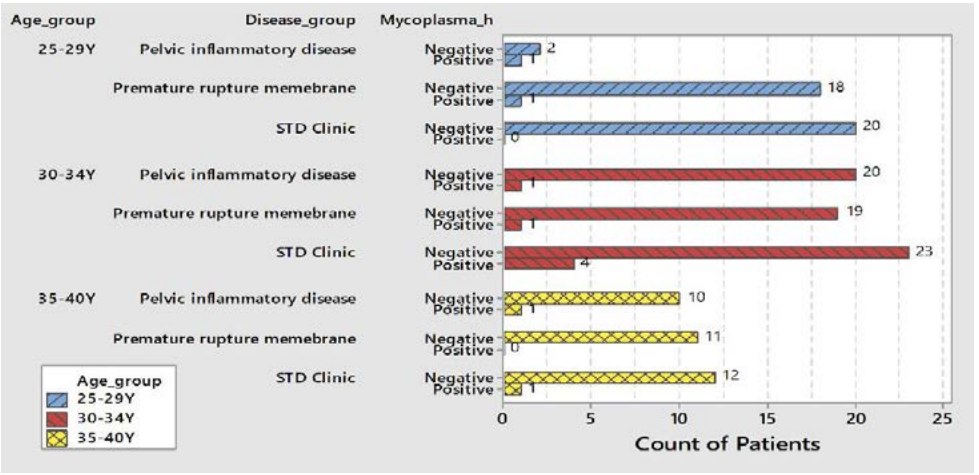

Fig. 5. Distribution of *Mycoplasma hominis* with different age groups and disease group

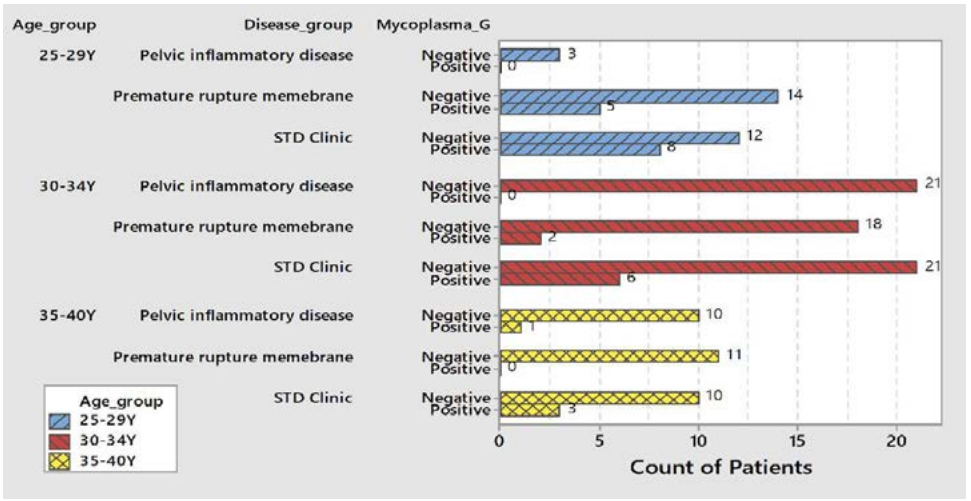

Fig. 6. Distribution of *Mycoplasma genitals* with different age groups and disease group

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 genitals* 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 individuals[3]. 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 study[10]. 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 age[11]. 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*infection[12]. As for M*ycoplasma 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 immuno suppressed patients[13].

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 group18—34 years. They concluded also that *U. urealyticum* was more frequently detected than *M. hominis*in both the controls and patients[9].

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 weight[14]. Bayraktar and coworkers stated that there is an association between *U. urealyticum* and preterm labor[9].

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 pregnancy[15]. 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 years[16].

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 Mycoplasmasis 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 load[18].

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.

REFERENCES

1. Baum SG, Edwards MS. *Mycoplasma hominis* and *Ureaplasma urealyticum* infections. Up-ToDate, Waltham, MA. 2017;1-46.
2. Waites KB, Schelonka RL, Xiao L, Grigsby PL, Novy MJ. Congenital and opportunistic infections: *Ureaplasma* species and *Mycoplasma hominis*. In Seminars in Fetal and Neonatal Medicine. 2009;14(4):190-199. WB Saunders. https://doi.org/10.1016/j.siny.2008.11.009
3. Taylor-Robinson D. Mollicutes in vaginal microbiology: *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum* and *Mycoplasma genitalium*. *Res Microbiol*. 2017;168:875-881. https://doi.org/10.1016/j.resmic.2017.02.009
4. Balkus JE, Manhart LE, Jensen JS, et al. *Mycoplasma genitalium* infection in Kenyan and US women. *Sexually Transmitted Diseases*. 2018;45(8):514-21. https://doi.org/10.1097/OLQ.0000000000000799
5. Unemo M, Jensen JS. Antimicrobial-resistant sexually transmitted infections: *gonorrhoea* and *Mycoplasma genitalium*. *Nat Rev Urol.* 2017;14:139-152. https://doi.org/10.1038/nrurol.2016.268
6. Kyndel A, Elmer C, Kallman O, Altman D. *Mycoplasmataceae* colonizationsin women with urethral pain syndrome: a case-control study. *J Low Genit Tract Dis.* 2016;20:272-274. https://doi.org/10.1097/LGT.0000000000000216
7. Michou IV, Constantoulakis P, Makarounis K, Georgoulias G, Kapetanios V, Tsilivakos V. Molecular investigation of menstrual tissue for the presence of *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis* collected by women with a history of infertility. *J ObstetGynaecol Res.* 2014;40:237-242. https://doi.org/10.1111/jog.12165
8. Jensen JS, Uldum SA, Sondergard-Andersen J, Vuust J, Lind K. Polymerase chain reaction for detection of *Mycoplasma genitalium* in clinical samples. *J Clin Microbiol*. 1991;29(1):46-50. https://doi.org/10.1128/JCM.29.1.46-50.1991
9. Bayraktar MR, Ozerol IH, Gucluer N, Celik O. Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women. *Int J Infect Dis*. 2010;14(2):e90-5. https://doi.org/10.1016/j.ijid.2009.03.020
10. Lokken EM, Balkus JE, Kiarie J, et al. Association of recent bacterial vaginosis with acquisition of *Mycoplasma genitalium*. *Am J Epidemiol*. 2017;186(2):194-201. https://doi.org/10.1093/aje/kwx043
11. Getman D, Jiang A, O'Donnell M, Cohen S. *Mycoplasma genitalium* prevalence, coinfection, and macrolide antibiotic resistance frequency in a multicenter clinical study cohort in the United States. *J Clin Microbiol*. 2016;54(9):2278-83. https://doi.org/10.1128/JCM.01053-16
12. Campos GB, Lobao TN, Selis NN, et al. Prevalence of *Mycoplasma genitalium* and *Mycoplasma hominis* in urogenital tract of Brazilian women. *BMC Infectious Diseases*. 2015;15(1):60. https://doi.org/10.1186/s12879-015-0792-4
13. Sarier M, Sepin N, Guler H, et al. Prevalence of Sexually Transmitted Disease in Asymptomatic Renal Transplant Recipients. *Exp Clin Transplant*. 2018. https://doi.org/10.6002/ect.2017.0232
14. Clegg A, Passey M, Yoannes M and Michael A. High rates of genital *Mycoplasma* infection in the highlands of Papua New Guinea determined both

by culture and by a commercial detection kit. *J Clin Microbiol.* 1997;35:197-200. https://doi.org/10.1128/JCM.35.1.197-200.1997

15. Luton D, Ville Y, Luton-Sigy A, et al. Prevalence and influence of *Mycoplasma hominis* and *Ureaplasma urealyticum* in 218 African pregnant women and their infants. *Eur J Obstet Gynecol Reprod Biol.* 1994;56:95-101. https://doi.org/10.1016/0028-2243(94)90263-1
16. Lillis RA, Martin DH, Nsuami MJ. Mycoplasma genitalium infections in women attending a sexually transmitted disease clinic in new orleans. *Clin Infect Dis*. 2019;69(3):459-65. https://doi.org/10.1093/cid/ciy922
17. Bao T, Chen R, Zhang J, et al. Simultaneous detection of *Ureaplasma parvum*, *Ureaplasma urealyticum, Mycoplasma genitalium* and *Mycoplasma hominis* by fluorescence polarization. *J Biotechnol.* 2010;150(1):41-43. https://doi.org/10.1016/j.jbiotec.2010.07.024
18. Sarier M, Kukul E. Classification of non-gonococcal urethritis: a review. *Int Urol Nephrol.*, 2019. https://doi.org/10.1007/s11255-019-02140-2