

# Prevalence of low genital tract colonization by mycoplasma, ureaplasma and bacterial vaginosis

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## Abstract

**Objectives:** To determine the prevalence of low genital tract colonization by *Mycoplasma*, *Ureaplasma* and its association with bacterial vaginosis

**Materials and Methods:** A cross-sectional study carry on 802 women who were visited at Vinmec International Hospital from January 2021 to January 2022. *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum* in low genital tract sample were detected by Realtime - PCR method. Diagnosis of bacterial vaginosis based on microbiological morphology according to Nugent score. Abnormal cases are treated and followed.

**Results:** The prevalence of *M. genitalium* was 1.6% (95%CI: 0.9 - 2.8), *M. hominis* was 5.0% (95%CI: 3.6 – 6.8), *U. urealyticum* was 13.1% (95%CI: 10.7 - 15.9) and *U. parvum* was 36.0% (95%CI: 32.0 - 40.4). *Mycoplasma* and *Ureaplasma* present in lower genital tract samples were not associated with clinical symptoms. The rate of bacterial vaginosis was 4.7% and it was not associated with the presence of *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum* in low genital tract.

**Conclusion:** The prevalence of *Mycoplasma* and *Ureaplasma* in the genital tract is relatively low and it was not associated with bacterial vaginosis.

**Keyword:** Vaginitis, cervicitis, bacterial vaginosis, *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum*, Nugent score.

## 1. INTRODUCTION

Mycoplasma and Ureaplasma belong to the class Mollicutes, including bacteria with very small size, simple structure, no cell wall and not detected by Gram stain. Mycoplasma and Ureaplasma have been discovered to include 4 orders, 5 families, 8 genera and nearly 200 species. About 17 species of Mycoplasma and 2 species of Ureaplasma have been isolated in humans [1]. Mycoplasma genitalium (*M. genitalium*) is a sexually transmitted agent that can be found in the urinary tract, genitals, oral cavity, and pharynx in both sexes. *M. genitalium* is associated with cervicitis, endometritis, increased risk of pelvic inflammatory disease, female infertility, and it is also associated with male urethritis [2]. Mycoplasma hominis (*M. hominis*), Ureaplasma urealyticum (*U. urealyticum*) and Ureaplasma parvum (*U. parvum*) are commonly found in the genitourinary tract in both healthy and symptomatic patients [3]. *M. hominis* and Ureaplasma spp can be considered as part of the vaginal microbiota of sexually active women.

The normal vaginal microbiota is characterized by a predominance of the family Lactobacillus, which can be described into five classes (Community State Type – CST): CSTI, CSTII, CSTIII, CSTIV, CSTV. In which, CST I, II, III, V is the normal microbiota patterns with dominant family Lactobacillus. And CSTIV (including 2

subclasses IV-A, IV-B) is characterized by the relatively high presence of species Atopobium, Prevotella, Sneathia, Gardnerella, Ruminococcaceae, Parvimonas, Mobiluncus and is associated with bacterial vaginosis [4]. These two microbiota pattern subclasses can still be found in healthy asymptomatic women with high Nugent scores. A high Nugent score has also been shown to be associated with an increased risk of sexually transmitted infections [4]. However, at present, there is not much clear evidence attributing the pathogenic role in the lower genital tract of Mycoplasma and Ureaplasma. These agents usually persist in the genitourinary tract without causing disease in healthy individuals and may be transient. In addition, it is difficult to distinguish with certainty the role of Mycoplasma and Ureaplasma as the main pathogen or co-presence of Mycoplasma and Ureaplasma, and in very few cases these bacteria have been isolated as the sole agent in specimens from symptomatic people [5]. In addition, there are still many difficulties in isolating Mycoplasma and Ureaplasma, which leads to limited research data on the association between these bacteria and lower genital tract infections.

Recommendations for performing screening tests for Mycoplasma and Ureaplasma vary by sex, age, sexual behavior [2]. The approach of screening for

these agents is not recommended for routine use in all populations and asymptomatic populations and may be considered after traditional low genital tract infections such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, etc. have been ruled out[6]. Selecting tests to identify *Mycoplasma* and *Ureaplasma* in group with symptoms such as increased vaginal discharge, urinary disorders, lower abdomen pain or pain during intercourse, possible pelvic inflammatory disease, etc. or in group of ineffective or recurrent vaginitis, cervicitis is still based on experience. Stemming from these issues, our study conducted with the aim of determining the prevalence of *Mycoplasma* and *Ureaplasma* in the genital tract and assessing the relationship between *Mycoplasma* and *Ureaplasma* with bacterial vaginosis.

## 2. MATERIALS AND METHODS

### 2.1. Objects

**Inclusion criteria:** All cases of gynecological examination at Women's Health Center, Vinmec Times City International Hospital.

**Sampling criteria:** No vagina douching for 24 hours,

no use of vaginal irritants, no sex within 24 hours, and having menstruation.

**Exclusion criteria:** individuals who are on antibiotics for an infection and do not give informed consent.

### 2.2. Methodology

Cross-sectional study design. The sample size was determined based on the objective of the study to estimate the prevalence of *M. genitalium* of 2.1% in vaginal fluid samples from couples who come to the infertility clinic at Hue University Hospital, announced in 2020 [7]. It was determined that the minimum sample required for the study was 632 women carrying *M. genitalium*.

The study used cluster sampling form during the data collection period from January 2021 to January 2022.

### 2.3. Steps to conduct research

The cases of gynecological examination at Women's Health Center, Vinmec Times City International General Hospital qualified for clinical examination, collecting samples of vaginal fluid for bacteriological testing, staining, and evaluating vaginal microbiota according to Nugent score.

**Table 1.** Nugent score.

Bacteria	Score				
	0	1+	2+	3+	4+
Lactobacilli	4	3	2	1	0
Gardnerella	0	1	2	3	4
Mobiluncus	0	1	1	2	2

Scoring method:

Normal vaginal microbiota: 0 - 3 points, intermediate microbiota (suspected dysbacteriosis): 4 - 6 points. Bacterial vaginosis: 7 - 10 points.

### Gynecological examination and testing for mycoplasma and ureaplasma:

Multi-agent assay including *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum* by Realtime - PCR method. Use Nimbus Automatic DNA Extractor and Seegene STARMag 96 X 4 Universal Cartridge Kit. Realtime PCR machine CFX96 and Seegene Allplex™ STI Essential Assay sexually transmitted pathogen test kit at Microbiology unit, Laboratory Department, Vinmec Times City International General Hospital. The machines and related equipment are checked every 6 months. The internal control process is regularly evaluated through each sample run, and the external control frequency is once a year.

Unusual cases detected are treated and monitored according to the recommended regimen [6].

### 2.4. Data analysis

Determine the prevalence of *M. genitalium*, *M.*

*hominis*, *U. urealyticum*, *U. parvum* and confidence intervals based on test results of positive vaginal discharge samples compared with total study samples. Compare the prevalence of *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum* between groups of normal and intermediate vaginal microbiota and diagnostic dysbacteriosis according to the Nugent vaginal microbiome score and associated assessment.

The level of statistical significance was chosen  $p < 0.05$ .

## 3. RESULTS

During the sampling period, 802 eligible cases were included in the analysis. The study samples had an average age of  $34.4 \pm 8.6$  years old, average BMI of  $20.9 \pm 2.4$  kg/m<sup>2</sup>, Vietnamese nationality accounted for 98.3%, resided in Hanoi (82.4%), their main occupations of officials and civil servants (67.2%), education concentrated in Universities and Colleges (79.3%), the majority of them got married (82.4%) and ever pregnant (71.7%).

**Table 2.** The prevalence of Mycoplasma and Ureaplasma.

	Quantity n	Prevalence %	95% CI
<b>Mycoplasma</b>	51	6.4	4.7 - 8.4
Mycoplasma:	51	6.4	4.7 - 8.4
M. genitalium	13	1.6	0.9 - 2.8
M. genitalium	13	1.6	0.9 - 2.8
M. hominis	40	5.0	3.6 - 6.8
M. hominis	40	5.0	3.6 - 6.8
<b>Ureaplasma</b>	370	46.1	41.5 - 51.1
Ureaplasma:	370	46.1	41.5 - 51.1
U. urealyticum	105	13.1	10.7 - 15.9
U. urealyticum	105	13.1	10.7 - 15.9
U. parvum	289	36.0	32.0 - 40.4
U. parvum	289	36.0	32.0 - 40.4
Sample	<b>802</b>	<b>100.0</b>	

The prevalence of *M. genitalium* was 1.6% and *M. hominis* was 5.0%, the prevalence of *U. urealyticum* was 13.1% and *U. parvum* was 36.0%.

**Table 3.** The prevalence of Mycoplasma and Ureaplasma according to clinical symptom groups.

	N (%)	M. genitalium	M. hominis	U. urealyticum	U. parvum
		n (%)	n (%)	n (%)	n (%)
No symptoms (*)	326 (40.6)	7 (2.1)	17 (5.2)	45 (13.8)	113 (34.7)
Vaginal discharge	404 (50.4)	5 (1.2)	26 (6.4)	62 (15.3)	160 (39.6)
Pain (or heaviness and tightness) in the lower abdomen	97 (12.1)	0 (0.0)	9 (9.3)	18 (18.6)	38 (39.2)
Itching vulva, perineum	143 (17.8)	3 (2.1)	8 (5.6)	23 (16.3)	51 (35.7)
Vaginal symptom	20 (2.5)	0 (0.0)	0 (0.0)	2 (10.0)	7 (35.0)
Cervical symptom	235 (29.3)	5 (2.1)	14 (6.0)	35 (14.9)	100 (42.6)
Urinary symptom	98 (12.2)	0 (0.0)	3 (3.1)	9 (9.2)	25 (25.5)
Other symptoms	27 (3.4)	0 (0.0)	3 (11.1)	4 (14.8)	10 (37.0)

(\*) Group of *M. genitalium*, *M. hominis* and *U. urealyticum*, *U. parvum* asymptomatic carriers used as a reference for comparison with bacterial prevalence in other symptomatic groups.

The prevalence of *M. genitalium*, *M. hominis* and *U. urealyticum*, *U. parvum* in groups with clinical symptoms including vaginal discharge, pain, itching and vulvar, vaginal, cervical and urinary tract symptoms were not different from those of the asymptomatic groups.

**Table 4.** Ureaplasma and bacterial vaginosis.

Nugent score	n	%	U. urealyticum			U. parvum		
			n	%	p	n	%	p
0 - 3 points	425	53.0	53	12.5	-	161	37.9	-
4 - 6 points	339	42.3	46	13.6	0.653	116	34.2	0.291
> 7 points	38	4.7	6	15.8	0.560	12	31.6	0.442

The group with the Nugent score of 7 - 10 points accounted for 4.7%, the group with 4 - 6 points was 42.3%.

The prevalence of *U. urealyticum* and *U. parvum* in the group with the Nugent score of 7 - 10 points was 15.8% and 31.6%, respectively, compared with the group with a Nugent score of 4 - 6 points and the group of 0 - 3 points, this difference is not statistically significant.

**Table 5.** Mycoplasma and bacterial vaginosis.

Nugent score	n	%	M. genitalium			M. hominis		
			n	%	p	n	%	p
0 - 3 points	425	53.0	6	1.4	-	17	4.0	-
4 - 6 points	339	42.3	6	1.8	0.660	19	5.6	0.300
7 - 10 points	38	4.7	1	2.6	0.560	4	10.5	0.065

The prevalence of *M. genitalium* and *M. hominis* in the group with the Nugent score of 7 - 10 points was 2.6% and 10.5% respectively, this prevalence in the group with the Nugent score of 4 - 6 points was 1.8% and 5.6% respectively, group of 0 - 3 points was 1.4% and 4.0% respectively, the difference in prevalence was not statistically significant among groups.

## 4. DISCUSSION

### 4.1. The prevalence of Mycoplasma and Ureaplasma

In our study, 51 women with genital tract samples confirmed to carry Mycoplasma, accounting for 6.4% (95% CI: 4.7 - 8.4), of which 13 cases carried *M. genitalium*, 1.6% (95%CI: 0.9 - 2.8). Collected samples were all cases of gynecological examination at Women's Health Center, Vinmec Times City International General Hospital, in which 40.6% of cases were asymptomatic and had gynecological examination periodically or for reasons unrelated to genitourinary symptoms. The proportions of Mycoplasma and Ureaplasma in vaginal discharge samples are suitable to assess the status in the study population characterized by the majority of cases with gynecological problems. For the groups of clinical manifestations, *M. genitalium* was identified in 2.1% of cases in the asymptomatic group, 1.2% of the cases of the vaginal discharge group, 2.1% of the cases of cervicitis group. Notably, there was no difference in the prevalence of *M. genitalium* in the symptomatic group and the asymptomatic group. The study found 40 cases carrying *M. hominis* accounted for 5.0%, (95%CI: 3.6 - 6.8), similar to the group of women carrying *M. genitalium*, vaginal discharge samples identified *M. hominis* also did not differ between symptomatic group and the asymptomatic group. Meanwhile, up to 370 women carry Ureaplasma, accounting for 46.1% (95% CI: 41.5 - 51.1), including 105 cases carrying *U. urealyticum*, accounted for 13.1% (95%CI: 10.7 - 15.9) and 289 cases carrying *U. parvum*, accounted for 36.0% (95% CI: 32.0 - 40.4).

In general, the prevalence of Mycoplasma and Ureaplasma in this study is relatively low compared to the general prevalence, including the groups with common clinical symptoms related to infection with

these bacteria such as vaginal discharge, cervicitis, lower abdomen, urinary symptoms. Notwithstanding this, published data on the prevalence of these bacteria are relatively variable from one study to another and are rather limited in our country. In Vietnam, a 2009 community study of 990 rural women aged 18 - 49 years showed that the prevalence of *M. genitalium* in vaginal discharge samples was only about 0.8% (95% CI: 0.25 - 1.35) [8], this study also found the prevalence of *M. genitalium* is relatively low in rural Vietnam. A study of infertile women, who are at higher risk for gynecological problems including genital infections, showed the prevalence of *M. genitalium* about 2.1% [7], which is similar to the prevalence in our study and quite low compared to general reports [6]. This study included the prevalence of *U. urealyticum* was 37.9%, while in our study, it is only 13.1%.

The prevalence of detectable *M. genitalium* is up to 10 - 30% in the group with cervicitis symptoms, ranging from 4 - 22% and possibly up to 60% in those with pelvic inflammatory disease. Presence of *M. genitalium* is associated with cervicitis, preterm birth, spontaneous abortion, and infertility [6]. The prevalence of *M. hominis* and Ureaplasma spp increases after puberty and is proportional to the number of sexual partners. By adulthood, up to 80% of healthy women have ever carried Ureaplasma spp and 50% of women have the presence of *M. hominis* in vaginal and cervical fluid [5]. Even the prevalence of *U. urealyticum* is determined by evaluation of IgG sera titres up to 93% [9]. As for men, about 25% of sexually active men are also regularly infected with asymptomatic *M. hominis* [6]. In a summary of the data in women of reproductive age, the prevalence of *U. parvum* is relatively high, followed by *U. urealyticum*, *M. hominis* and *M. genitalium* with a lower percentage. On the other hand, during pregnancy, most of the pooled data show similar prevalence of Mycoplasma and Ureaplasma in pregnant and non-pregnant women, varying between 7.7 and 88.0% for *U. urealyticum* and between 3 and 51% for *M. hominis* [9].

### 4.2. Relationship between Mycoplasma and Ureaplasma and bacterial vaginosis.

In this study, we used Nugent score to diagnose bacterial vaginosis. Bacterial vaginosis is characterized

by the replacement of *Lactobacillus* by anaerobic bacteria such as *Mobiluncus*, *Mycoplasma hominis*, *Bacteroides* species, *Gardnerella vaginalis*, of which more than 80% are *Gardnerella vaginalis* [9]. These bacteria can cause non-specific vaginitis, however, in some cases, they do not cause inflammation, produce many white blood cells, consistent with the term Bacterial vaginosis. The Nugent score gives scores Gram-stained samples from the vaginal smear, and the diagnosis of bacterial vaginosis is based solely on microbiological morphology, on the basis of the presence of large rod-shaped bacteria (*Lactobacillus*), the presence of small rod-shaped bacteria (*Gardnerella vaginalis*, *Atopobium vaginae*, *U. urealyticum*, *M. hominis*, *Prevotella*, *Peptoniphilus*, *Megasphaera*, *Mobiluncus*, etc.), the presence of curved rod-shaped bacteria and cocci (*Mobiluncus* spp). The total score of these parameters can be from 0 to 10, Nugent point from 7 to 10 is considered to have bacterial vaginosis. The Nugent score is considered to be the gold standard for diagnosing bacterial vaginosis with its low cost and high sensitivity, although it has some limitations such as time, equipment, and trained technicians to perform [10].

In our study, the bacterial vaginosis group accounted for 4.7%, this rate is relatively low. The group of 4 - 6 points is the group of suspected bacterial vaginosis or the intermediate group, with the rate of 42.3%, the rest, 53.0% of the cases have normal vaginal microflora. The prevalence of *M. genitalium* and *M. hominis* in the group with the Nugent score of 7 - 10 points was 2.6% and 10.5% respectively, this prevalence in the group with the Nugent score of 4 - 6 points was 1.8% and 5.6% respectively, group of 0 - 3 points was 1.4% and 4.0% respectively. The prevalence of *U. urealyticum* and *U. parvum* in the group with the Nugent score of 7 - 10 points was 15.8% and 31.6%, respectively, the group with the Nugent score of 4 - 6 points was 13.6% and 34.2%, and the group of 0 - 3 points was 12.5% and 37.9% respectively. Notably, the difference of the prevalence of *Mycoplasma* and *Ureaplasma* is not statistically significant in these groups, or women carrying *M. genitalium*, *M. hominis*, *U. urealyticum* and *U. parvum* were not associated with bacterial vaginosis as determined by the Nugent score. Although the study did not analyze the prevalence of *Mycoplasma* and *Ureaplasma* according to the low genital tract infection causes, the results show that the approach to testing for *Mycoplasma* and *Ureaplasma* should be considered. Overall, women who had *M. genitalium*, *U. urealyticum* and *U. parvum* in vaginal discharge samples are often accompanied by symptoms of dysuria, increased vaginal discharge, pain during intercourse and lower abdominal pain, severe lower abdominal pain, and abnormal vaginal discharge, vaginal discomfort, vaginal

pruritus, cervicitis, vulvovaginitis [6] and Nugent score  $\geq 8$  were associated with *M. genitalium* [11], however, has not ruled out these bacteria as pathogens or co-infections. Similarly, there are not many consistent data including case-control studies or evidence that *M. hominis*, *U. parvum* or *U. urealyticum* cause low genital tract infection. In some cases, symptoms were transient, while others were reported to complicate pelvic inflammatory disease (endometritis, fallopian tube infection), and tubal infertility [6]. In another aspect, the study of Lee SE et al. showed that vaginal samples were positive for *Mycoplasma*, high Nugent score did not increase the risk of spontaneous preterm birth, and this group of authors recommends that there is no need to spend resources to testing for bacteria *M. genitalium* in vaginal fluid to determine the risk of preterm birth [11]. The results of our study do not recommend testing for *Mycoplasma* and *Ureaplasma* in any subjects, but it showed that the presence of *Mycoplasma* and *Ureaplasma* was not associated with bacterial vaginosis and did not differ by clinical symptom group. We also agree that screening tests for *Mycoplasma* and *Ureaplasma* should be indicated for certain subjects such as those with recurrent cervicitis or considering testing in women with pelvic inflammatory disease [2], [6].

## 5. CONCLUSIONS

The prevalence of *Mycoplasma* and *Ureaplasma* in the genital tract is relatively low, the prevalence of *M. genitalium* was 1.6%, *M. hominis* was 5.0% and *U. urealyticum* was 13.1 %, *U. parvum* was 36.0%. Women carrying these bacteria are not associated with clinical manifestations.

Bacterial vaginosis group accounted for 4.7%, women carrying *M. genitalium*, *M. hominis*, *U. urealyticum* and *U. parvum* were not found to be associated with bacterial vaginosis as determined by the Nugent score.

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