Prevalence of *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Chlamydia trachomatis* in symptomatic and asymptomatic patients

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Abstract. The purpose of the present study was to assess the prevalence of *Ureaplasma urealyticum* (*U. urealyticum*), *Mycoplasma hominis* (*M. hominis*) and *Chlamydia trachomatis* (*C. trachomatis*) in a Romanian population considering the presence or absence of genital symptoms. Urethral and vaginal samples were collected from patients presenting at 'Ponderas' Academic Hospital (Bucharest, Romania) from January 2021 to December 2021. A total of 266 samples were obtained from two groups of patients: Symptomatic subjects with urethritis, prostatitis, vaginitis or both urethritis and prostatitis (n=59; 22%), and asymptomatic subjects (n=207; 78%). *Mycoplasma* and *Chlamydia* kits were used to assess the presence of *U. urealyticum* and *M. hominis*, and *C. trachomatis*, respectively. The symptomatic subjects comprised 27 patients with urethritis symptoms, of whom 4 (15%) were infected with *U. urealyticum* and 1 (4%) was infected with *C. trachomatis*. In addition, 23 (9%) of the patients had prostatitis-like symptoms, which in 3 (13%) of the patients was associated with *U. urealyticum* and in 1 patient (4%) was associated with *C. trachomatis*. None of the symptomatic patients were infected with *M. hominis*. By contrast, 29 (14%) of the asymptomatic patients were discovered to be infected with *U. urealyticum*, 13 (6%) were coinfected with both Mollicutes and 4 (2%) were infected with *C. trachomatis*; only 1 patient was positive for *M. hominis* alone. Two patients (14%) who presented with *U. urealyticum* and *M. hominis* coinfection were also infected with *C. trachomatis*. No patient with *U. urealyticum* or *M. hominis* alone was also positive for *C. trachomatis*. Therefore, the most frequently identified pathogen populating the genital tract in both males and females was *U. urealyticum*, followed by coinfection with *U. urealyticum* and *M. hominis*, and *C. trachomatis*. As these infections are asymptomatic in numerous cases, this suggests that a thorough screening should be mandatory.

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Introduction

Sexually transmitted diseases (STDs) occur worldwide and are an important public health problem. In developing countries, STDs rank among the five most frequent reasons for health services being sought (1). The term STDs refers to the various clinical symptoms generated by pathogenic microorganisms that are transmitted through sexual intercourse. Among the causative agents of STDs, *Chlamydia trachomatis* (*C. trachomatis*) is associated particularly with urethritis and cervicitis. *Mycoplasma hominis* (*M. hominis*) and *Ureaplasma* species are frequently found in the commensal microorganisms of the lower genital tract; however, their role in other sexually transmitted infections remains unclear. *Ureaplasma urealyticum* (*U. urealyticum*) was originally considered to have two biovars, biovar 1 and biovar 2, which were subsequently found to be separate species by polymerase chain reaction (PCR) and named *U. parvum* and *U. urealyticum*, respectively (2). *Ureaplasma* species are the most widely investigated pathogens associated with non-gonococcal urethritis, and the results are conflicting (3). *U. urealyticum* has been reported to cause infections in the lower genital tract, being a pathogen of male urethritis and a likely cause of bacterial vaginosis (2,3). *Ureaplasma* species, *M. hominis* and *C. trachomatis* can cause infertility in both men and women (4).

Mollicutes (*U. urealyticum* and *M. hominis*) and *Chlamydia*, when localized and colonized within certain anatomical sites, may cause pathological disorders, including urethritis in males and females, prostatitis and epididymitis in males, and vaginitis, endometriosis and salpingitis in females. Urethritis and vaginitis are characterized by discharge and/or dysuria, although they may also be entirely asymptomatic (5). *C. trachomatis* is the most common cause of non-gonococcal urethritis, with a prevalence in the general population of between 1 and 10% (6). The prevalence of *Ureaplasma* species and *M. hominis* has been reported to be 21 and 3%, respectively (7). These microorganisms can also cause sexually transmitted reactive arthritis (Reiter's Syndrome) (8). Regarding female patients, it is worthy of note that their role in

the etiology of pregnancy complications has been suggested, including the induction of preterm labor, infertility, spontaneous abortion, puerperal fever and pelvic inflammatory disease (5,8). Moreover, the transmission of *U. urealyticum* to the fetus or newborn may cause severe bronchopulmonary dysplasia and central nervous system (CNS) infections (5).

The objective of the present study was to estimate the prevalence of *U. urealyticum*, *M. hominis* and *C. trachomatis* in a Romanian population taking into consideration the presence or absence of genital symptoms.

Materials and methods

Patients. The present study is a retrospective, observational study, conducted from January 2021 to December 2021. The study was conducted at 'Ponderas' Academic Hospital (Bucharest, Romania) in the Dermato-venerology Department. Data on all 266 patients who provided urogenital samples for *Ureaplasma* and *Mycoplasma* detection by culture were collected and analyzed, as well as urogenital samples for *Chlamydia* detection by PCR. Specimens were obtained from two different groups of patients: Symptomatic subjects who reported urogenital symptoms and were categorized in four subgroups, namely urethritis, prostatitis, vaginitis, and urethritis with prostatitis; and completely asymptomatic subjects who came for microbiological screening for STDs, a number of whom reported sexual contact with infected individuals. All patients were sexually active. Only samples collected during the first visit were considered in the study, and specimens obtained during follow-ups of the same patient were excluded. All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

Urethral/vaginal swabs. Male patients were placed in the gynecological position and asked to retract the foreskin of the penis and keep it retracted throughout the procedure. The doctor used sterile cotton or gauze to clean the opening of the urethra at the tip of the penis. To facilitate sample collection and stimulate prostatic gland secretion, prostatic massage was performed prior to collection of the sample. Then, a first cotton swab was gently inserted ~2 cm into the urethra and rotated. To obtain a good sample, the test was performed ≥3 days from the last sexual intercourse and 2 h after urination. The swabs were placed in R1 broth from a Mycoplasma IST 2 kit (bioMérieux) to initiate the isolation of mycoplasmas. For female patients, the vaginal sample was taken by placing the patient in a gynecological position and carefully introducing a cotton swab into the vaginal canal. The use of commercial lubricants or antiseptics was avoided. The swabs were placed in R1 broth to initiate the isolation of Mollicutes. The liquid medium for *U. urealyticum* and *M. hominis* was a transport medium used for inoculation of a test strip. In order to perform the phenotypic identification of *U. urealyticum*, urea broth was used, which contained medium base (pleuropneumonia-like organism broth), yeast extract, horse serum and urea. To determine the growth of this microorganism, phenol red was added to the culture medium, as it changes from red to intense raspberry red in the presence of urease and ammonium production. The culture medium specific for *M. hominis* included arginine which, when metabolized, produces an alkaline compound that changes phenol red to a raspberry red color. The culture media were incubated at 37˚C until the phenol red indicator changed color. The Mycoplasma IST 2 kit was used according to the manufacturer's instructions as follows. As aforementioned, the sample-bearing swab was placed in the transport medium R1 broth (3 ml). The broth was mixed with the contents of the lyophilised R2 vial provided with the kit, which contained the substrates necessary for the development of microorganisms. A volume of 55 ml was added to each of the 22 domes in the test strip. Firstly, the phenotypic detection of *M. hominis* and *U. urealyticum* was performed. Secondly, the microorganisms were quantified, to determine whether the sample concentration was $>1x10^4$ change color-changing units (CCU), as this indicated an important presence of these microorganisms (positive result) (9).

A second swab was inserted in the urethra of male patients and the vaginal canal of women to collect urogenital samples for the detection of *C. trachomatis* by PCR. The samples were collected using a DNA collection device, comprising a cytobrush and DNA holder buffer (Specimen Transport Medium; Digene; Qiagen, Inc.), for the investigation of bacterial infections. Bacterial DNA samples were extracted from samples collected from the urogenital tract using an RTP®-Bacteria DNA kit (Invitek Diagnostics), according to the manufacturer's procedures and amplified using a 5TD6 ACE Detection kit (Allplex STI Essential Assay; Seegene, Inc.) for the detection of *C. trachomatis* by PCR. The PCR primer sequences are not disclosed by the manufacturer. The PCR conditions were as follows: 1 cycle of 94˚C for 15 min, 40 cycles of 94˚C for 30 sec, 63˚C for 90 sec and 72˚C for 90 sec, and 1 cycle of 72˚C for 10 min. The amplification of plasmidial DNA as an internal control occurred in the same reaction. The PCR product was subjected to electrophoresis on a gel containing 2% agarose stained with ethidium bromide. Amplification of the target was only observed when the respective bacterial DNA was present in the clinical sample (10). This method was applied to 30 samples. For the remaining 236 the testing method was changed due to financial issues at the laboratory. Images of the agarose gel are not available.

The subsequent method used for the detection of *C. trachomatis* involved the insertion of a swab in the urethra for male patients and the vaginal canal for women to collect urogenital samples for analysis by an alternative PCR method. The urethral sample was transferred into an Aptima Swab Specimen Transfer Tube (Hologic, Inc.). The samples were transported at a temperature of between 2 and 30˚C. Determination of *C. trachomatis* rRNA in the genital secretions was performed using the Panther® System analyzer (Hologic, Inc.), which is based on nucleic acid amplification testing with transcription-mediated amplification (TMA) and dual kinetic detection. TMA is an isothermal amplification method that uses RNA polymerase and reverse transcriptase. Since the amplification temperature is 37-42˚C, the technique does not require a thermocycler and can be performed using a thermoblock. TMA uses two primers that flank the region to be amplified: A promoter primer and a non-promoter primer with the same sense as the target. The 3' end of the promoter primer

is complementary to that of the target RNA and the 5' end is recognized by RNA polymerase. Amplification is initiated via the binding of the promoter primer to the target RNA, which is then reverse transcribed to generate cDNA. The DNA-RNA duplex is degraded, and the RNA released through the RNase H activity of the reverse transcriptase. The second primer binds to the cDNA and generates double-stranded molecules. Hundreds of copies of the RNA amplicons are thus transcribed by means of this DNA and each copy can be converted into new double-stranded DNA molecules. The amplification products are analyzed by hybridization with oligonucleotide probes labeled with chemiluminescent substances. Assay results were automatically interpreted by APTIMA Assay software (Panther System®; Hologic, Inc.) using the APTIMA Combo 2 protocol, and presented as individual CT test results. Based on the kinetic type and total relative light units (RLU) in the detection step, the test results were assigned as negative (RLU <25), equivocal (RLU <100), positive (RLU >100) or invalid (11).

Statistical analysis. Categorical variables are expressed as counts and percentages. Tests of association were performed using Chi-square or Fisher's exact tests, as appropriate. P<0.05 was considered to indicate a statistically significant result, at which the null hypothesis could be rejected. Data were analyzed with R Statistical Software version 4.1.1 (https://www.r-project.org).

Results

The study population consisted of 266 patients (225 males and 41 females) aged between 18 and 80 years, and 89% of the patients were aged 31-50 years. Among these 266 patients, 59 (22%) had STD symptoms while the other 207 patients (78%) had no STD symptoms at all. Regarding the symptomatic patients, 8 (14%) had *U. urealyticum*, 1 (2%) had *U. urealyticum* and *M. hominis* coinfection, 3 (5%) had *C. trachomatis*, and no symptomatic patients were infected with *M. hominis* alone (all P<0.001; Figs. 1-3; Table I).

Symptoms of urethritis were reported by 27/266 (10%) of the patients. Of those 27 patients, 4 (15%) had *U. urealyticum* and 1 (4%) had *C. trachomatis*, but none of the patients with symptoms of urethritis had *U. urealyticum* and *M. hominis* coinfection or were infected with *M. hominis* alone (all P<0.001; Figs. 4 and 5; Table II).

In the present study, 23/266 (9%) patients presented symptoms characteristic of prostatitis. These comprised 3 patients (13%) whose prostatitis was caused by *U. urealyticum* and 1 (4%) in which the prostatitis was caused by *C. trachomatis*, while prostatitis was caused neither by *U. urealyticum* and *M. hominis* coinfection, nor by *M. hominis* alone in these patients (all P<0.001; Figs. 6 and 7; Table II).

Symptoms of both urethritis and prostatitis were present in 6/266 (2%) of the patients. Only 1/6 patients with these symptoms (17%) was infected with *C. trachomatis* (P=0.1025; Table II). Neither of the Mollicutes was detected in patients with urethritis and prostatitis (P=0.003892).

The study population included 207/266 (78%) patients who were completely asymptomatic. Among these 207 patients, 29 (14%) were discovered to have *U. urealyticum*, 13 (6%) were coinfected with both Mollicutes, 1 (0%) was infected with *M. hominis* alone and 4 (2%) were infected with *C. trachomatis* (all P<0.001; Figs. 8-11; Tables I and II).

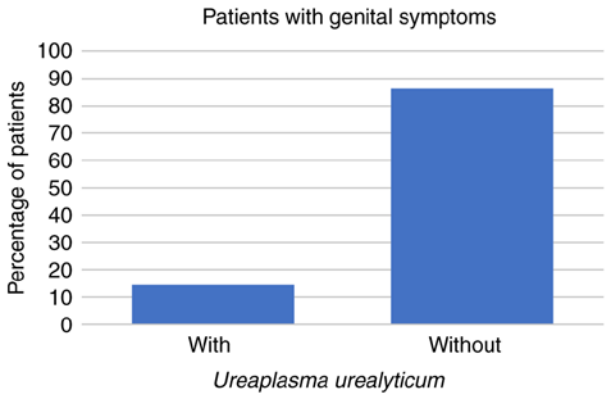

Figure 1. Genital infection with Ureaplasma urealyticum in all symptomatic patients.

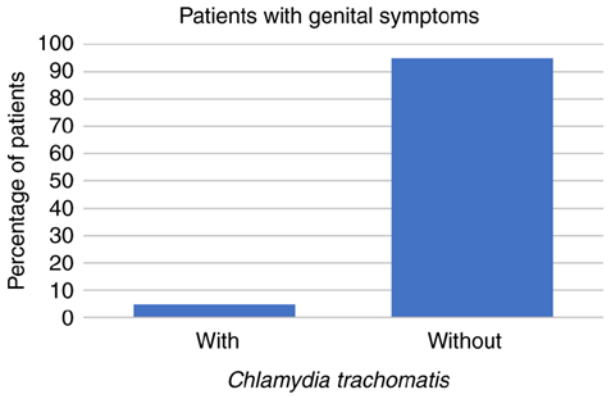

Figure 2. Genital infection with *Chlamydia trachomatis* in all symptomatic patients.

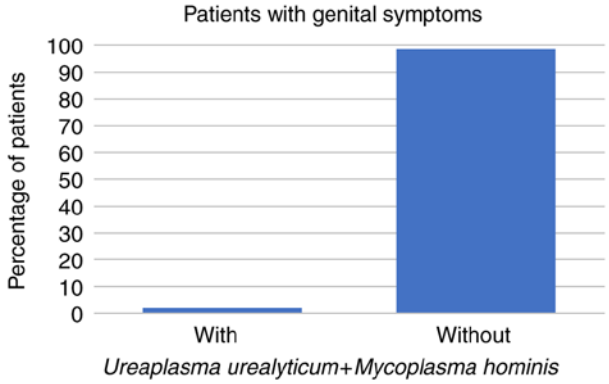

Figure 3. Genital coinfection with *Ureaplasma urealyticum* and *Mycoplasma hominis* in all symptomatic patients.

Regarding the female patients, there were only 3 cases of vaginitis, one of which was a symptomatic coinfection with Mollicutes. The remaining 38 women had asymptomatic genital infections.

The association between the Mollicutes and *C. trachomatis* was also evaluated. It was found that 2 patients (14%) with *U. urealyticum* and *M. hominis* coinfection were also

Table I. Distribution of patients according to the presence of symptoms and pathogens.

	Symptomatic, n (%)		Asymptomatic, n (%)	
Pathogens	Males	Females	Males	Females
U. urealyticum	7 (88)	1 (13)	21 (72)	8 (28)
M. hominis	0 (0)	0 (0)	1 (100)	0 (0)
Coinfection	1 (100)	0 (0)	5 (38)	8 (62)
C. trachomatis	3 (100)	0 (0)	2 (50)	2 (50)

U. urealyticum, *Ureaplasma urealyticum*; *M. hominis*, *Mycoplasma hominis*; coinfection, infection with *U. urealyticum* and *M. hominis*; *C. trachomatis*, *Chlamydia trachomatis*.

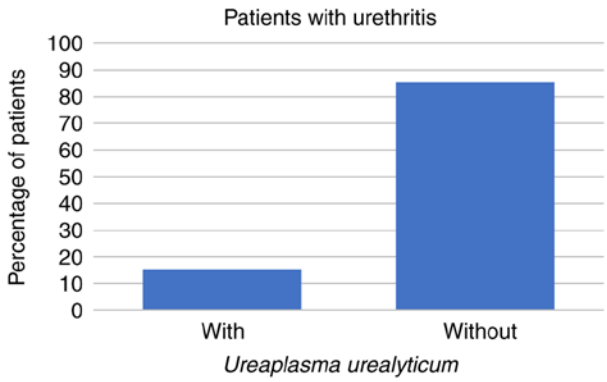

Figure 4. Genital infection with *Ureaplasma urealyticum* in patients with urethritis.

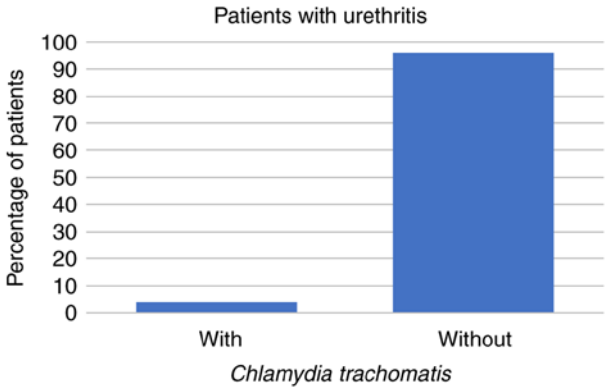

Figure 5. Genital infection with *Chlamydia trachomatis* in patients with urethritis.

infected with *C. trachomatis*, while none of the patients who were infected with *U. Urealyticum* or *M. hominis* alone were also positive for *C. trachomatis* (P=0.1071).

Discussion

The genital tract is a propitious area for the growth of numerous microorganisms, some of which may cause

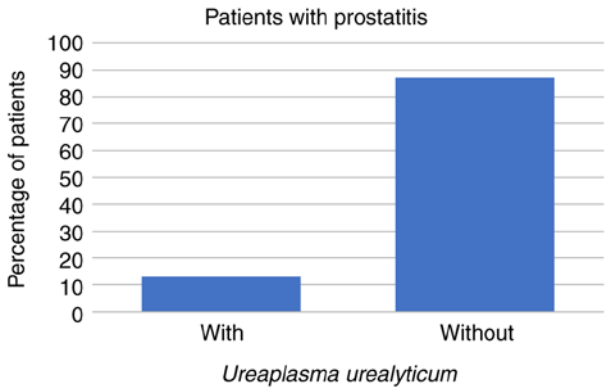

Figure 6. Genital infection with *Ureaplasma urealyticum* in patients with prostatitis.

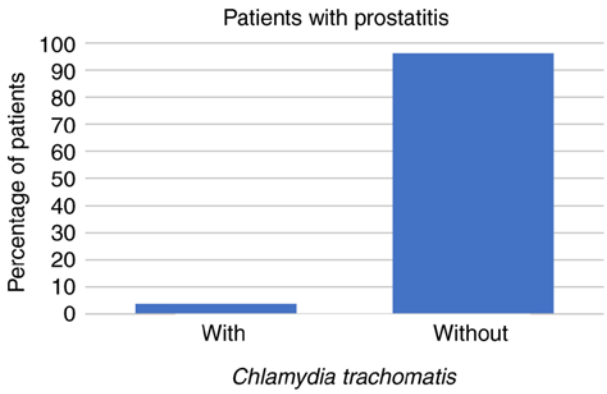

Figure 7. Genital infection with *Chlamydia trachomatis* in patients with prostatitis.

pathologies, including urethritis, endometriosis, epididymitis and salpingitis. Urethritis is characterized by discharge and/or dysuria, although it can also occur without any symptoms. Urethritis may be either gonococcal, when *Neisseria gonorrhoeae* is detected, or non-gonococcal (5). *M. hominis* and *U. urealyticum* are commonly found in the genitourinary tract as causative agents for several STDs. In men, *U. urealyticum* is a major cause of non-gonococcal urethritis, which may also be caused by *M. hominis* to a lesser extent (12). These microorganisms can cause sexually transmitted reactive arthritis (Reiter's syndrome), epididymitis and chronic prostatitis, and are suggested to play a role in pregnancy complications. In addition, *U. urealyticum* transmission to the fetus may cause bronchopulmonary dysplasia and CNS infections (5).

Although previous studies showed a greater prevalence of the Mollicutes and/or *Chlamydia* in women (13,14), mainly male patients are seen in the Dermato-venerology Department at 'Ponderas' Academic Hospital as women are usually referred to the Gynecology Department. Therefore, 225 of the 266 patients enrolled in the study were male. Similar to other studies (15,16), 207/266 (78%) were completely asymptomatic. This emphasizes the requirement for STD screening in sexually active individuals, since many modern STDs can be clinically silent, while their outcomes could be serious.

The most common symptom in all male patients was urethritis (10%), the main causes of which were *U. urealyticum*

Table II. Distribution of patients according to specific symptoms and pathogens.

	Symptoms, n (%)			
Pathogens	Urethritis	Prostatitis	Urethritis + prostatitis	Asymptomatic
U. urealyticum	4 (15)[a]	3 (13)[a]	0 (0)[a]	29 (14)[a]
M. hominis	0 (0)[a]	0 (0)[a]	0 (0)[a]	1 (0)[a]
Coinfection	0 (0)[a]	0 (0)[a]	0 (0)[a]	13 (6)[a]
C. trachomatis	1 (4)[a]	1 (4)[a]	1 (17)[b]	4 (2)[a]

[a]P<0.001 and [b]P=0.1025. *U. urealyticum*, *Ureaplasma urealyticum*; *M. hominis*, *Mycoplasma hominis*; coinfection, infection with *U. urealyticum* and *M. hominis*; *C. trachomatis*, *Chlamydia trachomatis*.

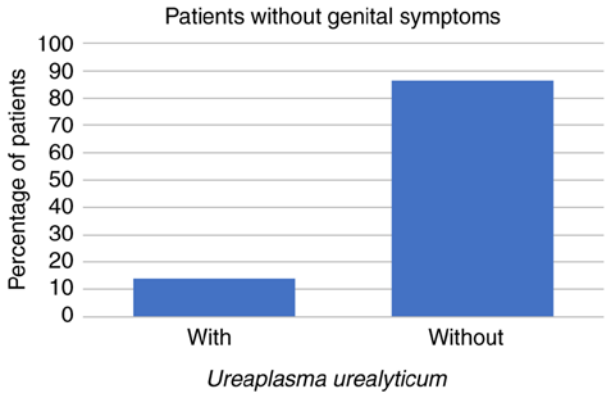

Figure 8. Genital infection with *Ureaplasma urealyticum* in asymptomatic patients.

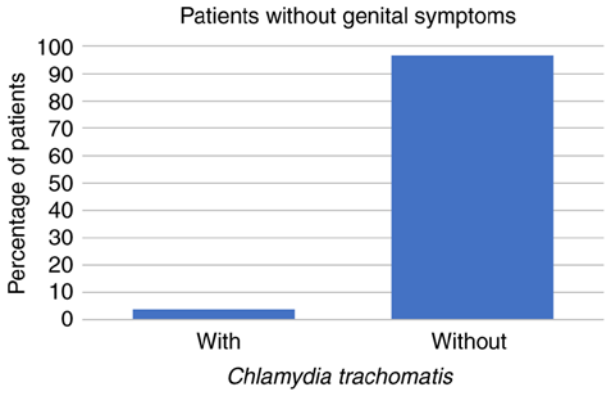

Figure 10. Genital infection with *Chlamydia trachomatis* in asymptomatic patients.

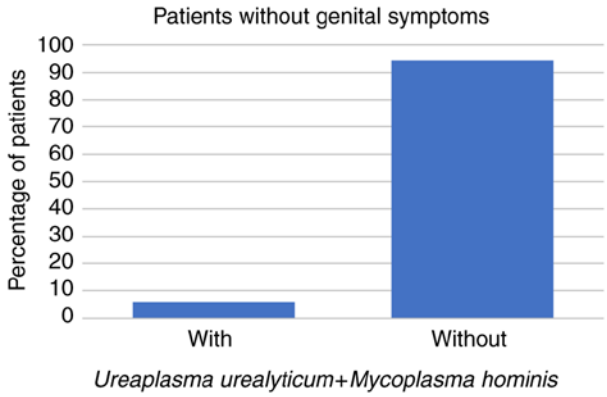

Figure 9. Genital coinfection with *Ureaplasma urealyticum* and *Mycoplasma hominis* in asymptomatic patients.

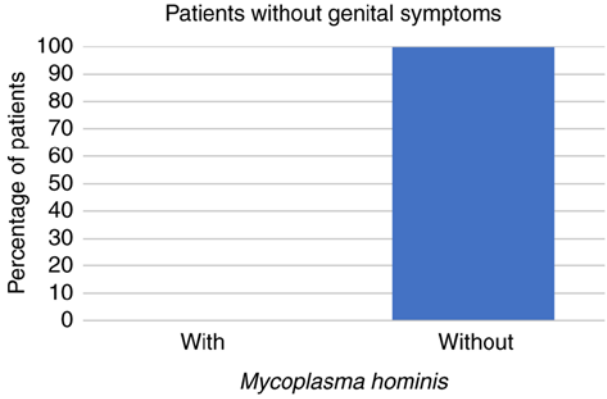

Figure 11. Genital infection with *Mycoplasma hominis* in asymptomatic patients.

(15%) and *C. trachomatis* (4%). In addition, 9% of the male patients had symptoms suggestive of prostatitis. Similar to urethritis, the causative agents of prostatitis were found to be *U. urealyticum* (13%) and *C. trachomatis* (4%). Both urethritis and prostatitis were present in 6 (2%) of the male patients, which was caused by *C. trachomatis* in 1 case. Regarding the asymptomatic patients, the main infective agent detected in the present study was *U. urealyticum* (14%), followed by coinfection (6%), *C. trachomatis* (2%), and *M. hominis* in a single patient (0%), which is similar to the pattern of infection in symptomatic patients: 14% *U. urealyticum*, 5% *C. trachomatis* and 2% coinfection. The small proportion of cases with *M. hominis*, only one asymptomatic male, may be attributed to the small number of women included, although it is consistent with literature data (17).

Recent studies have shown that the prevalence of *Ureaplasma* species and *M. hominis* is ~21 and 3%, respectively (7) and the prevalence of *C. trachomatis* is ~2.9% (18). In the present study, the percentages are consistent with the worldwide trend regarding Mollicutes, but differ slightly

regarding infection with *C. trachomatis*, with a prevalence of 2% for asymptomatic patients and 5% for individuals who presented with genital symptoms. A similar percentage to that in the present study has been identified in Latin America and regions of Africa, at 6.7 and 3.8%, respectively (18). Regional variations may be associated with social, cultural and economic conditions, differences in control policy and gender inequality, but those require examination in further studies (18). Moreover, it should be taken into account that, generally, women are more affected by these infections than men. Since the individuals enrolled in the present study were predominantly males, further studies are required in order to obtain an accurate percentage. In the future, statistics from the Dermato-venerology Department will be compared from those in the Gynecology Department to determine if there is an important difference regarding infection with Mollicutes and *C. trachomatis* between the sexes in Romania. However, many gynecologists in Romania do not include testing for Mollicutes and C. *trachomatis* in the basic screening process, unless the patients report urogenital symptoms. A study from Spain illustrated that the prevalence of *U. urealyticum* was 17.73%, and the prevalence of *M. hominis* and *C. trachomatis* was 10.64 and 26.95%, respectively, in men with and without symptoms of urethritis (19). In addition, studies of Chinese patients indicated that the overall prevalence of total *Ureaplasma* species and/or *M. hominis* was 38.1% from 2013 to 2019. *Ureaplasma* species were the most frequently isolated (overall prevalence, 31.3%), followed by *Ureaplasma* species/*M. hominis* coinfection (6.0%) and single *M. hominis* infection (0.8%) (20,21).

These marked differences between countries and regions could be due to the lack of sexual education in young individuals in developing countries, insufficient screening tests or poor technique during sample collecting. In addition, numerous physicians do not routinely perform tests for Mollicutes and/or *C. trachomatis*.

Notably, it is recommended that sampling should be performed by the physician, not a nurse, since the nurse may not have undergone adequate training in the collection of urethral swabs, resulting in false negative results. In the Dermato-venerology Department of 'Ponderas' Academic Hospital, the dermatovenerologists perform these maneuvers to enhance the validity of the tests. Moreover, urethral swabs are collected from male patients to search for *C. trachomatis*, instead of urine samples. This is because it is more convenient to collect all samples at once when performing a complete STD screen. However, this procedure is more uncomfortable for the patient.

Finally, it must be emphasised that *Ureaplasma* and *Mycoplasma* are opportunistic pathogens, frequently found in the commensal flora of the lower genital tract. The Mycoplasma kit used in the present study determined whether the sample concentration was $>1x10^4$ CCU in order to make a diagnosis of Mollicute infection. However, previously reported studies describe different techniques for Mollicute and *Chlamydia* detection. Some of these evaluated the microscopy of Gram-stained urethral smears in the diagnosis of non-gonococcal urethritis, and reported a threshold of ≥2 polymorphonuclear leukocytes/high power field as being indicative of a positive result (22-24). A comparison of the two diagnostic methods will be made in a future study.

In conclusion, the most prevalent pathogen populating the genital tract in both males and females is *U. urealyticum*, followed by *U. urealyticum* and *M. hominis* coinfection, and *C. trachomatis*. Numerous infections are asymptomatic, but should be screened for, since they can cause serious complications, most importantly infertility in men and women. Furthermore, the present study raises awareness of the importance of complete STD screening, regardless of the presence of symptoms.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

AC performed the statistical analysis, critically reviewed literature findings and revised the manuscript. DB collected the urethral and vaginal swabs from the patients and sent them to the laboratory, and conceived and designed the study. AC and DB confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of 'Ponderas' Academic Hospital (approval no. 509/16.02.2022). Written informed consent was obtained from all patients prior to publication.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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