BMC Infectious Diseases

RESEARCH

Open Access

U. Parvum serovars exhibit distinct pathogenicity in Chinese women of childbearing age: a multicentre cross-sectional study

Yingxuan Zhang[1†], Wei Qing[1†], Wenyu Mo[2†], Rongdan Chen[1], Zuyi Zhou[1], Yi Hou[1], Yiya Shi[1], Cancan Qi[1], Jinxia Ou[1], Longxu Xie[3], CALM 2004 Study Group, Yifeng Wang[2], Hongwei Zhou[1] and Muxuan Chen[1*]

Abstract

Background *Ureaplasma* spp. can be classified into different serovars. It is unknown whether distinct serovars are associated with clinical signs and symptoms.

Methods We conducted a multicentre cross-sectional study. *U. parvum* serovars were identified on the basis of their multiple-banded antigen (MBA) genes. After adjusting for demographic variables and other reproductive tract infections, the odds ratio (OR) and 95% confidence interval (CI) were calculated to determine the impact of *U. parvum* serovars on clinical symptoms.

Results Among 5,277 individuals, *U. parvum* serovars 3 and 6 were the most prevalent serovars (17.9% and 16.0%, respectively). Potential confounders, such as age, body mass index (BMI), ethnicity, education level, contraceptive methods, number of sexual partners, gravidity, parity, and other sexually transmitted infections (STIs) that are associated with clinical symptoms ($P < 0.1$) were adjusted for in the univariate analysis. *U. parvum* serovar 14 was strongly positively associated with certain clinical symptoms, including redness and swelling of the vaginal wall (crude OR: 3.53, 95% CI: 1.92–6.49; adjusted OR: 5.21, 95% CI: 2.56–10.58), cervical bleeding and swelling (crude OR: 3.89, 95% CI: 2.38–6.36; adjusted OR: 7.37, 95% CI: 3.82–14.23), and cervical ectropion (crude OR: 2.08, 95% CI: 1.25–3.45; adjusted OR: 3.04, 95% CI: 1.60–5.74). In contrast, *U. parvum* serovar 3 was negatively associated with a variety of clinical symptoms, whereas no correlations were detected between *U. parvum* serovars 1and 6 with clinical symptoms.

Conclusions Different *U. parvum* serovars exhibit distinct correlations with clinical symptoms, suggesting that *U. parvum* serovars are pathogenically heterogeneous and that further differentiation of serovars may be necessary.

Trial registration The study was registered with *ClinicalTrials.gov* (https://www.clinicaltrials.gov; ID: NCT04694495; Registration Date: 2021–01-05).

Keywords *U. parvum* serovars, Women of childbearing age, Clinical symptoms, Heterogeneity in pathogenicity

†Yingxuan Zhang, Wei Qing and Wenyu Mo contributed equally to this work.

*Correspondence:
Muxuan Chen
muxuanchen@126.com
Full list of author information is available at the end of the article

Background

Ureaplasma spp. is one of the most prevalent Mycoplasma species associated with urogenital tract infection in humans, and it can be classified into 2 biovars, *Ureaplasma parvum* (*U. parvum*) and *Ureaplasma urealyticum* (*U. urealyticum*) [1–3]. On the basis of multiple-banded antigen (MBA) genes, 4 serovars (serovars 1, 3, 6, and 14) belong to the *U. parvum* biovar, and the remaining serovars (serovars 2, 4, 5, 7–13) belong to the *U. urealyticum* biovar [4, 5]. *U. urealyticum* and *U. parvum* are prevalent in the reproductive tract of nonpregnant women of childbearing age, with estimated prevalence rates ranging from 7.6% to 28.5% for *U. urealyticum* and 38.3% to 60.6% for *U. parvum* [6–9]. *U. urealyticum* has been associated with a variety of medical problems, including spontaneous abortion and premature birth [10, 11]. In contrast, *U. parvum* is considered potentially harmless because of its high prevalence and weak correlation with disease [12]. Nevertheless, few studies have examined the possibility that *U. parvum* pathogenicity varies by serovar, which frequently leads to confusion among clinicians regarding the necessity of treating *U. parvum*-positive patients [11, 13]. Since distinct serovars may have varying degrees of pathogenicity, testing *U. parvum* as a whole rather than individual serovars may introduce confounding factors into the associations between *U. parvum.* and clinical complications.

The clinical presentation of symptoms and signs is the most straightforward indicator of pathological alterations. One study indicated that *U. parvum* was not associated with symptoms/signs in women [12]. Only a limited number of studies have identified diverse connections between different *U. parvum* serovars and symptoms [14, 15]. However, these studies focused only on *U. parvum* serovars, and no other sexually transmitted infections (STIs) were considered confounding variables. Furthermore, the sample size was restricted to only one centre. In addition, there is no clinical differentiation among serovars with respect to the treatment of *U. parvum* and *U. urealyticum* [16], but differences in drug susceptibility between *U. parvum* serovars have been demonstrated [17, 18]. Identifying variations in the pathogenicity of *U. parvum* serovars simultaneously assists clinicians in selecting the most appropriate antibiotic regimens.

Thus, we conducted a study to determine whether specific *U. parvum* serovars are related to specific symptoms and/or clinical indications in nonpregnant women, to explore the heterogeneity in the pathogenicity of *U. parvum* serovars.

Methods

Study design and population

For this cross-sectional study, we used baseline data (2020.11–2022.10) from the Chinese Association for cLinical Microbiome 2004 (CALM 2004) project. The CALM 2004 project is an ongoing prospective multicentre cohort study in China that aims to determine the relationship between the female genital tract microbiota and human papillomavirus (HPV) infection clearance, persistence, and progression to cervical intraepithelial neoplasia (CIN) or cervical cancer. All the participants who were routinely screened for cervical cancer were recruited from the gynaecological outpatient department of a class A tertiary hospital. The design was described previously [19]. The detailed inclusion, exclusion and elimination criteria of the study are outlined in Table S2. This analysis, which was based on baseline data from the CALM 2004 project, included participants who provided basic information, maintained exhaustive records of clinical signs and symptoms, and underwent thorough comprehensive testing for pathogens causing STIs. The final analysis included 38 centres and 5,277 participants. The sample sizes of each centre are presented in Table S3.

U. parvum serovar detection

Exfoliated cervical cells were collected from each individual by a clinician for *U. parvum* serovar testing, which was measured with an STIs detection kit (Hybribio, Guangdong, China) [20, 21]. In accordance with the manufacturer's instructions, 1 mL of cell preservation solution containing cervical cells was centrifuged at 7,000 ×*g* for 5 min. Then, the supernatant was discarded, 0.5 mL of cell preservation mixture was added, and the cells were resuspended and centrifuged again at 7,000 ×*g* for 1 min. The supernatant was discarded, 50 µl of cell lysate was added, the mixture was boiled for 10 min, and centrifuged at 7,000 ×*g* for 10 min, the supernatant was kept for DNA amplification. The serovars of *U. parvum* were identified (Hybribio, Guangdong, China) via PCR and flow-through hybridization using specific probes. *U. parvum* was divided into *U. parvum* serovars 1, 3, 6 and 14 according to the following probe sequences.

U. parvum serovar 1: 5'-TTACACATATTAAATAAA GACAATAAA-3'.

U. parvum serovar 3: 5'-TATGTAAGATTACCAAAT CTTAGTGTT-3'.

U. parvum serovar 6: 5'-ATTTTTTACTAGTATTAA ATTAAAAACAAT-3'.

U. parvum serovar 14: 5'-TATTAATCTTACATAATT TCTAC-3'.

Outcomes

The eligibility of individuals for the study was determined and evaluated by a gynaecologist and research nurse. All participants completed a questionnaire on genital symptoms, including vaginal itching, lower abdominal pain, pain or bleeding during sexual intercourse and irregular vaginal bleeding. The clinical signs of purulent cervical discharge, redness and swelling of the vaginal wall, vaginal odour, cervical bleeding and swelling, and cervical ectropion were also recorded by a clinician during a genital examination.

Covariate collection

We selected covariates associated with clinical symptoms and *U. parvum* infections, including those identified by previous studies such as socioeconomic information (age, ethnicity, education level, contraceptive methods, number of sexual partners, gravidity, and parity), pathogens causing genital tract infections, including *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *U. urealyticum*, *Mycoplasma hominis* (*M. hominis*), *Mycoplasma genitalium* (*M. genitalium*), Herpes simplex virus type 2 (HSV-2), *Chlamydia trachomatis* (*C. trachomatis*), HPV, *Trichomonas vaginalis* (*T. vaginalis*), *Candida*, and the Nugent and Donders scores [22, 23]. Socioeconomic information was compiled from a self-report questionnaire. Table S4 shows a detailed summary of the questionnaires. Ethnicity was a binary variable comprising Han or ethnic minorities, with ethnic minorities defined as ethnic groups other than the Han ethnic group. Education level was defined as below an undergraduate or an undergraduate or above. Contraceptive methods were divided into 4 categories: noncontraception, intrauterine device, contraceptives, and condom. Individuals using multiple contraceptive methods were excluded from the analysis. There were 2 categories for the number of sexual partners: 1 or ≥ 2 partners. We categorized gravidity and parity status into 4 groups accordingly: 0, 1, 2, and ≥ 3 pregnancies/births.

The methods used for the detection of *N. gonorrhoeae*, *U. urealyticum, M. hominis*, *M. genitalium*, HSV-2, and *C. trachomatis* were the same as those used for the detection of *U. parvum* serovars. Genotypes of HPV were determined according to the manufacturer's guidelines via an HPV GenoArray diagnostic kit (Hybribio, Guangdong, China). The presence or absence of *T. vaginalis* was determined via wet mount microscopy and *Candida* positivity was determined via both wet mount microscopy and culture methods for vaginal secretions [23]. The Nugent score was determined via Gram staining of the vaginal smear whereas the Donders score was determined via wet film microscopy of the vaginal secretions. The Nugent score was divided into 3 categories: bacterial vaginosis (BV), defined as a score ≥ 7; intermediate, defined as a score of 4–6; and normal, defined as a score of 3 [24]. Aerobic vaginitis (AV) was defined as a Donders score ≥ 3; normal was defined as a Donders score < 3 [25].

Data analysis

Continuous variables are reported as the means with standard deviations, and categorical variables are reported as frequencies and percentages. Univariate analyses were used to assess risk factors associated with clinical symptoms. The exposure factor was the *U. parvum* serovar, whereas the outcome variables consisted of numerous signs and symptoms of vaginal and cervical, for which data were collected via questionnaires and clinician examinations. The exposure and outcome variables were both classified as categorical variables. The correlation between *U. parvum* serovar and clinical symptoms were assessed using logistics regression. We constructed 3 models: 1) a crude model, which was unadjusted; 2) a minimally adjusted model, which was adjusted for basic demographic variables, including age, BMI and ethnicity; and 3) a fully adjusted model, which included the variables adjusted for in the minimally adjusted model and was additionally adjusted for potential confounders, such as education level, contraceptive methods, number of sexual partners, gravidity, parity and STIs with $P < 0.1$ in univariate analyses. If *U. parvum* serovars were the independent variables, other serovars were also adjusted for. Additional stratified analyses were conducted to evaluate the connection between *U. parvum* serovar 14 and cervical-related symptoms in different HPV infection statuses. Additionally, the *P*-value for the interaction between *U. parvum* serovar 14 and HPV was determined. Odds ratios (ORs) with 95% confidence intervals (CI) were calculated.

R 4.2.0 (http://www.R-project.org) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA) were used for the statistical analysis. Statistical significance was set at a *P* value < 0.05.

Results

Description of the study population

A total of 5,277 participants whose *U. parvum* serovars test results and self-reported clinical symptoms were available were included in this cross-sectional study (Fig. 1). *U. parvum* serovar 3 was the most prevalent serovar (17.9%, $n = 944$), followed by *U. parvum* serovar 6 (16.0%, $n = 845$), and *U. parvum* serovar 14 was the least prevalent (1.6%, $n = 87$). *U. parvum* serovar 1 was detected in 423 participants (8.0%) (Table 1).

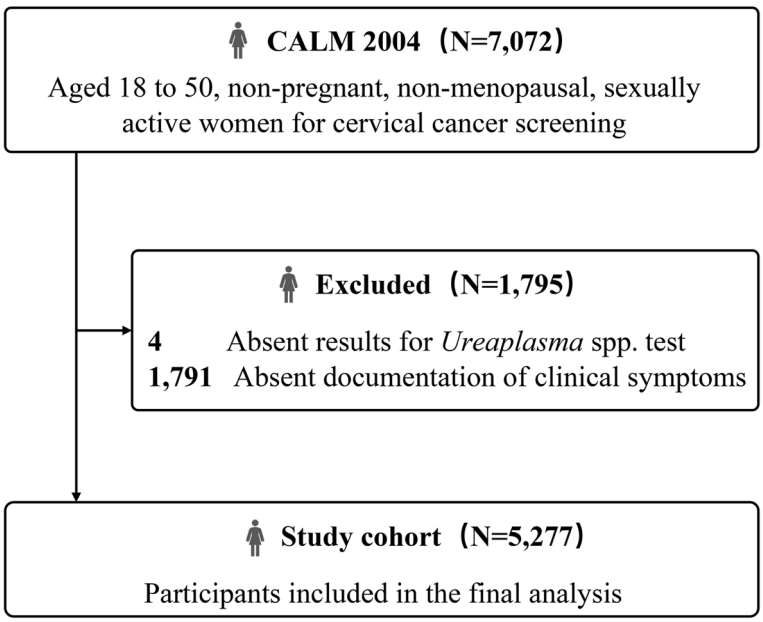

Fig. 1 Flow chart of the study

Baseline characteristics of the study population

There were no significant differences in age or BMI between individuals infected with *U. parvum* serovars and those who were not infected (Table 1). The participants infected with *U. parvum* serovar 14 were less educated than those who were not infected (Table 1). Compared with those who did not experience pregnancy and childbirth, women who had experienced pregnancy and childbirth were significantly less likely to have *U. parvum* serovar 3 infection (Table 1). In addition, various contraceptive techniques were associated with different *U. parvum* serovars, and infected women were also less likely to use condoms than uninfected women (Table 1). Additionally, comparisons of baseline characteristics were conducted between symptomatic and asymptomatic females (Table S5). Infections with *U. urealyticum*, *M. hominis*, *C. trachomatis* and HPV were more prevalent among symptomatic women, who also presented greater Nugent and Donder scores (Table S5).

Coinfection with *U. parvum* serovars and other STIs pathogens

Individuals with other genital infections, such as *M. hominis*, HSV-2, HPV and *C. trachomatis*, were more likely to be infected with specific *U. parvum* serovars simultaneously, whereas individuals with *T. vaginalis* and *U. urealyticum* infections had a lower incidence of *U. parvum* serovar 3 infection than those uninfected individuals did (Table 2). Individuals with *U. parvum* serovar 6 infection had higher Nugent scores but did not differ from negative *U. parvum* serovar 6-individuals in terms of *Candida* infection (Table 2). Additionally, we evaluated coinfection among different *U. parvum* serovars. Individuals infected with *U. parvum* serovars 1, 3 and 6 were more susceptible to infection with a single serovar than to coinfection with multiple serovars, but individuals infected with *U. parvum* serovar 14 had a greater probability of coinfection with *U. parvum* serovars 1, 3, and 6 than noninfected individuals did (Table S6).

Self-reported clinical symptoms associated with *U. parvum* serovars

Potential risk variables associated with clinical symptoms were determined via univariate analysis (Table S7). To identify the independent effects of different *U. parvum* serovars on clinical symptoms, we constructed a crude model, a minimally adjusted model (Table S8) and a fully adjusted model (Fig. 2). According to the fully adjusted model, *U. parvum* serovar 14 was strongly positively associated with redness and swelling of the vaginal wall (OR: 5.21, 95% CI: 2.56–10.58), cervical bleeding and swelling (OR: 7.37, 95% CI: 3.82–14.23) and cervical ectropion (OR: 3.04, 95% CI: 1.60–5.74). In contrast, the relationship between *U. parvum* serovar 3 and clinical symptoms produced opposite effects. Compared with uninfected women, *U. parvum* serovar 3-infected women were less likely to experience pain or bleeding during sexual intercourse (OR: 0.66, 95% CI: 0.47–0.92), irregular vaginal bleeding (OR: 0.66, 95% CI: 0.46–0.96), and cervical bleeding and swelling (OR: 0.64, 95% CI: 0.47–0.88).

Table 1 Baseline characteristics of study participants

Characteristic	*U. parvum* serovar 1			*U. parvum* serovar 3			*U. parvum* serovar 6			*U. parvum* serovar 14		
	Negative (*n* = 4854)	Positive (*n* = 423)	*P*	Negative (*n* = 4333)	Positive (*n* = 944)	*P*	Negative (*n* = 4432)	Positive (*n* = 845)	*P*	Negative (*n* = 5190)	Positive (*n* = 87)	*P*
Age, year	35.96 ± 7.32	36.67 ± 7.40	0.057	36.00 ± 7.31	36.13 ± 7.40	0.618	35.95 ± 7.33	36.39 ± 7.27	0.106	36.03 ± 7.33	35.26 ± 6.81	0.332
BMI, kg/m^2	22.16 ± 3.14	22.33 ± 3.15	0.293	22.17 ± 3.13	22.19 ± 3.19	0.861	22.19 ± 3.13	22.08 ± 3.18	0.401	22.16 ± 3.14	22.83 ± 3.27	0.087
Ethnicity			0.220			0.899			0.075			0.248
Han	4110 (94.24%)	356 (92.71%)		3679 (94.14%)	787 (94.03%)		3765 (94.38%)	701 (92.72%)		4407 (94.17%)	59 (90.77%)	
Ethnic minorities	251 (5.76%)	28 (7.29%)		229 (5.86%)	50 (5.97%)		224 (5.62%)	55 (7.28%)		273 (5.83%)	6 (9.23%)	
Education level			0.062			0.347			0.072			**0.033**
Below undergraduate	2392 (64.70%)	229 (69.82%)		2159 (64.80%)	462 (66.67%)		2185 (64.53%)	436 (68.23%)		2577 (64.93%)	44 (78.57%)	
Undergraduate and above	1305 (35.30%)	99 (30.18%)		1173 (35.20%)	231 (33.33%)		1201 (35.47%)	203 (31.77%)		1392 (35.07%)	12 (21.43%)	
Contraceptive methods			**0.002**			0.192			**0.009**			0.236
Noncontraception	692 (18.21%)	74 (22.63%)		618 (18.20%)	148 (20.19%)		624 (17.86%)	142 (22.40%)		758 (18.64%)	8 (12.90%)	
Intrauterine device	444 (11.68%)	48 (14.68%)		402 (11.84%)	90 (12.28%)		407 (11.65%)	85 (13.41%)		480 (11.81%)	12 (19.35%)	
Contraceptives	92 (2.42%)	15 (4.59%)		82 (2.42%)	25 (3.41%)		88 (2.52%)	19 (3.00%)		106 (2.61%)	1 (1.61%)	
Condom	2573 (67.69%)	190 (58.10%)		2293 (67.54%)	470 (64.12%)		2375 (67.97%)	388 (61.20%)		2722 (66.95%)	41 (66.13%)	
Number of sexual partners			0.590			0.113			0.865			0.125
1	4343 (96.60%)	367 (96.07%)		3895 (96.75%)	815 (95.66%)		3958 (96.54%)	752 (96.66%)		4645 (96.51%)	65 (100.00%)	
≥ 2	153 (3.40%)	15 (3.93%)		131 (3.25%)	37 (4.34%)		142 (3.46%)	26 (3.34%)		168 (3.49%)	0 (0.00%)	
Gravidity			0.635			**0.033**			0.275			0.504
0	609 (13.63%)	49 (12.86%)		518 (12.96%)	140 (16.43%)		554 (13.62%)	104 (13.32%)		652 (13.64%)	6 (8.82%)	
1	1015 (22.72%)	85 (22.31%)		920 (23.02%)	180 (21.13%)		929 (22.84%)	171 (21.90%)		1085 (22.70%)	15 (22.06%)	
2	1158 (25.92%)	91 (23.88%)		1045 (26.15%)	204 (23.94%)		1063 (26.14%)	186 (23.82%)		1227 (25.67%)	22 (32.35%)	
≥ 3	1685 (37.72%)	156 (40.94%)		1513 (37.86%)	328 (38.50%)		1521 (37.40%)	320 (40.97%)		1816 (37.99%)	25 (36.76%)	
Parity			0.621			**0.015**			0.230			0.130
0	784 (17.76%)	69 (18.16%)		674 (17.06%)	179 (21.18%)		701 (17.41%)	152 (19.79%)		847 (17.90%)	6 (9.38%)	
1	1974 (44.71%)	157 (41.32%)		1759 (44.53%)	372 (44.02%)		1784 (44.30%)	347 (45.18%)		2096 (44.30%)	35 (54.69%)	
2	1441 (32.64%)	134 (35.26%)		1313 (33.24%)	262 (31.01%)		1344 (33.37%)	231 (30.08%)		1553 (32.83%)	22 (34.38%)	
≥ 3	216 (4.89%)	20 (5.26%)		204 (5.16%)	32 (3.79%)		198 (4.92%)	38 (4.95%)		235 (4.97%)	1 (1.56%)	

Abbreviations: U. parvum Ureaplasma parvum, BMI body mass index

Means ± SDs for continuous variables, n (%) for categorical variables

Statistics with a *P*-value of 0.05 or less are shown in bold text

Table 2 Baseline microbiological factors of the study participants

Characteristic	*U. parvum* serovar 1			*U. parvum* serovar 3			*U. parvum* serovar 6			*U. parvum* serovar 14		
	Negative (*n* = 4854	Positive (*n* = 423)	*P*	Negative (*n* = 4333)	Positive (*n* = 944)	*P*	Negative (*n* = 4432)	Positive (*n* = 845)	*P*	Negative (*n* = 5190)	Positive (*n* = 87)	*P*
N. gonorrhoeae			0.746			0.543			0.397			**0.001**
Negative	4838 (99.67%)	422 (99.76%)		4320 (99.70%)	940 (99.58%)		4419 (99.71%)	841 (99.53%)		5175 (99.71%)	85 (97.70%)	
Positive	16 (0.33%)	1 (0.24%)		13 (0.30%)	4 (0.42%)		13 (0.29%)	4 (0.47%)		15 (0.29%)	2 (2.30%)	
U. urealyticum			0.057			**< 0.001**			**0.005**			0.162
Negative	4345 (89.51%)	391 (92.43%)		3860 (89.08%)	876 (92.80%)		3955 (89.24%)	781 (92.43%)		4654 (89.67%)	82 (94.25%)	
Positive	509 (10.49%)	32 (7.57%)		473 (10.92%)	68 (7.20%)		477 (10.76%)	64 (7.57%)		536 (10.33%)	5 (5.75%)	
M. hominis			**< 0.001**			0.092			**0.021**			0.237
Negative	4480 (92.30%)	371 (87.71%)		3996 (92.22%)	855 (90.57%)		4091 (92.31%)	760 (89.94%)		4774 (91.98%)	77 (88.51%)	
Positive	374 (7.70%)	52 (12.29%)		337 (7.78%)	89 (9.43%)		341 (7.69%)	85 (10.06%)		416 (8.02%)	10 (11.49%)	
M. genitalium			0.295			0.804			0.397			0.303
Negative	4801 (98.91%)	416 (98.35%)		4283 (98.85%)	934 (98.94%)		4384 (98.92%)	833 (98.58%)		5132 (98.88%)	85 (97.70%)	
Positive	53 (1.09%)	7 (1.65%)		50 (1.15%)	10 (1.06%)		48 (1.08%)	12 (1.42%)		58 (1.12%)	2 (2.30%)	
HSV-2			0.565			**0.010**			0.656			0.086
Negative	4790 (98.68%)	416 (98.35%)		4283 (98.85%)	923 (97.78%)		4371 (98.62%)	835 (98.82%)		5122 (98.69%)	84 (96.55%)	
Positive	64 (1.32%)	7 (1.65%)		50 (1.15%)	21 (2.22%)		61 (1.38%)	10 (1.18%)		68 (1.31%)	3 (3.45%)	
C. trachomatis			0.807			**0.004**			0.863			0.636
Negative	4635 (95.49%)	405 (95.74%)		4155 (95.89%)	885 (93.75%)		4232 (95.49%)	808 (95.62%)		4956 (95.49%)	84 (96.55%)	
Positive	219 (4.51%)	18 (4.26%)		178 (4.11%)	59 (6.25%)		200 (4.51%)	37 (4.38%)		234 (4.51%)	3 (3.45%)	
HPV			0.091			**0.007**			**< 0.001**			0.912
Negative	3896 (80.26%)	325 (76.83%)		3496 (80.68%)	725 (76.80%)		3644 (82.22%)	577 (68.28%)		4151 (79.98%)	70 (80.46%)	
Positive	958 (19.74%)	98 (23.17%)		837 (19.32%)	219 (23.20%)		788 (17.78%)	268 (31.72%)		1039 (20.02%)	17 (19.54%)	
T. vaginalis			0.401			**0.001**			0.213			0.347
Negative	4693 (98.80%)	405 (99.26%)		4187 (98.61%)	911 (99.89%)		4287 (98.76%)	811 (99.27%)		5024 (98.82%)	74 (100.00%)	
Positive	57 (1.20%)	3 (0.74%)		59 (1.39%)	1 (0.11%)		54 (1.24%)	6 (0.73%)		60 (1.18%)	0 (0.00%)	
Nugent score			0.988			0.408			**0.007**			0.779
≤ 3	3651 (76.72%)	313 (76.90%)		3265 (76.75%)	699 (76.64%)		3338 (76.75%)	626 (76.62%)		3907 (76.73%)	57 (77.03%)	
4–6	830 (17.44%)	71 (17.44%)		749 (17.61%)	152 (16.67%)		775 (17.82%)	126 (15.42%)		887 (17.42%)	14 (18.92%)	
≥ 7	278 (5.84%)	23 (5.65%)		240 (5.64%)	61 (6.69%)		236 (5.43%)	65 (7.96%)		298 (5.85%)	3 (4.05%)	
Donders score			0.227			0.846			0.145			0.730
< 3	3916 (84.67%)	346 (86.93%)		3516 (84.80%)	746 (85.06%)		3606 (85.17%)	656 (83.14%)		4205 (84.83%)	57 (86.36%)	
≥ 3	709 (15.33%)	52 (13.07%)		630 (15.20%)	131 (14.94%)		628 (14.83%)	133 (16.86%)		752 (15.17%)	9 (13.64%)	
Candida[a]			0.964			0.810			0.240			0.285
Negative	4173 (90.38%)	360 (90.45%)		3742 (90.34%)	791 (90.61%)		3810 (90.18%)	723 (91.52%)		4475 (90.44%)	58 (86.57%)	

Table 2 (continued)

Characteristic	*U. parvum* serovar 1			*U. parvum* serovar 3			*U. parvum* serovar 6			*U. parvum* serovar 14		
	Negative (*n* = 4854	**Positive (*n* = 423)**	***P***	**Negative (*n* = 4333)**	**Positive (*n* = 944)**	***P***	**Negative (*n* = 4432)**	**Positive (*n* = 845)**	***P***	**Negative (*n* = 5190)**	**Positive (*n* = 87)**	***P***
Positive	444 (9.62%)	38 (9.55%)		400 (9.66%)	82 (9.39%)		415 (9.82%)	67 (8.48%)		473 (9.56%)	9 (13.43%)	

Abbreviations: U. urealyticum Ureaplasma urealyticum, *U. parvum Ureaplasma parvum*, *N. gonorrhoeae Neisseria gonorrhoeae*, *M. hominis Mycoplasma hominis*, *M. genitalium Mycoplasma genitalium*, HSV-2 herpes simplex virus type 2, *C. trachomatis Chlamydia trachomatis*, HPV human papilloma virus, *T. vaginalis Trichomonas vaginalis*

N (%) for categorical variables

Statistics with a *P*-value of 0.05 or less were shown in bold text

[a] *Candida* positivity was based on the presence of visible pseudohyphae and/or budding yeasts via microscopy and culture positive

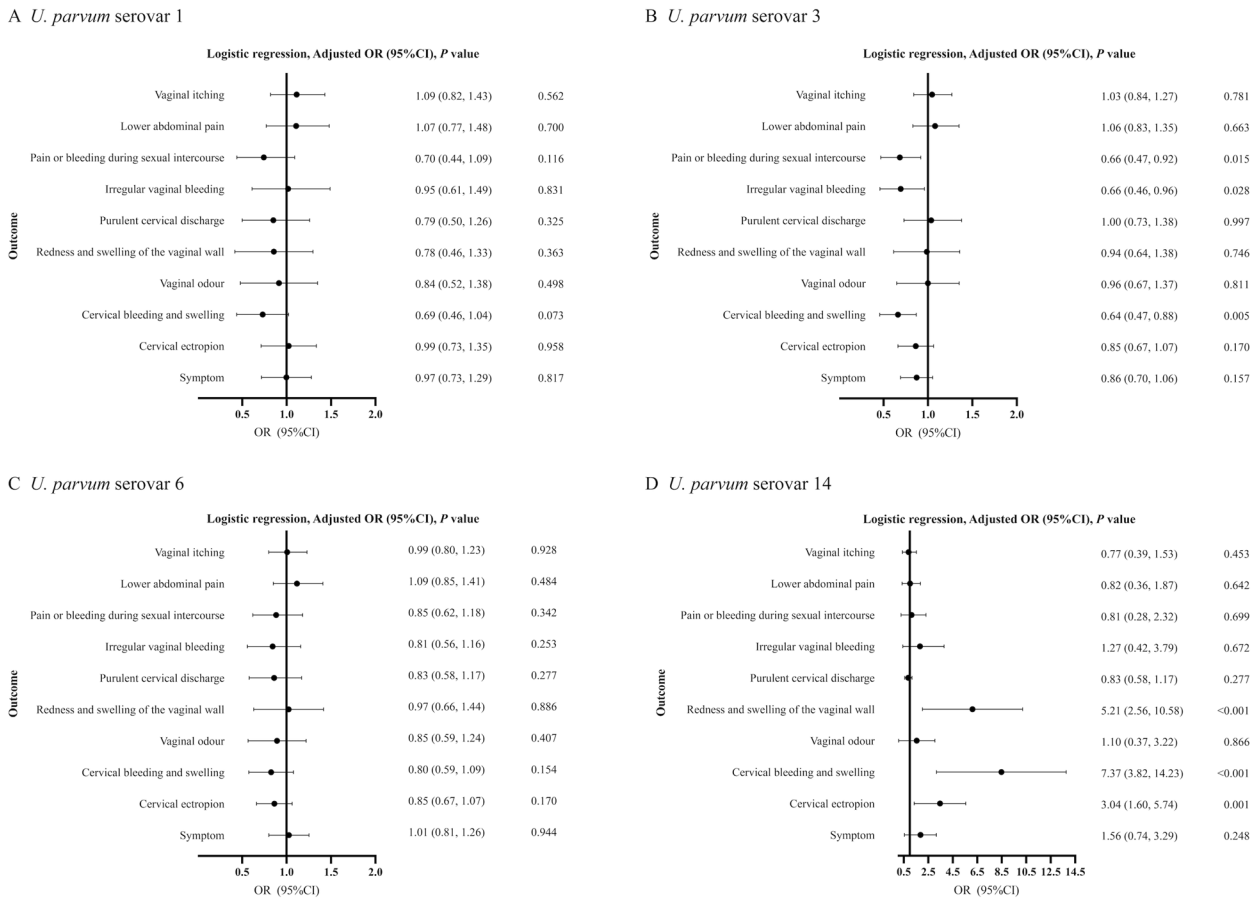

Fig. 2 Association of clinical symptoms with *U. parvum* serovars. Age, BMI, ethnicity, education level, contraceptive methods, number of sexual partners, gravidity, parity, and other STIs that were associated with clinical symptoms were adjusted for ($P < 0.1$ in univariate analysis)

Additionally, we observed that *U. parvum* serovar 1 and 6 infection was not associated with any clinical symptoms.

Stratified analysis

U. parvum serovar 14 is strongly correlated with symptoms associated with cervicitis, and HPV serves as a significant etiological factor for cervical cancer, which can manifest as cervical symptoms. We analysed the associations between *U. parvum* serovar 14 and cervicitis symptoms in individuals with or without HPV infection to further examine the correlations among *U. parvum* serovar 14, HPV, and symptoms of cervicitis. Among HPV-infected individuals, *U. parvum* serovar 14-infected women were more prone to cervical ectropion (OR: 2.59, 95% CI: 1.48–4.54), than HPV- uninfected women. *U. parvum* serovar 14-infected patients presented a significant positive association with cervical bleeding and swelling, regardless of their HPV status (OR: 3.95, 95% CI: 2.29–6.82 for HPV negative participants; OR: 3.74, 95% CI: 1.18–11.88 for HPV positive participants) (Table 3).

Discussion

In this study, we investigated the associations of *U. parvum* serovars with clinical symptoms in 5,277 Chinese women of reproductive age. Our results revealed that *U. parvum* serovars 3 and 6 were the most prevalent *U. parvum* serovars in Chinese women (17.9% and 16.0%, respectively). Even after adjusting for pathogens causing genital tract infection, such as BV, *U. parvum* serovar 14 was significantly positively related to clinical symptoms associated with cervicitis, including cervical bleeding and swelling and cervical ectropion independent of HPV infection. Notably, the findings indicated that *U. parvum* serovar 3 was significantly negatively associated with specific clinical symptoms, whereas no correlations were detected between *U. parvum* serovars 1, 6 and clinical symptoms. These findings suggest the relationships between different *U. parvum* serovars and clinical symptoms are heterogeneous.

Owing to the high detection rate of *U. parvum* and its presence in women with and without signs of genitourinary tract infections, it has been hypothesized that

Table 3 Effects of *U. parvum* serovar 14 on cervicitis-related symptoms in participants with or without HPV infection

Stratified		Cervical bleeding and swelling		Cervical ectropion	
		OR (95% CI)	Test for interaction[a]	OR (95% CI)	Test for interaction
HPV	Negative	3.95 (2.29, 6.82)**	0.935	2.59 (1.48, 4.54)**	0.085
	Positive	3.74 (1.18, 11.88)*		0.81 (0.22, 2.94)	

Abbreviations: U. parvum Ureaplasma parvum, HPV human papilloma virus

The data are presented as ORs (95% CIs), *$P < 0.05$, **$P < 0.01$

[a] Test for interaction between *U. parvum* serovar 14 (negative or positive) and HPV (negative or positive) on cervicitis-related symptoms

U. parvum is a natural part of the female genital tract flora [26–28]. In 2018, the Editorial Board of the European STIs issued a statement suggesting that there was no evidence of benefit in relation to routine testing and the treatment of *M. hominis*, *U. parvum* and *U. urealyticum* in adults based on existing available research [29]. The same conclusion was drawn in a cross-sectional investigation [12]. Notably, the recent relevant investigations did not adjust for genital pathogens and serovars of *U. parvum* were not considered. Recent research on the correlations between *U. parvum* serovars and clinical symptoms is conflicting and limited. Another study that compared *U. parvum* serovar infections in women with and without symptoms of genital tract infection reported that *U. parvum* serovar 3 or *U. parvum* serovar 14 infection was related to clinical symptoms, but that *U. parvum* serovar 6 infection was associated with asymptomatic women [15]. However, the sample size was limited in this study, and the *U. parvum* serovar 3/14 variable was used in the analysis instead of the individual serovar. Moreover, other genital tract pathogens were not adjusted for in this study. We found that the association between *U. parvum* serovar 3 and clinical symptoms was opposite that between *U. parvum* serovar 14 and clinical symptoms, suggesting heterogeneity in the pathogenicity of various *U. parvum* serovars.

Tetracyclines (particularly doxycycline) continue to be the first-line treatment for mycoplasma infections [16, 30]. As a consequence of the abuse of antibiotics, a separate study revealed that multidrug resistance among *Ureaplasma* spp. isolates has shown an increasing trend [31]. Compared with *U. urealyticum*, *U. parvum* has substantially higher drug resistance rates to ciprofloxacin and roxithromycin [17, 32]. In addition, different *U. parvum* serovars exhibit varying antibiotic sensitivities [18, 33]. According to our study, infection with various *U. parvum* serovars may result in various clinical signs. Therefore, it may be important to differentiate the serovars of *U. parvum*.

Notably, our study revealed a strong positive association between *U. parvum* serovar 14 and cervicitis-related symptoms, such as cervical bleeding and swelling and cervical ectropion. This contrasts with many studies and guidelines that have found no clear evidence of an association between *U. parvum* and cervicitis [7, 23, 29, 34, 35]. The differences observed could be because no discrimination between *U. parvum* serovars was performed in previous studies, potentially masking the significant association between *U. parvum* serovar 14 and the clinical symptoms. In contrast to *U. parvum* serovar 14, *U. parvum* serovar 3 was significantly negatively correlated with cervical bleeding, indicating that *U. parvum* might be a protective factor against cervical bleeding and swelling when the independent correlations between specific *U. parvum* serovars and symptoms were evaluated. The diversity of *U. parvum* serovars could be explained by genetic variations. Nevertheless, research comparing genomic differences across different *U. parvum* serovars is scarce. Undertaking comparative genomic analyses of *U. parvum* serovars could represent a fruitful avenue for future research.

Our study has several strengths, including its geographically diverse multicentre design with a large sample size. In this study, we were able to assess the associations between individual serovars of *U. parvum* and different clinical signs and symptoms. To evaluate the independent correlations between *U. parvum* serovars and clinical symptoms, we also adjusted for additional genital tract infections that could have confounded the results, which led to more robust results.

There are several limitations of this study. First, owing to the cross-sectional design, we were not able to assess the causal associations between *U. parvum* serovars and clinical symptoms in this study. Second, because the majority of participants in our study were Chinese women, the findings may not be applicable to women outside China. Third, as with any observational study, there may be other unobserved confounders that influence the results, despite adjusting for a considerable number of relevant variables known to affect clinical symptoms in our study. Fourth, we identified the serovar-specific pathogenicity of *U. parvum* in this study, and further investigation of *U. urealyticum* is needed. Finally, there may be selection bias, as our sample was recruited

from hospital outpatient clinics. We aspire to mitigate this bias in future research.

Conclusion

We identified heterogeneous associations between different *U. parvum* serovars and various clinical symptoms and that the lack of discrimination of *U. parvum* serovars could result in a percentage of infected patients not exhibiting clinical symptoms when patients are tested only *U. parvum.* These findings highlight the importance of testing for different *U. parvum* serovars. We also found that in contrast to *U. parvum* serovar 3, *U. parvum* serovar 14 had a strong positive association with cervicitis-related symptoms, indicating a specific pathogenic mechanism of *U. parvum* serovar 14 that needs further investigation. Owing to the cross-sectional nature of our study and the lack of applicability to women outside, further investigations need to be conducted with a prospective cohort consisting of multiple ethnic groups to verify the results of our study.

Abbreviations

U. urealyticum	*Ureaplasma urealyticum*
U. parvum	*Ureaplasma parvum*
MBA	Multiple-banded antigen
STIs	Sexually transmitted infections
HPV	Human papillomavirus
CIN	Cervical intraepithelial neoplasia
N. gonorrhoeae	*Neisseria gonorrhoeae*
M. hominis	*Mycoplasma hominis*
M. genitalium	*Mycoplasma genitalium*
HSV-2	Herpes simplex virus type 2
C. trachomatis	*Chlamydia trachomatis*
T. vaginalis	*Trichomonas vaginalis*
BV	Bacterial vaginosis
AV	Aerobic vaginitis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-10113-9.

Supplementary Material 1.

Acknowledgements

We thank for all participants, staff and collaborators who participated in the CALM 2004 project. We thank for all members of CALM 2004 study group.

Authors' contributions

Muxuan Chen, Hongwei Zhou and Yifeng Wang conceptualized and conducted the study. Rongdan Chen, Zuyi Zhou and Yi Hou were in charge of data collection. Longxu Xie, Wenyu Mo, Yiya Shi and Jinxia Ou contributed to the interpretation of data. Yingxuan Zhang, Wei Qing and Cancan Qi performed statistical analysis. Yingxuan Zhang wrote the original draft. Yingxuan Zhang, Wei Qing and Wenyu Mo reviewed and edited the manuscripts. All authors are members of CALM 2004 project and reviewed and approved the final version of the manuscript.

Funding

The study was supported by the National Natural Science Foundation of China (82205157, 81925026 and 82302610), the Guangdong Basic and Applied Basic Research Foundation (2021B1515230007), the China Postdoctoral Science Foundation (2022M721538) and the Guizhou High-level (BAI) Innovative Talents Project (QIANKehe Platform & Talents-GCC [2022]042–1).

Data availability

This study was a substudy of the ongoing CALM2004 project. Upon reasonable request, all the data and materials used in this study are available from the corresponding author.

Declarations

Ethics approval and consent to participate

This study was a substudy of the CALM2004 project, and all the individuals who participated in CALM2004 project provided written informed consent. The study was approved by the ethics committee of ZhuJiang Hospital of Southern Medical University (NO. 2020-KY-0711–02) and the ethics committees of the other collaborating research centres. The study has been registered with *ClinicalTrials.gov* (https://www.clinicaltrials.gov; ID: NCT04694495; Registration Date: 2021–01-05).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Microbiome Medicine Centre, Department of Laboratory Medicine, ZhuJiang Hospital, Southern Medical University, 253 Industrial Avenue, Haizhu District, Guangzhou 510260, Guangdong, China. [2]Department of Obstetrics & Gynecology, ZhuJiang Hospital, Southern Medical University, Guangzhou, Guangdong, China. [3]Guangzhou Hybribio Medicine Technology Ltd, Guangzhou, Guangdong, China.

Received: 25 July 2024 Accepted: 23 October 2024
Published online: 28 October 2024

References

1. Beeton ML, Payne MS, Jones L. The role of Ureaplasma spp. in the development of nongonococcal urethritis and infertility among men. Clin Microbiol Rev. 2019;32(4):e00137–18.
2. Shimizu T, Kida Y, Kuwano K. Ureaplasma parvum lipoproteins, including MB antigen, activate NF-κB through TLR1, TLR2 and TLR6. Microbiology. 2008;154(5):1318–25.
3. Razin S, Yogev D. Genetic relatedness among Ureaplasma urealyticum serotypes (serovars). Pediatr Infect Disease. 1986;5(6 Suppl):S300–4.
4. Fanrong K, James G, Zhenfang M, Gordon S, Wang B, Gilbert GL. Phylogenetic analysis of Ureaplasma urealyticum–support for the establishment of a new species, Ureaplasma parvum. Int J Syst Evol MicroBiol. 1999;49(4):1879–89.
5. Stellrecht KA, Woron AM, Mishrik NG, Venezia RA. Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. J Clin Microbiol. 2004;42(4):1528–33.
6. Kim Y, Kim J, Lee KA. Prevalence of sexually transmitted infections among healthy Korean women: implications of multiplex PCR pathogen detection on antibiotic therapy. J Infect Chemother. 2014;20(1–2):74–6.
7. Leli C, Mencacci A, Latino MA, Clerici P, Rassu M, Perito S, et al. Prevalence of cervical colonization by Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma hominis and Mycoplasma genitalium in childbearing age women by a commercially available multiplex real-time PCR: an Italian observational multicentre study. J Microbiol Immunol Infect. 2018;51(2):220–5.
8. Lobao TN, Campos GB, Selis NN, Amorim AT, Souza SG, Mafra SS, et al. Ureaplasma urealyticum and U. Parvum in sexually active women attending public health clinics in Brazil. Epidemiol Infect. 2017;145(11):2341–51.
9. Doroftei B, Ilie OD, Armeanu T, Anton E, Scripcariu I, Maftei R. The prevalence of Ureaplasma Urealyticum and Mycoplasma Hominis Infections in Infertile patients in the Northeast Region of Romania. Med (Kaunas). 2021;57(3):211.

10. Jonduo ME, Vallely LM, Wand H, Sweeney EL, Egli-Gany D, Kaldor J, et al. Adverse pregnancy and birth outcomes associated with Mycoplasma hominis, Ureaplasma urealyticum and Ureaplasma parvum: a systematic review and meta-analysis. BMJ open. 2022;12(8):e062990.
11. Rittenschober-Böhm J, Waldhoer T, Schulz SM, Pimpel B, Goeral K, Kasper DC, et al. Vaginal Ureaplasma parvum serovars and spontaneous preterm birth. Am J Obstet Gynecol. 2019;220(6):594. e1-. e9.
12. Plummer EL, Vodstrcil LA, Bodiyabadu K, Murray GL, Doyle M, Latimer RL, et al. Are Mycoplasma hominis, Ureaplasma urealyticum and Ureaplasma parvum Associated with specific genital symptoms and clinical signs in Nonpregnant women? Clin Infect Dis. 2021;73(4):659–68.
13. Zhao N, Li KT, Gao YY, Xu JJ, Huang DS. Mycoplasma Genitalium and Mycoplasma Hominis are prevalent and correlated with HIV risk in MSM: a cross-sectional study in Shenyang, China. BMC Infect Dis. 2019;19(1):494.
14. Kim MS, Lee DH, Kim TJ, Oh JJ, Rhee SR, Park DS, Yu YD. The role of Ureaplasma parvum serovar-3 or serovar-14 infection in female patients with chronic micturition urethral pain and recurrent microscopic hematuria. Transl Androl Urol. 2021;10(1):96–108.
15. De Francesco M, Negrini R, Pinsi G, Peroni L, Manca N. Detection of Ureaplasma biovars and polymerase chain reaction-based subtyping of Ureaplasma parvum in women with or without symptoms of genital infections. Eur J Clin Microbiol Infect Dis. 2009;28(6):641–6.
16. Krausse R, Schubert S. In-vitro activities of tetracyclines, macrolides, fluoroquinolones and clindamycin against Mycoplasma hominis and Ureaplasma ssp. isolated in Germany over 20 years. Clin Microbiol Infect. 2010;16(11):1649–55.
17. Ma H, Zhang X, Shi X, Zhang J, Zhou Y. Phenotypic antimicrobial susceptibility and genotypic characterization of clinical ureaplasma isolates circulating in Shanghai, China. Front Microbiol. 2021;12:724935.
18. Ghofran K, Al-khafaji S. Antimicrobial Resistance of Genital Ureaplasma Parvum. Nano Biomed Eng. 2017;9(3):236–41.
19. Zhou Z, Hou Y, Qing W, Shi Y, Zhang Y, Chen R et al. The association of HPV infection and vaginal microbiota of reproductive women in China: a multicenter cohort study protocol. 2023;15:100072.
20. Bai S, Li Y, Wan Y, Guo T, Jin Q, Liu R, et al. Sexually transmitted infections and semen quality from subfertile men with and without leukocytospermia. Reproductive Biology Endocrinol. 2021;19(1):92.
21. Zhao N, Li KT, Gao Y-y, Xu J-j, Huang D-S. Mycoplasma Genitalium and Mycoplasma Hominis are prevalent and correlated with HIV risk in MSM: a cross-sectional study in Shenyang, China. BMC Infect Dis. 2019;19:1–7.
22. Zhang N, Wang R, Li X, Liu X, Tang Z, Liu Y. Are Ureaplasma spp. a cause of nongonococcal urethritis? A systematic review and meta-analysis. PLoS ONE. 2014;9(12):e113771.
23. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al. Sexually Transmitted Infections Treat Guidelines 2021 MMWR Recomm Rep. 2021;70(4):1–187.
24. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol. 1991;29(2):297–301.
25. Donders GG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B. Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. BJOG: Int J Obstet Gynecol. 2002;109(1):34–43.
26. Robertson JA, Stemke GW, Davis JW, Harasawa R, Thirkell D, Kong F, et al. Proposal of Ureaplasma parvum sp. nov. and emended description of Ureaplasma urealyticumShepard (1974) Robertson 2001. Int J Syst Evol Microbiol. 2002;52(Pt 2):587–97.
27. Wetmore CM, Manhart LE, Lowens MS, Golden MR, Jensen NL, Astete SG, et al. Ureaplasma urealyticum is Associated with Nongonococcal Urethritis among men with fewer lifetime sexual partners: a case-control study. J Infect Dis. 2011;204(8):1274–82.
28. Kletzel HH, Rotem R, Barg M, Michaeli J, Reichman O. Ureaplasma urealyticum: the role as a Pathogen in Women's Health, a systematic review. Curr Infect Dis Rep. 2018;20(9):1–12.
29. Horner P, Donders G, Cusini M, Gomberg M, Jensen J, Unemo M. Should we be testing for urogenital Mycoplasma hominis, Ureaplasma parvum and Ureaplasma urealyticum in men and women?–a position statement from the European STI guidelines Editorial Board. J Eur Acad Dermatol Venereol. 2018;32(11):1845–51.
30. Wada K, Hamasuna R, Sadahira T, Araki M, Yamamoto S. UAA-AAUS guideline for M. genitalium and non-chlamydial non-gonococcal urethritis. J Infect Chemother. 2021;27(10):1384–8.
31. Song T, Ye A, Xie X, Huang J, Ruan Z, Kong Y, et al. Epidemiological investigation and antimicrobial susceptibility analysis of ureaplasma species and Mycoplasma hominis in outpatients with genital manifestations. J Clin Pathol. 2014;67(9):817–20.
32. Zhu C-t, Hu Z-y, Dong C-l, Zhang C-s, Wan M-z, Ling Y. Investigation of Ureaplasma urealyticum biovars and their relationship with antimicrobial resistance. Ind J Med Microbiol. 2011;29(3):288–92.
33. Boujemaa S, Mlik B, Ben Allaya A, Mardassi H, Mardassi BB. Spread of multidrug resistance among Ureaplasma serovars. Tunisia Antimicrob Resist Infect Control. 2020;9(1):1–10.
34. Lusk MJ, Garden FL, Rawlinson WD, Naing ZW, Cumming RG, Konecny P. Cervicitis aetiology and case definition: a study in Australian women attending sexually transmitted infection clinics. Sex Transm Infect. 2016;92(3):175–81.
35. 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.

Publisher's note