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Original Article

A study of the risk factors for *Ureaplasma urealyticum* infection and the predictive role of immunoinflammation

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Abstract

Introduction: Despite increasing awareness on the prevention of *Ureaplasma urealyticum* (*Uu*) infection, the high-risk factors responsible for infection in female patients in China are yet to be determined.

Methodology: The study included 3043 Chinese women. Cervical secretion samples were collected for *Uu* identification.

Results: Higher age groups (25–30, 30–35, 35–40, and >40 years) had a higher risk of *Uu* infection (OR = 1.46; OR = 1.51; OR = 1.71; OR = 2.49, respectively). Being literate, and use of intrauterine device (IUD), or other contraceptive methods could reduce the risk of *Uu* infection (OR = 0.64; OR = 0.79; OR = 0.76, respectively). Women with low level of cleanliness or promiscuous behavior had a higher risk of *Uu* infection (OR = 1.42; OR = 1.41, respectively). Among the *Uu*-positive patients, 66.84%, 24.81%, and 8.35% were infected with biovars 1, 2, and coinfection. The predominant subtypes were S6 serotypes (28.91%) in biovar 1 and S2' subtypes (62.73%) in biovar 2. The possibility of S1 + S6 infection was lower than that in S1 patients (OR = 0.529). C-reactive protein (CRP) and systemic immune inflammation index (SII) could be used to predict *Uu* infection (area under curve, AUC = 0.55; AUC = 0.68, respectively).

Conclusions: *Uu*-positive patients were infected with two biovars and multiple subtypes. Age, method of contraception, cleanliness, education level, promiscuity, and subtypes of *Uu* were factors influencing *Uu* infection. CRP and SII provide a new strategy for clinical diagnosis of *Uu* infection.

Key words: *Ureaplasma urealyticum*; biovars; serotypes; subtypes; immunoinflammation.

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Introduction

The global prevalence of infertility in women is currently estimated to be 10–15%, and this rate continues to increase [1]. Numerous studies have established an association between *Ureaplasma urealyticum* (*Uu*) and conditions such as female infertility and reproductive tract infections [2–4]. *Uu* is a bacterium that lacks a cell wall, has a small genome, and adheres to the mucous membranes of the genitourinary tract in adults or the respiratory tract in infants [5]. Studies have found that *Uu* is predominantly found in the genital tract of sexually experienced healthy adults, and its presence has been detected in 40–80% of healthy women [6–8]. However, many neonatal populations and adult genitourinary disorders are often associated with *Uu* [9,10]. *Uu* has been categorized into 2 biotypes and 14 subtypes, each exerting distinct effects on female reproductive tract diseases [11,12]. Evaluation of the pathogenicity of different subtypes in mice revealed that although all subtypes were pathogenic, the degree of pathogenicity was different [13]. Additionally, research has demonstrated that the prevalence of *Uu* in the female genital tract within a population is influenced by factors such as age, race, socioeconomic status, contraceptive use, menopausal changes, and pregnancy [14]. Although biovars and subtypes of Uu are known to exist, the population distribution of biotypes and subtypes of *Uu* among Chinese female patients is unclear, and the high-risk factors for infection are not known. Several studies have pointed out that *Mycoplasma deiureticum* can cause an inflammatory response in the patient's body, with levels of several inflammatory factors related to *Uu* infection [15–17]. A variety of inflammatory factor scores have been used in recent years to assess inflammation-related diseases—both to accurately

assess the course of the disease or treatment, and for economic convenience. The aim of this study was to analyze the data on the prevention and clinical treatment of female genital tract *Uu* infections by investigating the infection status and external factors affecting female genital tract infections, and by exploring the distribution of *Uu* subtypes that predispose females to infection in China.

Methodology

Participants

A total of 3,043 women admitted to Tangshan Workers' Hospital, Tangshan City Fengnan District Hospital, and Tangshan Center for Disease Control and Prevention, between January 2019 and January 2023, were included in the study. A total of 1,176 *Uu*-infected patients and 1,867 healthy controls were included. Patients co-infected with other pathogens (*Mycoplasma* and *Clamydia*) were excluded from this study. Inclusion and exclusion of the study population was shown in Figure 1.

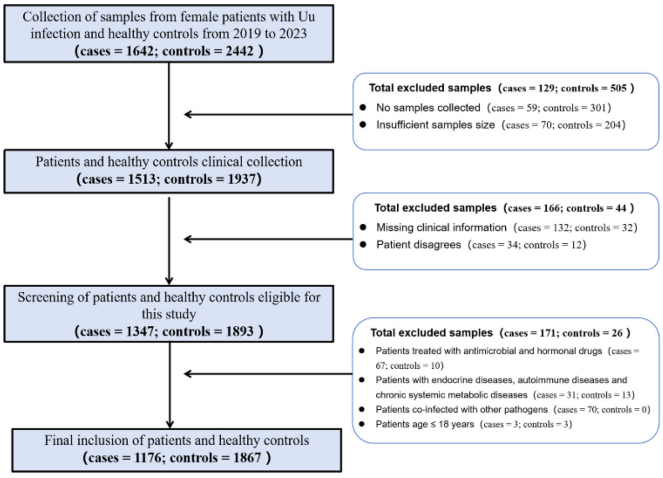
Clinical data

The basic clinical information of patients; including age, education level, living environment, age of menarche, age at first sexual intercourse, number of pregnancies, contraceptive methods, number of abortions, degree of vaginal cleanliness, and promiscuity; was collected.

Sample collection and testing

Samples collected from all subjects were preserved for testing within 48 hours. A sterile female swab was used to collect cervical secretions 1–2 cm inside the cervical canal. The samples were then aseptically sealed and sent for examination. A commercial *Uu* identification kit (Zhuhai Lizhu Reagent Co., Zhuhai, China) was employed to detect *Uu* infection in all subjects, following the manufacturer's instructions.

Figure 1. Flow chart for participants selection.



The biovars and subtypes of *Uu* were determined using polymerase chain reaction (PCR). The primers were designed based on the *Uu* multiple-banded antigen gene, using with Primer 3 software [18]. To ensure genotyping reliability, 15% of samples were randomly selected for repeat tests. The results of the repeat tests were entirely consistent with the original findings. The primer sequences used in this study are listed in Table 1.

PCR detection for Uu infections

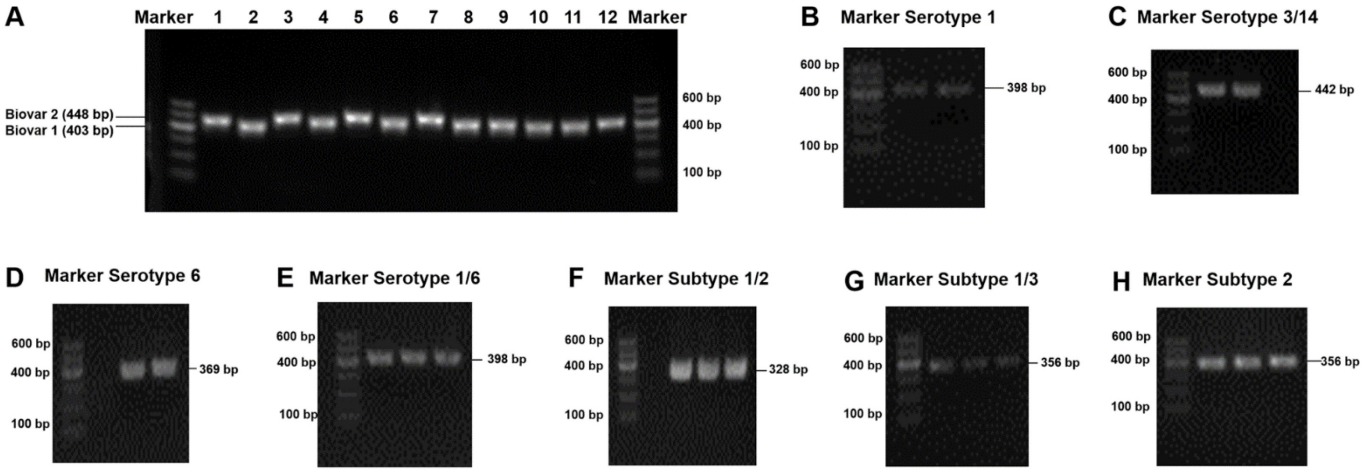
As described previously, PCR assay was performed to identify biovars and subtypes of *Uu* [19]. PCR was performed on patients who were positive for *Uu*, with amplified bands of 403 bp for biovar 1 and 448 bp for biovar 2 (Figure 2).

Table 1. Primer sequences.

Primer Names	Biovars and subtypes	Primer sequences (5'-3')
UPF	Biovar1	AAATCTTAGTGTTCATATTTTTTAC
UPR		GTAAGTGCAGCATAAATTCAATG
UUF	Biovar2	GAGTATGCAATCTTTATATGTTTTCG
UUR		GAGTTTGTTGTTGCGTTTTCTG
UMS83	UPS1	TACTGTAGAAATTATGTAAGATTGC
UMA269a		CCAAATGACCTTTTGTAACTAGAT
UMS125	UPS3 + S14	GTATTTGCAATCTTTATATGTTTTCG
UMA269		CTAAATGACCTTTTTCAAGTGTAC
UMA269a	UPS6	CCAAATGACCTTTTGTAACTAGAT
UMS54		CTTAGTGTTCATATTTTTTACTAG
UMS61	UUS1 + 2	TTTGCAAAACTATAAATAGACAC
UMA219		GTAATTGCAACATGCAATTCAGCTTCG
UMS112	UUS1 + 3	GATTAAACAAAATCTTAATGTTGTTA
UMA194		CGTTTAATGCTTTTTTATCATTTTCAG
UMS112a	UUS2	GATTAAACAAAATCTTAATGTTGTTG
UMS194a		CGTTTAATGCTTTTTTATCATTTTCAT

UPS1 refers to the biovar1 S1; UPS3 + S14 refers to the biovar1 S3 + S14; UPS6 refers to the biovar1 S6; UUS1 + 2 refers to the biovar2 S1 + S2'; UUS1 + 3 refers to the biovar2 S1 + S3'; UUS2 refers to the biovar2 S2'.

Figure 2. PCR amplified fragments for various Uu biovars, serotypes and subtypes.



A. Electrophoresis gel images for Uu Biovars, including biovar1 and biovar2. **B-E.** Electrophoresis gel pictures for serotypes in biovar 1, including (B) S1, (C) S3+S14, (D) S6, (E) S1+S6. **F-H.** Electrophoresis gel pictures for subtypes in biovar 2, including (F) S1'+S2', (G) S1'+S3', and (H) S2'.

Table 2. Baseline characteristics of study population.

Characteristic	Overall	*Uu* detection results		*p* value
		Non-infection	Infection	
	N = 3043	N = 1867	N = 1176	
Age (years), n (%)				
≤ 25	599 (19.7%)	431 (23.1%)	168 (14.3%)	< 0.001
25–30	609 (20%)	387 (20.7%)	222 (18.9%)	
30–35	591 (19.4%)	374 (20%)	217 (18.5%)	
35–40	523 (17.2%)	313 (16.8%)	210 (17.9%)	
> 40	721 (23.7%)	362 (19.4%)	359 (30.5%)	
Education level, n (%)				
Illiterate	581 (19.1%)	301 (16.1%)	280 (23.8%)	< 0.001
Literate	2462 (80.9%)	1566 (83.9%)	896 (76.2%)	
Area, n (%)				
Rural	1366 (44.9%)	839 (44.9%)	527 (44.8%)	0.946
Urban	1677 (55.1%)	1028 (55.1%)	649 (55.2%)	
Age of menarche, Mean (SD)	15.03 (2.32)	14.97 (2.3)	15.11 (2.33)	0.108
Age at first sexual intercourse, Mean (SD)	24.49 (24.49)	24.55 (4.47)	24.4 (4.44)	0.395
Number of pregnancies, n (%)				
0	1011 (33.2%)	628 (33.6%)	383 (32.6%)	0.830
1–2	1706 (56.1%)	1040 (55.7%)	666 (52.6%)	
≥ 3	326 (10.7%)	199 (10.7%)	127 (10.8%)	
Methods of Contraception, n (%)				
Condom	1842 (60.5%)	1210 (64.8%)	632 (53.7%)	< 0.001
Intrauterine device (IUD)	152 (5.0%)	88 (4.7%)	64 (5.4%)	
Others	454 (14.9%)	264 (14.1%)	190 (16.2%)	
No measures taken	595 (19.6%)	305 (16.3%)	290 (24.7%)	
Number of miscarriages, n (%)				
0	1993 (65.5%)	1233 (66%)	760 (64.6%)	0.779
1	560 (18.4%)	343 (18.4%)	217 (18.5%)	
2	438 (14.4%)	261 (14%)	177 (15.1%)	
3	52 (1.7%)	30 (1.6%)	22 (1.9%)	
Cleanliness, n (%)				
I	1394 (45.8%)	890 (47.7%)	504 (42.9%)	0.002
II	801 (26.3%)	497 (26.6%)	304 (25.9%)	
III	473 (15.5%)	280 (15%)	193 (16.4%)	
IV	375 (12.3%)	200 (10.7%)	175 (14.9%)	
Promiscuity, n (%)				
No	2292 (75.3%)	1451 (77.7%)	841 (71.5%)	< 0.001
Yes	751 (24.7%)	416 (22.3%)	335 (28.5%)	
***Uu* detection results, n (%)**				
Negative	1713 (56.3%)	1713 (91.8%)	0 (0.0%)	< 0.001
Positive	1330 (43.7%)	154 (8.2%)	1176 (100.0%)	

PCR typing of patients positive for biovar 1 of *Uu* showed amplification bands of 398 bp, 442 bp, 369 bp, and 398 bp for S1, S3 + S14, S6, and S1 + S6, respectively (Figure 2).

PCR typing was performed on patients positive for biovar 2 of *Uu*, and the amplified bands of S1 + S2', S1 + S3' and S2' were 328 bp, 356 bp and 356 bp, respectively (Figure 2).

Inflammatory metrics collection

About 2 mL of venous blood was collected from all study subjects—while they were in fasting state—in ethylene diamine tetra acetic acid (EDTA) anticoagulant vacuum tube, and routine blood tests were performed, including white blood cell (WBC), neutrophil (NE), lymphocyte (LY), platelet (P), and serum C-reactive protein (CRP). The SII score was calculated from the collected hematological indices, $SII = P \times NE \div LY$.

Statistical analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 23.0 software (IBM Corp, Armonk, NY, USA). Measurement data were expressed as x ± s, and the t-test was used for between-group comparisons. The data were presented as numbers or percentages, and the Chi square test was used for intergroup comparisons. Logistic regression was applied to analyze the factors affecting *Uu* positivity and the relationship between different subtypes and *Uu* susceptibility. A p value < 0.05 was considered statistically significant.

Results

Study population

The sociodemographic and clinical characteristics of the study population are summarized in Table 2. The age distribution and education level of patients with *Uu* infection differed from that of patients without *Uu* infection ($p < 0.05$). Among women with *Uu* infection, 632 (53.7%) used condoms, 64 (5.4%) used intrauterine devices (IUDs), 190 (16.2%) used other methods, and 290 (24.7%) used no contraceptive method. By contrast, among women without *Uu* infection, 1,210 (64.8%) used condoms for contraception, 88 (4.7%) used IUDs, 264 (14.1%) used other contraceptive methods, and 305 (16.3 %) used no contraception. The contraceptive methods of women were found to be related to *Uu* infection ($p < 0.05$). Among women with *Uu* infection, 504 (42.9%) were classified as cleanliness I, 304 (25.9%) as cleanliness II, 193 (16.4%) as cleanliness III, and 175 (14.9%) as cleanliness IV. Among the women without *Uu* infection, 890 (47.7%), 497 (26.6 %), 280 (15%), and 200 (10.7%) were categorized under cleanliness I, II, III, and IV, respectively. A significant correlation was observed between cleanliness levels and *Uu* infection ($p < 0.05$). There was a total of 416 (22.3%) cases of promiscuity among the uninfected population, and 335 (28.5%) cases of promiscuity among *Uu* infected patients. The proportion of promiscuity among *Uu* infected patients was higher ($p < 0.05$). Among women with *Uu* infection, 1,176 (100.0%) tested positive for *Uu*. Conversely, among women without *Uu* infection, 154 (8.3 %) tested positive and 1,713 (91.8 %) tested negative. Again, there was a correlation between

Table 3. Multifactor stepwise logistic regression analysis of factors influencing *Uu* infection.

Characteristic	all/case	OR	95% CI	*p* value
Age (years), n (%)				
≤ 25	599/168	-	-	-
25–30	609/222	1.46	1.14-1.87	0.003
30–35	591/217	1.51	1.18-1.94	0.001
35–40	523/210	1.71	1.33-2.21	<0.001
> 40	721/359	2.49	1.97-3.15	<0.001
Education level, n (%)				
Illiterate	581/280	-	-	-
Literate	2462/896	0.64	0.53-0.77	<0.001
Methods of contraception, n (%)				
No measures taken	595/290	-	-	-
Condom	1842/632	0.56	0.55-1.14	0.207
Intrauterine device (IUD)	152/64	0.79	0.59-0.98	0.031
Others	454 / 190	0.76	0.46-0.68	<0.001
Cleanliness, n (%)				
I	1394/504	-	-	-
II	801/304	1.07	0.89-1.29	0.469
III	473/193	1.11	0.87-1.42	0.399
IV	375/175	1.42	1.09-1.85	0.010
Promiscuity, n (%)				
No	2292/841	-	-	-
Yes	751/335	1.41	1.19-1.68	<0.001

OR: odds ratio; CI: confidence interval.

Table 4. Biovars and subtypes distribution of *Uu.*

Biovars	Subtypes	Isolates	Rates (%)
Biovar 1		889	
	S6	257	28.91
	S1	251	28.23
	S1 + S6	225	25.31
	S3 + S14	91	10.24
	S1 + S3 + S14	65	7.31
Biovar 2		330	
	S2'	207	62.73
	S1'+ S3'	69	20.91
	S1' + S2' + S3'	54	16.36
Biovar 1 + biovar 2		111	

negative and positive testing for *Uu* specimens and the presence of *Uu* infection ($p < 0.05$). However, there was no significant correlation between *Uu* infection and factors such as patients' geographical area, age of menarche, age at first sexual intercourse, number of pregnancies, and number of miscarriages ($p > 0.05$).

Factors associated with Uu infections

Multifactorial stepwise logistic regression analyses were conducted for the variables that had a statistically significant difference (age, contraceptive methods, personal hygiene, and promiscuity). Women aged 25–30, 30–35, 35–40, and > 40 years exhibited an increased risk of contracting *Uu* compared with women who were < 25 years (OR = 1.46, 95% CI [1.14–1.87]; OR = 1.51, 95% CI [1.18–1.94]; OR = 1.71, 95% CI [1.33–2.21]; OR = 2.49, 95% CI [1.97–3.15], respectively; Table 3). The literate population were associated with a lower likelihood of *Uu* infection (OR = 0.64, 95% CI [0.53–0.77]) than the illiterate people. Compared with no contraception, the use of IUD and others were associated with a lower likelihood of *Uu* infection (OR = 0.79, 95% CI [0.59–0.98] and OR = 0.76, 95 % CI [0.46–0.68], respectively). Women with degree IV cleanliness were more susceptible to *Uu* infection than those with degree I cleanliness (OR = 1.42, 95% CI [1.09–1.85]). In addition, women who engaged in promiscuous behavior are more likely to be infected with *Uu* (OR = 1.41, 95% CI [1.19–1.68]). Therefore, emphasizing personal hygiene and strengthening contraception use can effectively reduce the risk of contracting *Uu*.

Distribution of biovars, serotypes and subtypes in women with Uu infection

Among the 1,330 patients, 66.84% (889/1330) were infected with *Uu* biovar 1, 24.81% (330/1330) were infected with biovar 2, and 8.35 % (111/1330) had coinfections (Table 4). The serotypes of biovar 1 were S6 (28.91%), S1 (28.23%), S6 (28.68%), mixed S1 + S6 (25.31%), mixed S3 + S14 (10.24%), and mixed S1 + S3 + S14 (7.31%). Biovar 2, subtypes mainly included S2' (62.73%), and mixed S1' + S3' (20.91%) and mixed S1' + S2' + S3' (16.36%).

Association between various subtypes and Uu susceptibility

The above findings indicate that patients with different subtypes of *Uu* exhibit varying levels of resistance to different drugs. Therefore, the relationship between *Uu* subtypes and the risk of morbidity in *Uu* patients was further investigated (Table 5). In the biovar 1 population, the risk of S1 + S6 diseases was significantly lower than that in S1 patients (OR = 0.529, 95% CI [0.288–0.973]). However, in the biovar 2 population, no statistically significant difference in the risk of S2', S1' + S3', and S1' + S2' + S3' diseases was observed ($p > 0.05$).

Table 5. Association between subtypes and *Uu* infection.

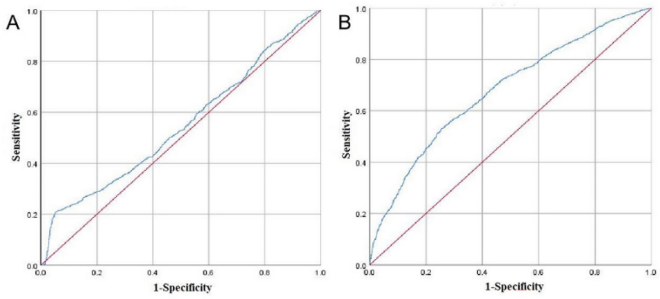
Biovars	Subtypes	Uu detection results		OR	95% CI	*p* value
		Non-infection	Infection			
Biovar1	S1	20 (19.23%)	231 (29.43%)	Reference	Reference	Reference
	S1 + S6	31 (29.81%)	194 (24.71%)	0.529	0.288-0.973	0.040
	S6	29 (27.88%)	228 (29.04%)	0.720	0.390-1.326	0.291
	S3 + S14	13 (12.5%)	78 (9.94%)	0.463	0.214-1.003	0.051
	S1 + S3 + S14	11 (10.58%)	54 (6.88%)	0.449	0.197-1.026	0.058
Biovar2	S2'	24 (60.00%)	183 (63.11%)	Reference	Reference	Reference
	S1' + S3'	8 (20.00%)	61 (21.03%)	1.537	0.599-3.947	3.947
	S1' + S2' + S3'	8 (20.00%)	46 (15.86%)	1.832	0.579-5.794	5.794

OR: odds ratio; CI: confidence interval. Logistic regression was adjusted for age, education level, contraceptive methods, personal hygiene, common pathogens and promiscuity of the infection.

The relationship between immune inflammation levels and Uu infection

When a patient is infected with *Uu*, the body responds with a series of inflammatory responses. Therefore, high levels of relevant inflammatory markers can be used to assess *Uu* infection in female patients. The levels of common inflammatory indicators (CRP, WBC, NE, LY, SII) of all women were collected measured and analyzed, and it was found that female patients infected with *Uu* had higher levels of CRP and higher SII scores (Table 6). The receiver operating characteristic (ROC) model was constructed based on the above results, and the diagnostic value of CRP and SII for *Uu* infection in women was further evaluated (Figure 3). Using CRP values, the area under curve (AUC) of *Uu* infection in women was 0.55 (95% CI: 0.53–0.57, $p < 0.05$). Using SII values, the AUC of *Uu* infection in women was 0.68 (95% CI: 0.66–0.70, $p < 0.05$).

Figure 3. ROC curve analysis of inflammatory markers to predict Uu infection in female patients.



ROC curves to evaluate the predictive ability regarding **A.** C-reactive protein (CRP) and **B.** systemic immune inflammation index (SII) for Uu infection.

Discussion

Uu is associated with various reproductive tract diseases in humans; including urethritis, prostatitis, cervicitis, pelvic inflammatory disease, adverse pregnancy outcomes, and fatal hyperammonemia in adults. Our analysis of 3,043 female patients showed that the risk of *Uu* infection increases with age in women. Focus on self-cleaning (high level of cleanliness) and the use of condoms for contraception significantly reduced the risk of *Uu* infection. Furthermore, patients with different biovars and subtypes exhibited variations in their proportions, morbidity risks. CRP, and SII indicators can be used to assess whether a patient is infected with *Uu*.

There are numerous factors influencing *Uu* infection in women [20–22]. We found that the risk of infection increases with age; so, we urge older women to pay more attention to their health and seek medical care. Low cleanliness was considered a risk factor for *Uu* infection in female patients. Women who used 'other contraceptives or IUDs had a lower risk of *Uu* infection ($p < 0.05$). Our research concluded that promiscuity increases the risk of *Uu* infection ($p < 0.05$). Other studies have reported that the number of sexual partners, a history of sexual abuse, and inappropriate use or non-use of condoms are all associated with contracting reproductive tract diseases [23,24]. Therefore, focusing on one's own hygiene and cleanliness as well as on contraceptive methods can be effective in avoiding infections of the reproductive tract. In addition, steps should be taken to increase awareness of gynecological hygiene among women.

The pathogenicity of *Uu* is closely associated with its subtypes [25,26]. Different *Uu* biotypes and subtypes lead to different sites of lesions in women [27,28]. In this study, 66.84% of *Uu*-positive patients were infected with biovar 1, 23.57% with biovar 2, and 8.34% with both biovar 1 and biovar 2. In contrast, the subgroup of *Uu*-positive patients among Mexican women exhibited a more even distribution, with 48% infected with biovar 1 and 28% with biovar 2 [29]. Another study found that 81% of the patients were infected with either biovar 1 or a mixture of biovar 1 and biovar 2, which is consistent with our study's results [30]. However, the percentage of people infected with *Uu* subtypes varies by region and among sex workers [30,31]. We found that the risk of infection was significantly lower in S1+S6 patients than in S1 patients in the biovar 1 population. Since different organisms and subtypes have different pathogenicity in the population, clinical treatment needs to be symptomatic.

A series of inflammatory reactions occur in the body of patients with *Uu* infections. *Uu* infection promotes endometriosis by increasing inflammatory mediators, adhesion molecules, and matrix

Table 6. Comparison of immunoinflammation between Uu-infected and non-infected groups ($\bar{x} \pm s$).

Immunoinflammation	Non-infection (N = 1867)	Infection (N = 1176)	*t*	*p* value
C-reactive protein (CRP)	5.94 ± 3.46	3.51 ± 2.16	8.491	< 0.001
White blood cell count (WBC)	5.48 ± 2.14	5.55 ± 2.25	– 0.374	0.709
Neutrophilic granulocyte (NE)	3.71 ± 1.13	3.87 ± 1.12	– 1.624	0.105
Lymphocyte (LY)	2.5 ± 0.74	2.38 ± 0.71	1.943	0.052
Systemic immune inflammation index (SII)	3.11 ± 2.35	4.71 ± 3.11	– 7.642	< 0.001

metalloproteinase 2 (*MMP-2*) expression in peripheral mononuclear cells (PMC) through toll like receptor 2 (TLR2) signaling [16].

Mycoplasma hyopneumoniae regulates cytokine and chemokine responses in human brain microvascular endothelial cells [32]. Perinatal exposure to *Mycoplasma solani* is associated with an increased risk of delayed sepsis and inflammatory imbalance in preterm infants and may exacerbate lung injury [33]. Therefore, we tried to evaluate the infection *Uu* in female patients by looking for indicators of inflammation as an evaluation index. In addition, inflammatory factors are important indicators of the body's disease progression; these tests can be cost-effective and can efficiently be used to assess patients. However, further extensive data are needed to support this. Pregnant patients were not excluded from this study population. It is possible that pregnant patients may have recurrent *Uu* infections due to the instability of their body functions; and this will be the direction of our future research.

This study analyzed and identified the risk factors of *Uu* in women by collecting a large number of samples from patients suffering from *Uu* in China. The aim was to develop resources for targeted prevention of the disease in the female population, and provide new ideas for clinical treatment by analyzing the subtype percentage of *Uu* in women as well as evaluate their immune indexes. However, our study population was limited to China, and validation in more areas is warranted. In addition, we excluded patients who were co-infected with *Mycoplasma* to improve accuracy of the results. Our study did not detect other pathogens.

Conclusions

Based on our results, improving awareness on hygiene, appropriate contraceptive methods, and cleanliness are effective ways of reducing the risk of contracting *Uu*, and should be emphasized among older women. Biovar 1 was predominant among *Uu*-positive patients and the risk varied by subtypes in the population. Meanwhile, the immune-related indicators, CRP and SII, had a possible role in the diagnosis of *Uu*. We identified the risk factors affecting *Uu* infection in women and also provided some suggestions to strengthen prevention methods.

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References

1. Fantus RJ, Alter K, Chang C, Ambulkar SS, Bennett NE, Helfand BT, Brannigan RE, Halpern JA (2021) Characterizing the epidemiology and provider landscape of male infertility care in the United States. Urology 153: 169–174. doi: 10.1016/j.urology.2021.04.008.
2. Dhawan B, Malhotra N, Sreenivas V, Rawre J, Khanna N, Chaudhry R, Mittal S (2012) *Ureaplasma* serovars and their antimicrobial susceptibility in patients of infertility and genital tract infections. Indian J Med Res 136: 991–996.
3. Rana C, Forsyth J, Semmens K (1978) Non-specific (non-gonococcal) urethritis. Med J Aust 1: 409–410. doi: 10.5694/j.1326-5377.1978.tb141908.x.
4. Viscardi RM, Hasday JD (2009) Role of *Ureaplasma* species in neonatal chronic lung disease: epidemiologic and experimental evidence. Pediatr Res 65: 84r–90r. doi: 10.1203/PDR.0b013e31819dc2f9.
5. Sulyok KM, Bekő K, Kreizinger Z, Wehmann E, Jerzsele Á, Rónai Z, Turcsányi I, Makrai L, Szeredi L, Jánosi S, Nagy S, Gyuranecz M (2018) Development of molecular methods for the rapid detection of antibiotic susceptibility of *Mycoplasma bovis*. Vet Microbiol 213: 47–57. doi: 10.1016/j.vetmic.2017.11.026.
6. Hoxha I, Lesiak-Markowicz I, Walochnik J, Stary A, Fürnkranz U (2023) The prevalence of genital mycoplasmas and coinfection with *Trichomonas vaginalis* in female patients in Vienna, Austria. Microorganisms 11: 933. doi: 10.3390/microorganisms11040933.
7. Leli C, Mencacci A, Latino MA, Clerici P, Rassu M, Perito S, Castronari R, Pistoni E, Luciano E, De Maria D, Morazzoni C, Pascarella M, Bozza S, Sensini A (2018) 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 51: 220–225. doi: 10.1016/j.jmii.2017.05.004.
8. McCormack WM, Almeida PC, Bailey PE, Grady EM, Lee YH (1972) Sexual activity and vaginal colonization with genital mycoplasmas. JAMA 221: 1375–1377. doi: 10.1001/jama.1972.03200250017004.
9. Jonduo ME, Vallely LM, Wand H, Sweeney EL, Egli-Gany D, Kaldor J, Vallely AJ, Low N (2022) Adverse pregnancy and birth outcomes associated with *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum*: a systematic review and meta-analysis. BMJ Open 12: e062990. doi: 10.1136/bmjopen-2022-062990.
10. Sprong KE, Mabenge M, Wright CA, Govender S (2020) *Ureaplasma* species and preterm birth: current perspectives. Crit Rev Microbiol 46: 169–181. doi: 10.1080/1040841X.2020.1736986.
11. Taylor-Robinson D (2017) Mollicutes in vaginal microbiology: *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, and *Mycoplasma genitalium*. Res Microbiol 168: 875–881. doi: 10.1016/j.resmic.2017.02.009.
12. Watanabe T, Matsuura M, Seto K (1983) Isolation and serological typing of *Ureaplasma* in human saliva. Arch Oral Biol 28: 545–548. doi: 10.1016/0003-9969(83)90188-7.
13. Zhu GX, Lu C, Chen CJ, Feng PY, Ma H, Lu RB, Yuan YL (2011) Pathogenicity of *Ureaplasma urealyticum* and *Ureaplasma parvum* in the lower genital tract of female BALB/c mice. Can J Microbiol 57: 987–992. doi: 10.1139/w11-098.

14. Donders GGG, Ruban K, Bellen G, Petricevic L (2017) *Mycoplasma*/*Ureaplasma* infection in pregnancy: to screen or not to screen. J Perinat Med 45: 505–515. doi: 10.1515/jpm-2016-0111.
15. Bender RA, Gundogdu C (2022) Cytological diagnosis of genital *Ureaplasma urealyticum* and its importance in cervical inflammation. Eur Rev Med Pharmacol Sci 26: 7912–7917.
16. Noh EJ, Kim DJ, Lee JY, Park JH, Kim JS, Han JW, Kim BC, Kim CJ, Lee SK (2019) *Ureaplasma urealyticum* infection contributes to the development of pelvic endometriosis through toll-like receptor 2. Front Immunol 10: 2373. doi: 10.3389/fimmu.2019.02373.
17. Jensen JS, Cusini M, Gomberg M, Moi H, Wilson J, Unemo (2022) 2021 European guideline on the management of *Mycoplasma genitalium* infections. J Eur Acad Dermatol Venereol 36: 641–650. doi: 10.1111/jdv.17972.
18. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3--new capabilities and interfaces. Nucleic Acids Res 40: e115. doi: 10.1093/nar/gks596.
19. Gupta V, Dhawan B, Khanna N, Agarwal N, Bhattacharya SN, Sreenivas V, Chaudhry R (2008) Detection and biovar discrimination of *Ureaplasma urealyticum* in Indian patients with genital tract infections. Diagn Microbiol Infect Dis 60: 95–97. doi: 10.1016/j.diagmicrobio.2007.07.004.
20. Pella-Saavedra P, Ramos-Vallejos F, Del Valle-Mendoza J, Becerra-Goicochea L, Silva-Caso W, Pinillos-Vilca L, Cornejo-Tapia A, Martins-Luna J, Aquino-Ortega R, Carrillo-Ng H, Aguilar-Luis MA (2023) Prevalence of coinfections in a cross-sectional cohort of women screened for multiple pathogens in Peru. Heliyon 9: e14257. doi: 10.1016/j.heliyon.2023.e14257.
21. Wang QY, Li RH, Zheng LQ, Shang XH (2016) Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in female outpatients, 2009-2013. J Microbiol Immunol Infect 49: 359–362. doi: 10.1016/j.jmii.2014.06.007.
22. Vitlic A, Lord JM, Phillips AC (2014) Stress, ageing and their influence on functional, cellular and molecular aspects of the immune system. Age (Dordr) 36: 9631. doi: 10.1007/s11357-014-9631-6.
23. Jongen VW, Schim van der Loeff MF, Botha MH, Sudenga SL, Abrahamsen ME, Giuliano AR (2021) Incidence and risk factors of *C. trachomatis* and *N. gonorrhoeae* among young women from the Western Cape, South Africa: The EVRI study. PLoS One 16: e0250871. doi: 10.1371/journal.pone.0250871.
24. Menezes LJ, Pokharel U, Sudenga SL, Botha MH, Zeier M, Abrahamsen ME, Glashoff RH, Engelbrecht S, Schim van der Loeff MF, van der Laan LE, Kipping S, Taylor D, Giuliano AR (2018) Patterns of prevalent HPV and STI co-infections and associated factors among HIV-negative young Western Cape, South African women: the EVRI trial. Sex Transm Infect 94: 55–61. doi: 10.1136/sextrans-2016-053046.
25. Masover GK, Sawyer JE, Hayflick L (1976) Urea-hydrolyzing activity of a T-strain mycoplasma: *Ureaplasma urealyticum*. J Bacteriol 125: 581–587. doi: 10.1128/jb.125.2.581-587.1976.
26. Larsen B, Hwang J (2010) *Mycoplasma*, *Ureaplasma*, and adverse pregnancy outcomes: a fresh look. Infect Dis Obstet Gynecol 2010: 521921. doi: 10.1155/2010/521921.
27. Deguchi T, Yoshida T, Miyazawa T, Yasuda M, Tamaki M, Ishiko H, Maeda S (2004) Association of *Ureaplasma urealyticum* (biovar 2) with nongonococcal urethritis. Sex Transm Dis 31: 192–195. doi: 10.1097/01.OLQ.0000114653.26951.71.
28. Yoshida T, Ishiko H, Yasuda M, Takahashi Y, Nomura Y, Kubota Y, Tamaki M, Maeda S, Deguchi T (2005) Polymerase chain reaction-based subtyping of *Ureaplasma parvum* and *Ureaplasma urealyticum* in first-pass urine samples from men with or without urethritis. Sex Transm Dis 32: 454–457. doi: 10.1097/01.olq.0000158932.78183.95.
29. Karim S, Bouchikhi C, Banani A, Fatemi HEL, Souho T, Erraghay S, Bennani B (2020) Detection of *Ureaplasma* biovars and subtyping of *Ureaplasma parvum* among women referring to a university hospital in Morocco. Infect Dis Obstet Gynecol 2020: 7286820. doi: 10.1155/2020/7286820.
30. Abele-Horn M, Wolff C, Dressel P, Pfaff F, Zimmermann A (1997) Association of *Ureaplasma urealyticum* biovars with clinical outcome for neonates, obstetric patients, and gynecological patients with pelvic inflammatory disease. J Clin Microbiol 35: 1199–1202. doi: 10.1128/jcm.35.5.1199-1202.1997.
31. Zhang Y, Hua C, Li SL (2018) The relationship between the biovars and the antimicrobial resistance of *Ureaplasma urealyticum* in female patients with urogenital infections. J Clin Lab Anal 32: e22211. doi: 10.1002/jcla.22211.
32. Silwedel C, Speer CP, Haarmann A, Fehrholz M, Claus H, Schlegel N, Glaser K (2019) *Ureaplasma* species modulate cytokine and chemokine responses in human brain microvascular endothelial cells. Int J Mol Sci 20: 3583. doi: 10.3390/ijms20143583.
33. Glaser K, Gradzka-Luczewska A, Szymankiewicz-Breborowicz M, Kawczynska-Leda N, Henrich B, Waaga-Gasser AM, Speer CP (2019) Perinatal *Ureaplasma* exposure is associated with increased risk of late onset sepsis and imbalanced inflammation in preterm infants and may add to lung injury. Front Cell Infect Microbiol 9: 68. doi: 10.3389/fcimb.2019.00068.

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