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Prevalence and correlates of *Mycoplasma genitalium* infection among prostatitis patients in Shanghai, China

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Abstract. ***Background*:** *Mycoplasma genitalium* (*M. genitalium*) has been shown to be involved in chronic non-gonococcal urethritis (NGU). However, the prevalence and determinants of this emerging sexually transmissible infection among prostatitis patients remain obscure. ***Methods*:** Two hundred and thirty-five patients diagnosed with prostatitis and 152 health controls from sexually transmitted diseases (STD) clinics in Shanghai, China, were selected. *M. genitalium* was detected in the initial voided urine (VB1), midstream of urine (VB2), expressed prostatic secretion (EPS) and the opening urine after massage (VB3) by quantitative polymerase chain reaction (Q-PCR) targeting the *Mycoplasma genitalium* adhesion protein (MgPa). An infection of the prostate was considered positive if a uropathogen was found only in the EPS sample or VB3, or if it was at least four-fold greater in EPS or VB3 than in VB1 or VB2. The prostatitis patients with *M. genitalium* infection were treated with azithromycin. ***Results*:** The prevalence of *M. genitalium* infection was significantly higher among the prostatitis group than the control group (10 vs 3%, $P = 0.005$). Among the prostatitis group, *M. genitalium* infection was significantly associated with those patients who received treatment for genitourinary infection previously than those patients who did not (17 vs 6%; adjusted OR, 4.011; 95% CI, 1.562–10.300). The symptoms were totally or partially improved in 83% per cent (19/23) of prostatitis patients with *M. genitalium*, positive in EPS and *M. genitalium* turned negative after azithromycin treatment. ***Conclusions*:** *M. genitalium* was prevalent in the patients with prostatitis, particularly in those who received ineffective antibiotic treatment for the bacterium, and was identified as having a significant association of prostatitis.

Additional keywords: diagnosis, PCR, transmissible infection.

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Introduction

Prostatitis is a common syndrome that involves inflammation in the prostate gland. It is one of the most widely diagnosed conditions affecting men; it affects men in a wide age range and has a significantly negative impact on the quality of life. There are at least four identified types of prostatitis, including acute bacterial prostatitis, chronic bacterial prostatitis, chronic pelvic pain syndrome and asymptomatic inflammatory prostatitis.[1] Prostatitis, especially bacterial prostatitis, is a life-threatening disease that needs prompt recognition and treatment with antibiotics. The prevalence of prostatitis is relatively high (ranging from 3 to 16 per cent) in the world. However, due to the complex and multifactorial origin of this condition, the etiology of prostatitis is still poorly understood.[2,3]

Mycoplasma genitalium (*M. genitalium*) was first isolated in 1980 from the urethral swabs of two symptomatic men with non-gonococcal urethritis (NGU).[4] Since *M. genitalium* was discovered, it has been documented to associate with many urogenital consequences such as male and female urethritis, balanoposthitis, cervicitis, pelvic inflammatory disease (PID) and both male and female infertility.[5–8] Despite *M. genitalium* being shown to be involved with chronic NGU, there is little evidence indicating the associations with prostatitis.[9,10]

M. genitalium was not tested routinely as a pathogen in sexually transmissible diseases (STD) clinics and the precise role of this mycoplasma in the aetiology of urogenital infection has not been established because of the immense difficulty in isolating it from clinical samples. Culturing is extremely difficult and not routinely performed, while serological methods are weakly sensitive and have poor specificity.[11,12] The direct microbiological detection is done mostly by polymerase chain reaction (PCR).[13,14] So far, several PCR techniques have been

developed by using the *M. genitalium* MgPa adhesion gene (mgpB) or the 16S rRNA gene as targets.[15–18] The results from different studies have demonstrated that both mgpB and the 16S rRNA gene were sensitive and equally suitable for the detection of *M. genitalium*.[17,19,20]

Due to the fact that some prostatitis patients usually show the signs and symptoms of infection in clinic practice, such as dysuria, urgency, burning and pain or discomfort in the pelvic region, we speculated that *M. genitalium* could be a potential causal agent and play a role in the development of infectious prostatitis. In this study, we investigated the prevalence of *M. genitalium* among patients with prostatitis in Shanghai, China, and evaluated the correlation between *M. genitalium* and prostatitis, as well as the potential treatment strategy for *M. genitalium*-related prostatitis.

Methods

Study population

Our study was carried out in identified patients who were diagnosed by the same urologist from all participating STD clinics The prostatitis patients were over 20 years old (to limit the study subjects to an adult population), and encompassed chronic bacterial prostatitis and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). We retrieved controls from the STD clinic for a comparison to prostatitis patients in terms of health examination. We randomly selected controls to match the prostatitis patients in terms of age. We determined that the selected controls had no suggestive symptoms of genitourinary infection or chronic pain conditions. We excluded those subjects who had a history of mental abnormalities or a substance abuse-related disorder, or those subjects infected by *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *Candida albicans* (*C. albicans*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) or *Chlamydia trachomatis* (*C. trachomatis*).

Clinical procedures

Subjects were recruited from patients attending STD clinics at Shanghai Dermatology Hospital. Detailed epidemiological investigation, such as age, education status, regular partner status and antibiotic treatment, was made before sample collection. Then, the diagnosis of prostatitis was made according to the '2009 Chinese Diagnosis and Treatment of Prostatitis Guide' proposed by the Urological surgery branch of the Chinese Medical Association. The presence of bacteria or pathogens in the prostate was assessed by the four-glass Meares–Stamey test.[21] Expressed prostate secretion (EPS) and urine specimens were collected, and were stored at −80°C for PCR testing. Meanwhile, the numbers of red blood cells (RBC), lecithin body and leukocytes in EPS were counted under microscopy. *N. gonorrhoeae*, *C. albicans*, *E. coli* and *S. aureus* were ruled out by smear microscopy detection and culture. *C. trachomatis* was excluded by using a colloidal gold immunoassay for EPS samples (Acom, Hangzhou, China).

The localising and positive infection of *M. genitalium* was determined when a uropathogen was absent in the initial voided urine (VB1) and midstream of urine (VB2), but present in EPS or the opening urine after massage (VB3), or when a uropathogen was present in EPS or VB3 in DNA copy number at least four-fold greater than that observed in VB1 or VB2. *M. genitalium*-positive men received the following antibacterial therapies according to the '2009 Chinese Diagnosis and Treatment of Prostatitis Guide'. A PCR-based assay was performed to evaluate the efficacy of microbiological eradication in these patients.

The protocol was approved by the Shanghai Dermatology Hospital Human Subjects Committee, and the study was conducted in compliance with the ethical principles of the Declaration of Helsinki. All patients provided written informed consent before the study procedure proceeded.

Quantitative polymerase chain reaction (Q-PCR)

We performed PCR for *M. genitalium* detection by using primers previously validated. First, *M. genitalium* was detected in VB1, VB2, EPS and VB3 by using Synergy Brands, Inc. (SYBR) Green real-time PCR assay targeting the *M. genitalium* adhesion protein (MgPa). Second, all positive results were confirmed by PCR analysis using 16S rRNA-gene-targeted primers for mycoplasmas.[22,23] The SYBR Green real-time PCR assay was carried out by using a Taq PCR Core Kit following the manufacturer's protocol and using an ABI Prism 7300 (Applied Biosystems, California, USA). *Ureaplasma parvum* (*U. parvum*), *Ureaplasma urealyticum* (*U. urealyticum*), herpes simplex viruses 2 (HSV-2) and human cytomegalovirus (HCMV) were also tested by using a SYBR Green real-time PCR assay for mixed infection among the subjects, as previously described.[24]

Statistical analysis

Data were entered and managed in Microsoft Access (Microsoft Corporation, Redmond, WA, USA). The database was then transferred into the Statistical Package for Social Science (SPSS) software 20.0 (IBM Corp., Armonk, NY, USA) for statistical analysis. McNemar's tests were used for comparison of pathogens in EPS and VB3. Univariate analysis was initially used to calculate crude odds ratios (ORs) and corresponding 95% confidence intervals (CIs), followed by multiple logistic regression analysis to control for potential confounding factors. All statistical tests were two-sided, and results were considered significant at a *P*-value level of 0.05.

Results

A total of 387 men recruited from STD clinics in the Shanghai Dermatologic Hospital, over the period from May 2013 to December 2014, were included in the study. Among these, 235 men were defined as patients who had symptoms of prostatitis including dysuria, urgency, burning and pain or discomfort in the pelvic region. The control group included 152 men who attended the clinics during the same study period for an urology check-up and were clinically asymptomatic for any genitourinary infection. The mean age of the 235 men with prostatitis was 38.3 years (range 20–68; s.d. ± 10.31), and the mean age of the control group was 37.9 years (range 21–65, s.d. ± 11.58). The demographic characteristics of the study participants are shown in Table 1.

Q-PCR results for the detection of five pathogens and localisation data in EPS and VB3 are shown in

Table 1. Sociodemographic, sexual behaviour and antibiotic treatment of the study population

Variable	No. (%)	
	Controls ($n=152$)	Cases ($n=235$)
Sociodemographic characteristics		
Age, years	$P=0.832$	
20–29	35 (23)	51 (22)
30–39	51 (34)	81 (34)
40–49	46 (30)	65 (28)
≥50	20 (13)	38 (16)
Education, highest level obtained	$P=0.371$	
Primary completed	47 (31)	83 (35)
Higher than primary	105 (69)	152 (65)
Having a regular partner	$P=0.168$	
Yes	50 (33)	62 (26)
No	102 (67)	173 (74)
Antibiotic treatment		
Treatment for genitourinary infection	$P<0.001$	
Yes	21 (14)	93 (40)
No	131 (86)	142 (60)

Table 2. Outcome of Q-PCR assays for the detection of *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, HSV-2 and HCMV in the prostatitis group and the control group

The localising and positive infection of uropathogens was determined in two ways. For definition 1, the test was localising when a pathogen was absent in the initial voided urine (VB1) and midstream of urine (VB2), but present in EPS or the opening urine after massage (VB3). For definition 2, when pathogens were present in EPS or VB3, copy numbers of DNA were at least four-fold greater than that observed in VB1 or VB2. Q-PCR, quantitative polymerase chain reaction; HSV-2, herpes simplex virus 2; EPS, expressed prostatic secretion; HCMV, human cytomegalovirus; NA, not available; Neg, negative Data represent *n*

	No. of Neg	No. of VB3 alone	No. of EPS alone	No. of EPS and VB3	*P*-value
Mycoplasma genitalium					
Q-PCR results	334	1	5	47	0.219
Definition 1	368	0	2	17	NA
Definition 2	378	0	1	8	NA
Ureasplasma urealyticum					
Q-PCR results	321	4	6	56	0.754
Definition 1	369	0	3	15	NA
Definition 2	375	2	1	9	1.000
Ureaplasma parvum					
Q-PCR results	334	1	3	49	0.625
Definition 1	379	0	0	8	NA
Definition 2	372	0	1	14	NA
HSV-2					
PCR results	366	0	2	19	NA
Definition 1	385	0	0	2	NA
Definition 2	384	0	1	2	NA
HCMV					
Q-PCR results	370	0	4	13	NA
Definition 1	382	0	0	5	NA
Definition 2	384	0	0	3	NA

Table 2. Prevalence of *M. genitalium* and *U. urealyticum* infection was significantly higher in the prostatitis group than in the control group [10 vs 3% ($P=0.005$), 11 vs 2% ($P=0.001$), respectively]. No significant difference was detected between the prostatitis and control groups in terms of *U. parvum*, HSV-2 and HCMV infection status (Table 3).

The recorded numbers of RBC, lecithin body and leukocytes in EPS were calculated for all the participants by using microscopic examination. Among 387 men, 15 out of 28 patients with a *M. genitalium*-positive infection had more than 10 leukocytes per high power field (HPF) in EPS. A reduction of the amount of lecithin body in EPS was detected in 61% (17/28) of men. The values of RBC, lecithin body and leukocyte counts were not significantly related to the detection of *M. genitalium* infection in EPS.

The prevalence of *M. genitalium* infection was significantly higher among the patients who previously received treatment for genitourinary infection than those patients who did not (17 vs 6%; adjusted OR, 3.535; 95% CI, 1.750–7.142; Table 4). There was little evidence of an association of other sociodemographic factors (including age, education and sexual partner) with biological factors (including *U. urealyticum*, *U. parvum*, HSV-2 and HCMV).

In the follow-up examination, 23 men who tested positive for *M. genitalium* in the EPS samples were re-examined retrospectively. Among all the follow-up patients, prostatitis-related symptoms were eliminated in 16 patients, and three patients experienced symptom relief; however, the remaining patients had no symptom improvement. The symptom improvement rate was 83% (19/23) for patients receiving azithromycin. Notably, all patients had negative *M. genitalium* PCR tests at the end of their follow-up visit.

Discussion

It was reported that almost half of the study patients experienced symptoms of prostatitis at some point in their lives, but the aetiology remains unknown for most patients.[2] *N. gonorrhoeae*, *C. albicans*, *E. coli*, *S. aureus* or *C. trachomatis* are the most common causes of urinary tract infection. Symptom surveys alone may have difficulty in distinguishing patients with prostatitis from patients with other urological disorders. Among the infectious bacteria causing prostatitis, few studies have been conducted to elaboration on the association between *M. genitalium* and prostatitis.

The aim of this study was to investigate clinical characterisation and corresponding therapeutic response towards azithromycin in the prostatitis patients with *M. genitalium* infection. In comparison with a biopsy tissue sampling approach for the diagnosis of prostatitis, an EPS test would instead allow for a painless and fast diagnosis approach for the prostatitis patients. The results showed that *M. genitalium* infection was common in men from the STD clinics, and also showed that the men with prostatitis should be investigated for infection with *M. genitalium*, especially for those who received experiential antibiotic treatment without bacteriological test confirmation. However, the weakness of this study is that, despite 387 men being selected, the study population is relatively small and no distinction was made in terms of the symptoms of prostatitis. In addition, it should be

Table 3. Prevalence of *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Mycoplasma genitalium*, HSV-2, and HCMV infections in prostatitis patients and the control group

Neg, negative; Pos, positive; HSV-2, herpes simplex virus 2; HMCV, human cytomegalovirus

Variable	No. (%)		
	Controls ($n=152$)		Cases ($n=235$)
Mycoplasma genitalium		$P=0.005$	
Neg	148 (97)		211 (90)
Pos	4 (3)		24 (10)
Ureaplasma urealyticum		$P=0.001$	
Neg	149 (98)		208 (89)
Pos	3 (2)		27 (11)
Ureaplasma parvum		$P=0.182$	
Neg	146 (96)		218 (93)
Pos	6 (4)		17 (7)
HSV-2		$P=0.669$	
Neg	151 (99)		231 (98)
Pos	1 (1)		4 (2)
HCMV		$P=0.639$	
Neg	150 (99)		229 (97)
Pos	2 (1)		6 (3)

noted that all the control subjects were selected according to the results of clinical routine testing, and the absolute healthy control could not be found clinically, because they might have been infected with other species of bacteria. Therefore, the results could not be extrapolated to prostatitis in clinically asymptomatic patients and in the normal, healthy individual.

Although *M. genitalium* has recently been recognised as a cause of urethritis, little is known about the prognosis of *M. genitalium* infection in the upper genital tract.[9,10] To the best of our knowledge, this is the first study to reveal an association between *M. genitalium* and prostatitis, namely that *M. genitalium* was associated with prostatitis in men from STD clinics. In contrast, *U. parvum*, HSV-2 and HCMV were not associated with prostatitis, although *U. urealyticum* was also associated significantly with prostatitis. Consistently, some reports showed that the infection with *M. genitalium* and *U. ureaplasma* in men was tightly associated with NGU.[25–27] In contrast to these previous reports, the rate of *M. genitalium* infection among men with prostatitis was higher in the present study. The reason for this different infection rate could be due to the fact that some patients have received experiential but

Table 4. Sociodemographic characteristics, antibiotic treatment and biological factors associated with *Mycoplasma genitalium* (MG) infections among prostatitis patients in Shanghai, China

CI, confidence interval; OR, odds ratio; Neg, negative; Pos, positive; HSV-2, herpes simplex virus 2; HMCV, human cytomegalovirus

Variable	*N*	MG+ *n* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
	235	24 (10)		
Sociodemographic characteristics				
Age, years			$P=0.807$	$P=0.505$
20–29	51	3 (6)	1.0	1.0
30–39	81	10 (12)	1.007 (0.212–4.791)	1.584 (0.282–8.886)
40–49	65	7 (11)	0.609 (0.157–2.353)	0.587 (0.145–2.381)
≥50	38	4 (11)	0.710 (0.172–2.927)	0.768 (0.177–3.322)
Education, highest level obtained			$P=0.829$	$P=0.359$
Primary completed	83	7 (8)	1.0	1.0
Higher than primary	152	17 (11)	0.907 (0.371–2.217)	0.635 (0.240–1.678)
Having a regular partner			$P=0.234$	$P=0.237$
Yes	62	4 (6)	1.0	1.0
No	173	20 (12)	0.528 (0.173–1.609)	0.488 (0.148–1.604)
Antibiotic treatment				
Treatment for genitourinary infection			$P=0.005$	$P=0.004$
Yes	93	16 (17)	1.0	1.0
No	142	8 (6)	3.481 (1.424–8.508)	4.011 (1.562–10.300)
Biological factors				
Ureaplasma urealytalium			$P=0.241$	$P=0.503$
Neg	205	19 (9)	1.0	1.0
Pos	30	5 (17)	1.958 (0.672–5.708)	1.493 (0.462–4.821)
Ureaplasma parvum			$P=0.332$	$P=0.197$
Neg	218	21 (10)	1.0	1.0
Pos	17	3 (18)	2.010 (0.534–7.568)	2.650 (0.603–11.647)
HSV-2			$P=0.394$	$P=0.138$
Neg	231	23 (10)	1.0	1.0
Pos	4	1 (25)	3.014 (0.301–30.181)	8.021 (0.512–125.756)
HCMV			$P=0.624$	$P=0.470$
Neg	229	23 (10)	1.0	1.0
Pos	6	1 (17)	1.791 (0.201–16.003)	2.372 (0.228–24.664)

unsuccessful antibiotic treatment in clinical therapies for co-infection of *M. genitalium* and other pathogenic infections.

In clinical practice, most men with prostatitis are usually treated with antibiotics such as trimethoprim and cephalexin; however, these antibiotic treatments in our clinical findings only worked for a few patients.[28] In addition, due to the lack of a cell wall, the Mycoplasmas are susceptible to tetracyclines instead of penicillin or cephalosporin. Furthermore, because *M. genitalium* grows very slowly, a prolonged antibiotic course would be required to eradicate this pathogen.[29,30] Notably, it was evaluated by both a *M. genitalium* PCR assay and clinical symptoms that *M. genitalium* was eradicated from prostatitis patients treated with azithromycin. Given that the treatment regimens of prostatitis for *M. genitalium* are not mentioned in the '2009 Chinese Diagnosis and Treatment of Prostatitis Guide', the findings in this study could be helpful in establishing optimal treatment for *M. genitalium*-positive prostatitis patients, although the number of study subjects was limited by the relative small number of patients with prostatitis recruited from the STD clinics and by the absence of testing for other microorganisms such as *C. trachomatis* or *N. gonorrhoeae* co-infection on which we might propose our future research focus on.

In conclusion, the present study results provided evidence that *M. genitalium* was detected frequently in patients with prostatitis, particularly in those who received antibiotic treatment without bacteriological efficacy. *M. genitalium* was therefore identified as having a significant association with prostatitis. We suggest that routine testing for *M. genitalium* should be undertaken for the diagnosis of prostatitis in patients attending STD clinics to ensure that treatment is guided by etiologic diagnosis.

Conflicts of interest

None declared.

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