Open Forum Infectious Diseases

BRIEF REPORT

Prevalence of *Mycoplasma penetrans* in Urogenital Samples From Men Screened for Bacterial Sexually Transmitted Infections

Marie Gardette,[1] Arabella Touati,[1] Cécile Laurier-Nadalié,[1] Cécile Bébéar,[1,2] and Sabine Pereyre[1,2]

[1]Bacteriology Department, Bordeaux University Hospital, National Reference Center for Bacterial Sexually Transmitted Infections, Bordeaux, France, and [2]Bordeaux University, Centre National de Recherche Scientifique, Unité Mixte de Recherche 5234 Fundamental Microbiology and Pathogenicity, Bordeaux, France

Mycoplasma penetrans prevalence was assessed in urogenital samples from men screened for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Prevalence was 3.5% among men who have sex with men and 5.3% among human immunodeficiency virus (HIV)–positive patients, significantly higher than in HIV-negative individuals (0.4%, $P = .0016$). No association was found between *M. penetrans* and urogenital symptoms.

Keywords. HIV; men who have sex with men; *Mycoplasma penetrans*; prevalence; urethritis.

Mycoplasma penetrans is a genital mycoplasma discovered in the 1990s. Early studies suggested that this bacterium was associated with human immunodeficiency virus (HIV) and progression to AIDS using indirect detection of anti–*M. penetrans* antibodies in serum samples [1–5]. After the development of *M. penetrans*–specific polymerase chain reaction (PCR), the prevalence of *M. penetrans* in first-void urine samples of men with HIV was estimated to be between 1.4% and 2.5% [6–8], but no comparison with the prevalence of *M. penetrans* in HIV-negative individuals was performed. Two studies reported a higher prevalence of *M. penetrans* among HIV-positive patients compared to HIV-negative individuals, but only 1 showed a significant prevalence difference [9, 10]. Recently, *M. penetrans* was associated with nongonococcal urethritis (NGU) in men who have sex with men (MSM) but not in men who have sex with women (MSW), and no information on HIV status was available [11].

To assess the prevalence of *M. penetrans* in relation to HIV status and sexual behavior, we searched *M. penetrans* by real-time PCR in men screened for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

METHODS

Between 1 February and 31 May 2021, all male specimens submitted for *C. trachomatis* and *N. gonorrhoeae* PCR detection to the Bacteriology Department of Bordeaux University Hospital, France, were systematically and prospectively collected if they met the following inclusion criteria: urethral swab or first-void urine sample from men aged >18 years received by the bacteriology department for detection of *C. trachomatis* and *N. gonorrhoeae* by PCR, whatever the reason for seeking medical care. Clinical data, HIV status, sexual orientation, and *C. trachomatis* and *N. gonorrhoeae* detection results obtained with the Cobas CT/NG kit (Roche Diagnostics) were collated before anonymization. The extraction and inhibition real-time PCR internal control (DICD-CY-L100, Diagenode Diagnostics) was added to specimens prior to DNA extraction. A 100-µL DNA extract was obtained from 200 µL of sample using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Diagnostics). Real-time PCR targeting the 16S ribosomal RNA gene of *M. penetrans* was performed as previously described [11, 12] using 5 µL of DNA extract. *M. penetrans*–positive results were confirmed by Sanger sequencing using the same primers.

RESULTS

A total of 444 specimens (436 first-void urine specimens and 8 urethral swabs) were collected from 429 men. Two men had concurrent first-void urine and urethral swab and 13 men had 2 subsequent first-void urine during the collection period. Among the 429 included men, 295 (68.8%) visited the Department of Infectious Diseases, 77 (17.9%) visited the healthcare access center for people in need, 34 (7.9%) visited the penitentiary center, and 13 (3.0%) visited the emergency ward (Table 1). In the studied population, 34 men (7.9%) had urogenital symptoms (12 dysuria, 6 dysuria and urethral discharge, 3 urethral discharge, 1 urethral discharge and urethral itching, 1 urethral irritation, 6 testicle pain, 4 genital lesions, and 1 hematuria). Among the 34 men with genital symptoms, 18 (4.2%) patients with dysuria and/or urethral discharge received a diagnosis of urethritis from the physician who managed them. Among the 305 men for whom sexual orientation

Received 01 February 2023; editorial decision 28 March 2023; accepted 03 April 2023; published online 4 April 2023

Correspondence: Sabine Pereyre, PharmD, PhD, National Reference Center for Bacterial Sexually Transmitted Infections, CHU Bordeaux, Place Amélie Raba Léon, 33076 Bordeaux Cedex, France (sabine.pereyre@u-bordeaux.fr).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofad180

Table 1. Characteristics of the Studied Population and *Mycoplasma penetrans*–Positive and –Negative Patients

Characteristic	Population Studied (n = 429)	*M. penetrans*–Positive Patients (n = 9)	*M .penetrans*–Negative Patients (n = 420)	*P* Value[a]
Age, y				
Range	6–86	28–60	6–86	
Mean	37.1	44.2	37.0	.14
Median	34	43	34	
Sample collection sites				
Infectious disease department	295 (68.8)	9 (100)	286 (68.1)	.06
Healthcare access center for people in need	77 (17.9)	0	77 (18.3)	
Penitentiary center	34 (7.9)	0	34 (8.1)	
Emergency ward	13 (3.0)	0	13 (3.1)	
Other[b]	10 (2.3)	0	10 (2.4)	
Genital symptoms[c]				
No symptoms	395 (92.1)	9 (100)	386 (91.9)	1
≥1 symptom	34 (7.9)	0	34 (8.1)	
Urethritis	18 (4.2)	0	18 (4.3)	
Sexual orientation				
MSM	255 (59.4)	9 (100)	246 (58.6)	.36
MSW	50 (11.7)	0	50 (11.9)	
Unknown	124 (28.9)	0	124 (29.5)	
HIV serology				
Positive	152 (35.4)	8 (88.9)	144 (34.3)	.0016
Negative	267 (62.2)	1 (11.1)	266 (63.3)	
Unknown	10 (2.3)	0	10 (2.4)	
Positivity for other STI agents				
Chlamydia trachomatis[d]	14 (3.3)	0	14 (3.3)	
Neisseria gonorrhoeae	12 (2.8)	0	12 (2.9)	

Abbreviations: HIV, human immunodeficiency virus; MSM, men who have sex with men; MSW, men who have sex with women; STI, sexually transmitted infection.

[a] *P* values were calculated using Student, χ^2, or Fisher exact test as appropriate between *M. penetrans*–positive and *M. penetrans*–negative patients.

[b] Geriatric, intensive care, neurology, pediatric, and rheumatology departments.

[c] Genital symptoms include dysuria, urethral discharge, urethral itching, urethral irritation, genital lesions, hematuria, and testicle pain.

[d] Two patients with subsequent first-void urine samples during the collection period were *C. trachomatis* positive in the first urine sample and *C. trachomatis* negative in the second urine sample. They were counted as *C. trachomatis* positive.

Table 2. *Mycoplasma penetrans* Prevalence in the Study Population and Different Subpopulations

Population	*M. penetrans* Prevalence, % (*M. penetrans*–Positive Patients/Study Population)	(95% CI)
Study population	2.1 (9/429)	(1.1–3.9)
MSM	3.5 (9/255)	(1.9–6.6)
MSW	0 (0/50)	(0–7.1)
HIV positive	5.3 (8/152)	(2.7–10.0)
HIV negative	0.4 (1/267)	(.1–2.1)
No symptoms	2.3 (9/395)	(1.2–4.3)
≥1 genital symptom	0 (0/34)	(0–10.2)

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; MSM, men who have sex with men; MSW, men who have sex with women.

information was available, 255 (83.6%) were MSM; of these, 128 (50.2%) were HIV positive. Among the 419 men for whom HIV status was available, 152 (36.3%) were HIV positive and of these, 128 (84.2%) were MSM. Nine urine samples from 9 patients were *M. penetrans* positive, resulting in a prevalence of *M. penetrans* infection of 2.1% (95% confidence interval [CI], 1.1%–3.9%) in the study population (Table 2). All *M. penetrans*–positive patients were MSM. The prevalence of *M. penetrans* infection was 3.5% (95% CI, 1.9%–6.6%) among MSM compared to zero among MSW. The prevalence of *M. penetrans* infection among HIV-positive patients was 5.3% (95% CI, 2.7%–10.0%), significantly higher than 0.4% (95% CI, .1%–2.1%) among HIV-negative patients ($P = .0016$, Fisher exact test). The 8 HIV-positive and *M. penetrans*–positive patients consulted for follow-up of their HIV infection, whereas the HIV-negative *M. penetrans*–positive patient consulted for the follow-up of HIV preexposure prophylaxis. All *M. penetrans*–positive patients were asymptomatic, and all were *C. trachomatis* and *N. gonorrhoeae* negative.

DISCUSSION

In the 1990s, *M. penetrans* had been associated with HIV infection using enzyme-linked immunosorbent assay methods that detected antibodies targeting membrane-associated antigens, mainly the P38 and P42 proteins [3]. However, subsequent

research revealed cross-reactions with serum samples from patients infected with *Mycoplasma hominis*, which is another common urogenital commensal mycoplasma [2]. This finding might question the previously established association. When PCR specifically targeting *M. penetrans* was developed, the prevalence of *M. penetrans* was estimated in men with HIV [6–10]. Only 2 of these studies compared the *M. penetrans* prevalence between HIV-positive and HIV-negative individuals; 1 study showed a significant difference while the other did not [9, 10]. In the present study, we confirmed, using specific real-time PCR, the higher prevalence of *M. penetrans* in urogenital samples among HIV-positive patients compared to HIV-negative individuals.

Some authors have suggested that the presence of *M. penetrans* was linked to the progression to AIDS, with high titers of *M. penetrans* antibodies being associated with a decline of the CD4/CD8 lymphocyte ratio [3, 5]. However, this link remains controversial as others found no correlation between *M. penetrans* antibodies and the CD4/CD8 ratio [2]. In the present study, 6 of the 8 *M. penetrans*– and HIV-positive patients were evaluated for their HIV type 1 viral load and CD4/CD8 lymphocyte ratio at the time of the urogenital sample collection. Four of these patients had a CD4/CD8 ratio >1, while the remaining 2 had ratios of 0.5 and 0.6. All 6 patients had an undetectable viral load (data not shown). If a higher susceptibility of HIV-positive patients to *M. penetrans* infection may be considered, the reasons for higher *M. penetrans* prevalence in HIV-positive patients remain largely unknown. It was shown that *M. penetrans* induces $CD4^+$ and $CD8^+$ lymphocyte T activation [13]. However, the proliferative response to *M. penetrans* was not statistically different between HIV-positive patients and healthy donors [13]. Recently, potential virulence factors such as greater sialic acid–dependent binding to erythrocytes, gliding motility speed, and hydrogen peroxide production were suggested in 4 *M. penetrans* isolates from HIV-negative men with NGU [14]. The authors, however, stressed the current absence of a model for comprehending the impact of these potential factors on infection.

In the present study, all *M. penetrans*–positive patients were MSM, in accordance with the high prevalence previously reported among MSM [7, 11, 15]. Only 1 study reported a higher prevalence of *M penetrans* in MSM compared to MSW, with a prevalence of 4.9% (7/144) in the MSM population, which was similar to the proportion of 3.5% (9/255) found in our study [7]. As suggested by the spread of the sexually transmitted agent *Mycoplasma genitalium*, the dense connectivity of sexual networks in the MSM community may result in a higher prevalence of *M. penetrans* [16]. Indeed, the sexual transmission of *M. penetrans* through male homosexual practices, such as anal sex, is strongly suggested [4, 7, 12], as the rectal site may be a preferred site of infection. *M. penetrans* has been detected at the rectal site in MSM [15] and was found in 13.4% of male *C. trachomatis*–positive rectal samples submitted for lymphogranuloma venereum typing [12]. Unfortunately, the detection of *M. penetrans* at the rectal site in conjunction with the urogenital site was not feasible in our cohort.

In our study, no association was found between the presence of *M. penetrans* and urethritis or urogenital symptoms. This is in accordance with a study performed by PCR among 108 male patients with NGU, for whom no *M. penetrans* infections were detected [17], but contrasts with the recent association of *M. penetrans* with NGU found among MSM [11]. In our study, all *M. penetrans*–positive patients were asymptomatic, suggesting that this bacterium might behave like other genital *Mollicutes* species, such as *M. hominis* and *Ureaplasma parvum*. These species are not associated with urogenital symptoms in men, even though their colonization rate can be as high as 20% [18]. However, a limitation of this study is the low number of patients with NGU, which prevents us from drawing definite conclusions about the involvement of *M. penetrans* in NGU. Another limitation here is that the study population does not represent the general population but rather a high-risk group in which HIV-positive status and MSM behavior are intimately associated. Additionally, *M. genitalium* and *Trichomonas vaginalis* could not be searched in this study.

In conclusion, this study found a higher prevalence of *M. penetrans* infection in urogenital samples from people with HIV using a specific PCR method, confirming previous studies that used indirect detection of anti–*M. penetrans* antibodies in serum. The question of whether *M. penetrans* could act as a bystander, more frequently circulating among MSM in connection with their sexual practices, and more specifically in HIV-positive MSM patients due to a higher susceptibility for an as-yet unknown reason, may be raised and will have to be further evaluated in larger studies that include a higher proportion of symptomatic patients and a clinical follow-up.

Notes

Author contributions. M. G. and A. T. performed the experimental assays. C. L.-N. performed statistical analysis. S. P. designed the study and wrote the manuscript. C. B. reviewed the manuscript. All authors approved the submitted version.

Acknowledgments. We thank Laura Albucher for technical support.

Patient consent. The design of the work conformed to standards currently applied in France and was approved by the Bordeaux University Hospital committee under number CHUBX2022RE0306. According to the French regulations, this study does not include factors necessitating patient consent. Remnants of specimens were preserved at the Centre de Ressource Biologique-Bordeaux Biothèque Santé of Bordeaux University Hospital under collection number BB-0033-00094 and authorization AC-2014-2166 from the French Ministry of Higher Education and Research with no information regarding patient identity. All patient data were anonymously reported.

Financial support. This study received no external funding.

Potential conflicts of interest. All authors: No reported conflicts.

References

1. Wang RY, Shih JW, Grandinetti T, et al. High frequency of antibodies to *Mycoplasma penetrans* in HIV-infected patients. Lancet **1992**; 340:1312–6.
2. De Cordova CMM, Takei K, Rosenthal C, Miranda MARB, Vaz AJ, Dacunha RAF. Evaluation of IgG, IgM, and IgA antibodies to *Mycoplasma penetrans*

detected by ELISA and immunoblot in HIV-1-infected and STD patients, in Sao Paulo, Brazil. Microbes Infect **1999**; 1:1095–101.
3. Grau O, Tuppin P, Slizewicz B, et al. A longitudinal study of seroreactivity against *Mycoplasma penetrans* in HIV-infected homosexual men: association with disease progression. AIDS Res Hum Retroviruses **1998**; 14:661–7.
4. Wang RY, Shih JW, Weiss SH, et al. *Mycoplasma penetrans* infection in male homosexuals with AIDS: high seroprevalence and association with Kaposi's sarcoma. Clin Infect Dis **1993**; 17:724–9.
5. Grau O, Slizewicz B, Tuppin P, et al. Association of *Mycoplasma penetrans* with human immunodeficiency virus infection. J Infect Dis **1995**; 172:672–81.
6. Chen LS, Wu JR, Wang B, et al. Epidemiology of *Mycoplasma* acquisition in male HIV-1 infected patients: a multistage cross-sectional survey in Jiangsu, China. Epidemiol Infect **2015**; 143:3327–34.
7. Jian-Ru W, Bei W, Hao C, Jin-Shui X, Xi-Ping H. Mycoplasmas in the urine of HIV-1 infected men. Epidemiol Infect **2012**; 140:1141–6.
8. Wu JR, Wang B, Chen LS, et al. Alarming incidence of genital mycoplasmas among HIV-1-infected MSM in Jiangsu, China. Eur J Clin Microbiol Infect Dis **2014**; 33:189–95.
9. Wang B, Wu JR, Guo HJ, et al. The prevalence of six species of Mycoplasmataceae in an HIV/AIDS population in Jiangsu Province, China. Int J STD AIDS **2012**; 23: e7–10.
10. Cordova CMM, Blanchard A, Cunha RAF. Higher prevalence of urogenital mycoplasmas in human immunodeficiency virus–positive patients as compared to patients with other sexually transmitted diseases. J Clin Lab Anal **2000**; 14:246–53.
11. Srinivasan S, Chambers LC, Tapia KA, et al. Urethral microbiota in men: association of *Haemophilus influenzae* and *Mycoplasma penetrans* with nongonococcal urethritis. Clin Infect Dis **2021**; 73:e1684–93.
12. Perez-Prieto I, Skafte-Holm A, Jensen JS. High prevalence of *Mycoplasma penetrans* in *Chlamydia trachomatis* positive rectal samples from men: a brief report. Front Microbiol **2022**; 13:914874.
13. Sasaki Y, Blanchard A, Watson HL, et al. In vitro influence of *Mycoplasma penetrans* on activation of peripheral T lymphocytes from healthy donors or human immunodeficiency virus-infected individuals. Infect Immun **1995**; 63:4277–83.
14. Schwab NR, Young NE, Nzenwata DU, et al. Characterization of virulence-associated traits in *Mycoplasma penetrans* strains acting as likely etiological agents of idiopathic non-gonococcal urethritis [manuscript published online ahead of print 2 January 2023]. J Infect Dis **2023**. https://doi.org/10.1093/infdis/jiac505
15. Taylor-Robinson D, Gilroy CB, Keane FE. Detection of several *Mycoplasma* species at various anatomical sites of homosexual men. Eur J Clin Microbiol Infect Dis **2003**; 22:291–3.
16. Guiraud J, Helary M, Le Roy C, Elguero E, Pereyre S, Bébéar C. Molecular typing reveals distinct *Mycoplasma genitalium* transmission networks among a cohort of men who have sex with men and a cohort of women in France. Microorganisms **2022**; 10:1587.
17. Deguchi T, Gilroy CB, Taylor-Robinson D. Failure to detect *Mycoplasma fermentans*, *Mycoplasma penetrans*, or *Mycoplasma pirum* in the urethra of patients with acute nongonococcal urethritis. Eur J Clin Microbiol Infect Dis **1996**; 15:169–71.
18. Horner P, Donders G, Cusini M, Gomberg M, Jensen JS, 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**; 32:1845–51.