ORIGINAL RESEARCH ARTICLE

Prevalence of *Mycoplasma genitalium* among HIV-infected men in São Paulo city detected by realtime polymerase chain reaction

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Summary: Genital mycoplasmas are natural inhabitants of the male urethra and are potentially pathogenic species playing an aetiological role in both genital infections and male infertility. This study aims to determine the presence of *Mycoplasma genitalium* DNA in urine samples of HIV-1-infected men in São Paulo city. Realtime polymerase chain reaction (PCR) was performed using the primers My-ins and Mgso-2 and the Taqman probe Mgen-P1 as described previously. A total of 223 HIV-1-infected men were tested with a mean age of 44 years. Thirteen (5.8%) presented *M. genitalium* in urine and the co-infection was more common among homosexual men (76.9% versus 51.9%, $P < 0.26$). In conclusion, realtime PCR was a useful and rapid method for detecting *M. genitalium* DNA in urine samples. Further studies should be conducted to assess the clinical significance of these results on HIV transmission and its impact on HIV viral load.

Keywords: *Mycoplasma genitalium*, HIV-1, realtime PCR, São Paulo, Brazil

INTRODUCTION

Mycoplasmas and ureaplasmas, belonging to the family Mycoplasmataceae and Mollicutes class, are widely distributed in humans, mammals, birds, reptiles, fish, and other vertebrates as well as in plants.[1] *Mycoplasma genitalium* is the smallest bacterium of the Mollicutes class, with a genome size of 580 kb,[2–4] and it was the second genome to be fully sequenced.[2,5] This group of bacteria is characterized by the absence of a cell wall and is closely related to *Mycoplasma pneumoniae. M. genitalium*, one of the 14 species of *Mycoplasma* that infect humans, was first isolated from two men with non-gonococcal urethritis.[5,6]

M. genitalium is a human pathogen that has been isolated from both the urogenital and the respiratory tracts.[2] In a clinical study, approximately 40% of infants born to infected mothers became infected with these bacteria, and colonization of the respiratory tract of infants was associated with pneumonia and meningitis.[7] More recently, this bacterium has been described among human immunodeficiency virus type-1 (HIV-1)-infected patients.[2,5,6] In fact, non-ulcerative sexually transmitted diseases (STDs) play an important role in HIV transmission and the existence of STD Control Programmes may reduce the HIV incidence.[8] Acute urethritis is an STD commonly diagnosed in men worldwide[8,9] and *M. genitalium* has been detected in 11–20% of men with urethritis; however, in patients without urethritis, the prevalence varied from 0% to 8.5% among HIV-1-seronegative subjects.[5]

Further studies of *M. genitalium* have been inhibited because it is difficult grow this agent in culture; the procedure is time consuming and takes many months for isolation.[7,9] The polymerase chain reaction (PCR) has been successfully used to detect *M. genitalium* in clinical studies.[4] This method is able to detect *M. genitalium* DNA in symptomatic and asymptomatic individuals, thus playing an important clinical role.[10]

Some methods have been developed using urine as a biological sample for the detection of *M. genitalium* DNA.[11] Using realtime PCR to detect *M. genitalium* DNA from first urine, Yoshida *et al.* found that 3.6% of patients with gonococcal urethritis and 19.6% of patients with non-chlamydial and non-gonococcal urethritis were infected with *M. genitalium.* The aim of the present study was to determine the presence of *M. genitalium* DNA in urine samples of HIV-1-infected subjects in São Paulo city using a novel realtime PCR assay.

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MATERIAL AND METHODS

Patients

Patients were recruited from two sites. One site was the Center of Reference on HIV/AIDS (CRT-SP), which takes care of approximately 3000 HIV-1-infected subjects; 148 men were invited when they were on urological visits for screening, such as for prostate cancer or sexual impotence. An additional

75 men of 400 HIV-1-infected patients from our HIV out-clinic (ADEE3002/HC/FMUSP) in a general hospital in São Paulo city were also studied. All the patients included in this study had HIV-positive serology. The Ethical Research Board of both institutions, CRT-SP and the Hospital of Clinics of School Medicine of São Paulo University (HC/FMUSP), approved this study. After signing the informed consent, each subject was submitted to a standardized interview on epidemiological factors, including ethnic background, age, educational level, STD history and sexual behaviour, and underwent urological examination. The HIV plasma viral load, CD4+ T cells, count and time of first HIV serology were obtained from the hospital records. Thus, 60 mL of urine samples were collected from 223 HIV-1-seropositive men for this study. Patients who presented any lesion or current symptoms of an STD were not included in the analysis, but asymptomatic subjects were included in our study for assessing the sensibility of the method.

METHODOLOGY

DNA extraction

The urine samples were stored at −70°C until DNA extraction, which was performed using a commercially available kit (GFX Genomic Blood DNA Purification Kit, Amersham Pharmacia Biotech, Inc, Piscataway, NJ, USA).

To determine the presence of human β-globin gene, DNA samples were amplified by PCR using the oligonucleotides GH20 (5′-GAA GAG CCA AGG ACA GGT AC-3′) and PCO4 (5′-CAA CTT CAT CCA CGT TCA CC-3′), resulting in a fragment of approximately 268 pb.[12]

Amplification of the *M. genitalium* gene was performed by realtime PCR using the primers My-ins (5′-GTAATACATAGG TCGCAAGCGTTATC-3′) and Mgso-2 (5′-CACCACCTGTCACT CGGTTAACCTC-3′) and the Taqman probe Mgen-P1 (5′-FAM-CTGTCGGAGCGATCCCTTCGGTA-TAMRA-3′).[11] Each PCR reaction contained 1× buffer, 5 mmol/L $MgCl_2$, 10 ng each primer, 50 ng Taqman probe, 0.2 mol/L each dNTP, 1 U/mL Taq platinum and 5 μL DNA in a final volume of 50 μL. The cycle conditions follow an initial denaturation at 95°C for 10 minutes and 45 subsequent cycles at 95°C for 15 seconds and at 66°C for 1 minute. Pooled samples from 10 patients were screened and specimens from positive pools were tested individually.

PCR products were detected by 2% agarose gel electrophoresis with ethidium bromide staining under ultraviolet light to confirm the expected size (550 bp) of the *M. genitalium* gene amplicon.

RESULTS

A total of 223 HIV-1-infected patients were tested and *M. genitalium* DNA was detected in 13 (5.8%) individuals by realtime PCR.

Table 1 shows the demographic data of patients. *M. genitalium* positive individuals had a mean age of 43 years, and negative patients had a mean age of 44 years. According to their marital situation, 53.8% of *M. genitalium* positive men were living in the same house with stable partners compared with only 26.2% of negative subjects ($P = 0.06$). About 77% of the *M. genitalium* positive patients self-reported being men who have sex only with men (MSM), in contrast with 52% *M. genitalium* negative subjects ($P = 0.26$).

Table 1 **Age and sexual behaviour of the patients**

Characteristics	*Mycoplasma genitalium* (+) ($n = 13$)	*Mycoplasma genitalium* (−) ($n = 210$)	*P* value
Age (years)			
<40	5 (38.5%)	51 (24.3%)	0.20
≥40	8 (61.5%)	159 (75.7%)	
Marital status			
Living together with stable partner	7 (53.8%)	55 (26.2%)	0.06
Single or separate	6 (46.2%)	140 (66.7%)	
Bachelor or widower	0 (0%)	5 (2.4%)	
Unknown	0 (0%)	10 (4.8%)	
Route of infection			
Men who have sex with women (MSW)	1 (7.7%)	34 (16.2%)	0.26
Men who have sex with men (MSM)	10 (76.9%)	109 (51.9%)	
Users of injected drugs	0 (0%)	7 (3.3%)	
Transfusion	0 (0%)	3 (1.4%)	
Unknown	2 (15.4%)	57 (27.1%)	
Practice of oral sex			
No practice	4 (30.8%)	78 (37.1%)	0.06
Always	0 (0%)	27 (12.9%)	
Sometimes	5 (38.5%)	35 (16.7%)	
Never	3 (23.1%)	60 (28.6%)	
No answer	1 (7.7%)	10 (4.8%)	

The results of the sexual history of patients showed that in the *M. genitalium* positive group, 46.2% had their first sexual relation before the age of 15 years and 69.2% had sexual relations only with men in the last 12 months. The *M. genitalium* negative group showed that 37% had their first sexual relation before the age of 15 years ($P = 0.53$) and 57.1% had sexual relations only with men in the last 12 months ($P = 0.84$). The *M. genitalium* positive patients reported greater early sexual activity and the practice of sex exclusively with men in relation to negative *M. genitalium* patients (data not shown).

The practice of oral sex was a risk factor to acquiring *M. genitalium* in our cohort. While all *M. genitalium*-infected subjects reported that they did not always use the condom at this practice against 12.9% in the negative group, about 39% of patients reported that they sometimes practised oral sex using a condom, against only 17% of negative patients ($P = 0.06$).

Regarding clinical and laboratory characteristics, 61.5% of *M. genitalium* positive patients reported the absence of urethral discharge and 57.1% in the negative group ($P = 0.48$) (data not shown). No statistically significant differences were noted with respect to the undetectable HIV plasma viral load (VL < 1000 copies/mL; 38.5% versus 59%; $P = 0.34$) or the T CD4+ cell counts in the two groups (CD4 < 200 cells/mm^3; 23.1% versus 30.5%; $P = 0.85$) (Table 2).

DISCUSSION

M. genitalium is commonly associated with acute non-gonococcal urethritis in humans.[7] The *M. genitalium* is normally harboured in urethral tracts of healthy adults, but data show that this agent can be detected in a larger proportion of AIDS patients and asymptomatic HIV-1-infected patients.[13] In a study of the general population, the prevalence of *M. genitalium* in women and men was 2.3% and 1.1%, respectively. In both genders, increasing numbers of sexual partners

Table 2 **Clinical and laboratory characteristics of the patients**

Characteristics	*Mycoplasma genitalium* (+) (*n* = 13)	*Mycoplasma genitalium* (−) (*n* = 210)	*P* value
Current HIV viral load (last viral load)			
<1000 copies/mL	5 (38.5%)	124 (59%)	0.34
≥1000 copies/mL	6 (46.2%)	63 (30%)	
No data	2 (15.4%)	23 (11%)	
Current T CD4+ cells (cells/mm^3) (last CD4+ count available)			
<200	3 (23.1%)	64 (30.5%)	0.85
≥200	9 (69.2%)	131 (62.4%)	
No data	1 (7.7%)	15 (7.1%)	

were associated with a greater risk of being infected with *M. genitalium*.[14]

Our study showed a prevalence of 5.8% of *M. genitalium* among 223 HIV-infected men who were analysed. Immunodeficiency associated with HIV could be a factor of predisposition for mycoplasma infection. Ten of the 13 men infected had homosexual practices, perhaps indicating a risk factor for the spread of infection. This prevalence is similar to the results of Taylor-Robinson and Horner (2005)[5] and Anagrius *et al.* (2000),[15] who found 8.5% of *M. genitalium* among HIV-seronegative individuals and 6.3% of prevalence in partners of *M. genitalium*-infected patients, respectively.

In a similar study, carried out in São Paulo, Cordova *et al.* (2000)[6] detected *M. genitalium* in only 0.9% urine samples from HIV-infected subjects. However, other *Mycoplasma* species, such as *M. fermentans* and *M. penetrans*, were described at higher rates in these patients, reaching 7.5% of prevalence. Two explanations may justify these results: (a) the immunodeficiency associated with HIV may be a predisposition factor of mycoplasma infection or (b) homosexual practice is suggested as a risk factor for the infection.[6] In our study, the majority of patients are homosexual men, showing that this practice may increase the risk of mycoplasma transmission, but it was not statistically significant in our cohort.

In conclusion, 5.8% *M. genitalium* DNA, mainly among urologically asymptomatic men living with HIV/AIDS, in São Paulo were noted in our study. This rate was similar to that found among HIV-1-infected subjects from the AIDS Clinical Center (ACC), Tokyo, Japan, which used similar methods.[16] There is a strong linking of *M. genitalium* with non-gonococcal and non-chlamydial urethritis. Finally, the realtime PCR revealed a useful and fast tool for *M. genitalium* detection in a large number of urine samples. However, further studies should be conducted to assess the clinical significance on HIV transmission, impact on HIV viral load and its use in the clinical practice setting.

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