RELEVANT PREVALENCE OF *Mycoplasma hominis* AND *Ureaplasma urealyticum* SEROGROUPS IN HIV-1 INFECTED MEN WITHOUT URETHRITIS SYMPTOMS

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**SUMMARY**

*M. hominis* and *U. urealyticum* are the better-known mycoplasma species pathogenic to the human genitourinary tract, causing mainly urethritis, bacterial vaginosis and pregnancy complications. In HIV-infected patients, the prevalence and role of these species is still not well known. The aim of this work was to determine the prevalence of these species in this group of male patients (HIV group), in comparison to a group of men with clinical symptoms of urethritis (STD group). *M. hominis* was isolated from 7.5% patients (8/106) and *U. urealyticum* from 18.9% patients (20/106) from the HIV group, being among these 62.5% and 85% in significant concentrations, respectively. In the STD group these rates were 0.9% (1/110) for *M. hominis* and 13.6% (15/110) for *U. urealyticum*, being 100% and 93.3% in significant concentrations, respectively. We could demonstrate infection rates by these mycoplasma species in the HIV group as high as the one found in the STD one, what may indicate the occurrence of opportunistic infections in our population. This fact is discussed here because in immunosuppressed patients, specially *M. hominis* have been reported causing severe infections, even systemically.

**KEYWORDS:** Mycoplasma; *M. hominis*; *U. urealyticum*; HIV; Sexually transmitted diseases; Urethritis; PCR.

**INTRODUCTION**

*M. hominis* and *U. urealyticum* are the better-known mycoplasma species pathogenic to the human genitourinary tract. *U. urealyticum* is one of the major causes of nongonococcal urethritis in men, being isolated from about 12% of these cases. It has also been reported causing epididimitis, infectious kidney stones, sexually transmitted reactive arthritis (Reiter’s Syndrome) and arthritis in hypogammaglobulinemic patients. In women, its role in the etiology of pregnancy complications is suggestive, inducing pre-term labor, spontaneous abort, infertility, puerperal fever and Pelvic Inflammatory Disease. Transmission of *U. urealyticum* to the fetus or newborn may cause severe broncopulmonar displasias, and even central nervous system (CNS) infections. However, a difference of pathogenicity among its 14 serotypes has been reported, by frequency of isolation from different clinical manifestations, as well as different in vitro properties.

*M. hominis* may also cause urethritis in men, but in a lesser extension: about 3-4% of the cases. It is known as the most important agent of bacterial vaginosis, being involved in Pelvic Inflammatory Disease, pre-term labor, puerperal fever, and respiratory tract diseases in neonates, infecting the CNS in some cases.

In HIV-infected patients, the prevalence and role of these species is still not well known. However, there have been several reports about *M. hominis* systemic infections in immunosuppressed patients, sometimes severe, as well as for *U. urealyticum*. Therefore, we decided to investigate the prevalence of this species among HIV-1 infected patients without symptoms of urethritis, compared to HIV-1 negative individuals presenting these symptoms.

**MATERIALS AND METHODS**

**Patients and samples:** *HIV Group*; urethral swabs and urine samples were obtained from 106 adult male patients infected by HIV-1, without clinical symptoms of urethritis. In this group, CD4+ T cells could be obtained from 58 (54.7%) patients. Six patients had CD4+ T cells lower than 50/mm³ (10.3%), 26 between 50 and 250 (44.8%), 17 between 250 and 500 (29.3%), and 9 above 500 (15.5%). Patients with CD4+ T cells count lower than or equal to 250/mm³ were receiving prophylactic preventive therapy.

**STD Group:** urethral swabs and urine samples were obtained from 110 adult HIV-negative men attending a STD clinic, with symptoms of urethritis. Patients under antibiotic therapy were excluded from this study. In this group, 47 (42.8%) were infected with *Chlamydia trachomatis*, 7 (6.4%) with *Neisseria gonorrhoeae*, and 5 (4.5%) with other microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus spp.*, and *Trichomonas spp.*
Urines samples were concentrated ten fold by centrifugation and, as urethral swab samples, were maintained in 3 ml of A_XB modified transport medium at 4 °C for 2 hours at most until inoculation in culture media.46

**Mycoplasma culture**: from each sample in transport medium, 0.1 ml was inoculated in A7 solid media, and 0.5 ml in tubes containing 2 ml of Arginine broth, 2 ml of U10 broth, or 2 ml of SP-4 broth. From each of these tubes, serial ten fold dilutions were made three times in fresh media, resulting in culture of samples in final dilutions of 1:5, 1:50 and 1:500. Cultures were incubated at 37 °C in 2-3% CO₂ for two weeks, with the exception of SP-4 cultures which were incubated under aerobic conditions, for at least one month, with blind passages every week to new tubes, and also replicated to plates with solid SP-4 medium. Cultures with suspected *M. fermentans* growth were also incubated in Glucose broth under anaerobic conditions, using the GasPack® System of 299 bp and another of 290 bp.

**Quantification of isolates**: quantification of *M. hominis* and *U. urealyticum* present in urethral swab samples was performed by the microtiter plate technique, once this methodology is well standardized for this kind of material.46 Briefly, in a 96-well sterile plate, 30 µl of sample in transport medium were inoculated in 270 µl of broth media, namely U10 for *U. urealyticum* or Arginine for *M. hominis*, and serial ten fold dilutions from 10⁻¹ were performed. The higher dilution which changed the color of the indicator present in the broth represents the number of microorganisms in the sample, in CCU (Color Changing Units) for mL.

**Mycoplasma identification**: Sample preparation for PCR: samples were treated according to the procedure described by BARBEYRAC et al.² Briefly, 1 ml of mycoplasma culture medium was centrifuged for 15 minutes at 13,000 x g, resuspended in 0.5 ml of lysis buffer (1 mM EDTA, 10 mM Tris-HCl, 0.1% Triton X-100, 200 µg/ml Proteinase K, pH=8.0), incubated at 56 °C for 60 minutes, and at 100 °C for 10 minutes. Samples were extracted with phenol and phenol/chloroform/isoamyl alcohol. DNA was precipitated with ethanol and dissolved in 100 µl of T.E. buffer (1 mM EDTA, 10 mM Tris-HCl, pH=8.0).

*M. hominis*: identification of *M. hominis* was performed in urethral swab samples which changed the color of the indicator in Arginine broth and showed typical *Mycoplasma* spp. colonies in A7 solid medium. Identification was done by the Ouchterlony’s immunodiffusion technique¹² with polyclonal anti-*M. hominis* rabbit serum (kindly produced by Dr. Célia Otsuka Takiy, PROBAC, São Paulo, Brazil), and by the PCR technique⁴, for which two µl of Arginine broth treated as described above were added to the reaction mix. PCR was done in a final volume of 50 µl, with 1x Taq Reaction Buffer (Pharmacia Biotech, Uppsala, Sweden), 25 mM dNTP solution (Gibco BRL, Gaithersburg, MD, U.S.A.), 1 µl of each primer at 30 µM (HOM1: 5’-tgc.acc.acg.ata.tgc.cgt.ac-.3’ and UNI-1: 5’-act.acg.gga.agg.acg.cgt.a-.3’), and 1 U of Taq DNA Polymerase (Pharmacia Biotech, Uppsala, Sweden). Amplification included initial denaturation step at 95 °C for 15 minutes, followed by 30 cycles with denaturation at 95 °C for 30 seconds, primers annealing at 58 °C for 1 minute and 30 seconds, and a final extension step at 72 °C for 10 minutes. PCR products were detected by agarose gel electrophoresis at 0.8% with ethidium bromide staining under U.V. light. As a positive control we used DNA from the strain ATCC 23114 of *M. hominis*. The specificity of *M. hominis* DNA amplification was evaluated by analysis of the PCR products restriction patterns with the enzyme *Kpn* I, which yielded one fragment of 299 bp and another of 290 bp.

**U. urealyticum**: samples that showed characteristic *Ureaplasma* colonies in A7 solid medium and changed the color of the indicator in U10 broth were tested using a specific PCR assay for *U. urealyticum*. The procedure was the same described above for *M. hominis*, with utilization of different primers (Uu3: 5’-gat.ggt.aag.tta.gtt.gct.gac-3’ and Uu4: 5’-aag.acg.tct.ata.agc.aca-3’). Another set of primers (Uu1: 5’- cac.aga.tgt.cct.tga.tgt.ac-3’ and Uu2: 5’-cac.aga.tgt.cct.tga.tgt.ac-3’), specific for *U. urealyticum* strains of biotype 2, was also used.⁴ As positive control of *U. urealyticum* biotype 1 and 2, we used DNA from the strain ATCC 27618 of *U. urealyticum* and from a strain isolated in our laboratory, respectively.

**Statistical analysis**: data obtained were analysed by the Fisher’s exact Test, using the GraphPad InStat™ software (San Diego, CA, USA).

**RESULTS**

*M. hominis* and *U. urealyticum* isolation and identification from clinical samples

In the group of HIV-infected patients, 21/106 (19.8%) had a positive culture for mollicutes, in urethral swabs as well as in urine samples (Table 1). From these, *U. urealyticum* was isolated in 20 (18.9%), and *M. hominis* in 8 (7.5%). In the STD group, *M. hominis* isolation rate was lower (0.9% [1/110]; p=0.02), but there were no significant difference with *U. urealyticum* (13.6% [15/110]; p=0.37). All of the strains isolated with presumptive identification of *M. hominis* were confirmed by the

Table 1

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<th>HIV group</th>
<th>STD group</th>
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<tr>
<td></td>
<td>Isolation</td>
<td>Relevant concentrations¹</td>
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<tr>
<td>M. hominis</td>
<td>8/106 (7.5%)*</td>
<td>5/8(62.5%)&lt;br&gt;1/110 (0.9%)</td>
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<tr>
<td>U. urealyticum</td>
<td>20 /106 (18.9%)</td>
<td>17/20(85%)&lt;br&gt;15/110 (13.6%)</td>
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¹Relevant concentrations were considered ≥10⁶ CCU/mL for *U. urealyticum* and ≥10⁶ CCU/mL for *M. hominis*. The statistical difference between the results obtained with the two groups of patients is indicated: *p ≤ 0.05.
infection rates observed in our study, these microorganisms may be playing a
more important role in HIV-infected individuals in our population than
the most part of clinicians and investigators currently suspect. Therefore,
its participation in some severe syndromes in this group of patients,
may have a possible implication regarding the transmissibility of HIV.
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concentrations of a lesser pathogenic serotype of *U. urealyticum* is more significant than a lower concentration of a more pathogenic serotype. The extension of these pathogenicity differences among *M. hominis* strains is still not known. Therefore, only studies including determination of *U. urealyticum* serotypes or *M. hominis* strains differences may help us to correctly evaluate their role in human diseases, specially in immunosuppressed patients infections.

Furthermore, beyond the fact that the defective immune response of these individuals may favour their establishment, these microorganisms, mainly *M. hominis*, may be playing more relevant pathogenic roles even in progression of AIDS disease. This can be hypothesised once they have, at least in vitro, properties that can modulate the host immune system\(^4\), such as arginine depletion, cytotoxicity toward lymphoid cells, activation of monocytes and polymorphonuclear cells, induction of cytoamines production, etc. However, the extend that *M. hominis* interaction may have systemically in modulating the immune system is still not known. As we can see, in spite of the evolution that science as a whole could accomplish in the last years, there are several aspects in human mycoplasma infections that remain unclear. We believe that the prevalence and the role of these microorganisms in immunosuppressed patients must be more extensively investigated.

**RESUMO**

Prevalência relevante de *Mycoplasma hominis* e sorogrupos de *Ureaplasma urealyticum* em homens infectados pelo HIV-1 sem sintomas de uretrite

*M. hominis* e *U. urealyticum* são as espécies de micoplasmas mais conhecidas como patogênicas para o trato geniturinário humano, causando principalmente uretrite, vaginose bacteriana e complicações da gravidez. Em pacientes infectados pelo HIV, a prevalência e o papel destas espécies ainda não está bem estabelecido. O objetivo do presente estudo foi comparar a prevalência das espécies acima referidas na uretra de pacientes masculinos portadores do vírus HIV (Grupo HIV) com a prevalência das mesmas entre homens não portadores do HIV mas com sintomas de uretrite (grupo DST). No grupo HIV, *M. hominis* foi isolado de 7,5% pacientes (8/106) e *U. urealyticum* de 18,9% pacientes (20/106) em concentrações significativas de 62,5% e 85% respectivamente. No grupo DST estas taxas foram 0,9% (1/110) para *M. hominis* e 13,6% (15/110) para *U. urealyticum*, sendo 100% e 93,3% em concentrações significantes, respectivamente. Pode-se demonstrar que no grupo HIV as taxas de infecção por estes micoplasmas foram tão elevadas quanto as observadas no grupo DST. Tais achados podem indicar a ocorrência de infecção oportunista, o que, em pacientes imunocomprometidos representa risco de desenvolvimento de infecções sistêmicas.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


