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Molecular Detection of *Mycoplasma hominis* in Cervicovaginal Samples by qPCR

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ABSTRACT

Introduction: *Mycoplasma hominis* is one of the microorganisms associated with vaginal microbiota dysbiosis and infertility, and its prevention and investigation are important criteria for monitoring gynecological health during the reproductive age. **Objective:** To determine the prevalence of *M. hominis* in the cervicovaginal samples of women receiving care through the Unified Health System (SUS) and relate it to sociodemographic, behavioral, and clinical characteristics. **Methods:** Cross-sectional study carried out in a Gynecology Outpatient Clinic. The research for *M. hominis* was performed using the Real-Time Polymerase Chain Reaction (qPCR) technique in samples of cervicovaginal samples of women undergoing the Pap smear. Sociodemographic and clinical data were collected through a questionnaire, and such information was related to the chi-square (χ^2) test or Fisher's exact test, with a significance of 5%. **Results:** A total of 182 women were included, with a mean age of 43.1 ± 10.9 years, White (64.8%), with a predominance of ≤4 years of schooling (46.7%), and with paid work (59.3%). The positivity for *M. hominis* in the samples was 7.7%. The presence of the microorganism studied was positively related to having had a Pap smear more than 36 months ago ($p<0.05$). **Conclusion:** The women of reproductive age who did not undergo cervical cancer screening in the last 3 years should be considered when monitoring genital health and identifying possible pathogens that may participate in microbiota dysbiosis.

Keywords: microbiota; vagina; Real-Time PCR; Pap test; vaginal smear; Gynecology.

INTRODUCTION

Most species of Lactobacilli residing in the genital tract are responsible for maintaining a vaginal pH below 4.5 and producing lactic acid through a process of symbiosis in the vaginal microenvironment. An imbalance of these bacterial species can characterize a clinical syndrome known as bacterial vaginosis (BV), resulting in an increased abundance of bacteria such as Prevotella sp., Mobiluncus sp., *Atopobium vaginae*, *Gardnerella vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, among other fastidious anaerobic microorganisms[1]. Among these pathogens, *Mycoplasma hominis* stands out, a bacterium of the Mollicutes class, microaerophiles, which has no cell wall and is isolated from the genital tract[2]. Approximately 25.0% of women with BV have an increase in this microorganism, while *M. hominis* is detected in less than 10% of women without this clinical condition[3]. However, the role of this bacterium in vaginal dysbiosis is not yet fully understood in the literature.

BV is the most common disorder of the lower genital tract in women of reproductive age and the most prevalent cause of foul-smelling vaginal discharge. This clinical condition increases the risk of acquiring sexually transmitted infections (STIs) and can make pregnancy difficult, in addition to being related to pelvic inflammatory disease. These factors highlight the importance of investigating potential etiological agents of BV, in the case of the present study, *M. hominis*[4].

Mycoplasma spp. are capable of producing and releasing various components into their environment, including polypeptides, exopolysaccharides, and extracellular vesicles. Due to the pathogenic potential of this bacterium, both in genital and neonatal infections, clinicians must be aware of the possibility of its presence in the body and the growing resistance of *M. hominis* as a global concern[5]. At the same time, it is up to professionals to avoid routine investigations and treatments, which are contraindicated by European guidelines on sexually transmitted infections. Since the role of *M. hominis* in the vaginal microbiota is not fully understood, as it

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can colonize the genital tract of asymptomatic women and promote other infections, further research is needed in this field to provide adequate evidence[6].

The purpose of this study was to investigate the prevalence of *M. hominis* in women treated by the Brazilian Unified Health System (SUS) and to determine their sociodemographic and clinical characteristics, relating them to *M. hominis* positivity.

METHODS

This was a cross-sectional study conducted at the teaching clinic of the Federal University of Southern Frontier (UFFS) in Passo Fundo, RS, which covers the specialty of gynecology and obstetrics. Data collection was carried out from November 2020 to December 2023, and the non-probabilistic convenience sample consisted of women aged between 18 and 64 years, treated at the aforementioned outpatient clinic through the Unified Health System (SUS), who underwent cytopathological examination of the cervix (Pap smear) with registration in the Cancer Information System (SISCAN). Patients whose samples were not suitable for molecular analysis were excluded.

This study was approved by the UFFS Research Ethics Committee, under approval number 3,736,932, in accordance with Resolution 466/2021 of the National Health Council. Patients who agreed to participate in the study were informed of its objectives and signed the Informed Consent Form (ICF).

Cervicovaginal samples were collected using sterile equipment, with an Ayre spatula for collecting samples from the ectocervix and a cervical brush for collecting endocervical cells. Both samples were placed in a liquid-based cytology (LBC) vial containing CellPreserv® preservative fluid (Kolplast). A 1 mL aliquot of this material was reserved and stored at -20°C until its use in the detection of *M. hominis* DNA. In addition, an interview was conducted to

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answer a previously prepared questionnaire. The patients' medical records were also accessed for the collection of clinical data.

For the *M. hominis* research, total DNA was extracted from the samples using the NucleoSpin® Blood Kit (Macherey-Nagel, Düren, GE), following the manufacturer's guidelines. To verify the validity of DNA extraction, endogenous beta-globin gene amplification was performed using primers GH20 and PC04 [7].

Once beta-globin positivity was confirmed, these samples were subjected to real-time PCR testing for *M. hominis* using the following nucleotide sequences: 5'-CAATGGCTAATGCCGGATACGC-3' (sense primer, M1) and 5'-GGTACCGTCAGTCTGCAAT-3' (antisense primer, M2)[8], which were obtained from Integrated DNA Technologies®. DNA extracted from *M. hominis* originating from the American Type Culture Collection (ATCC #14027) was used as a positive control.

The reaction was performed in a QIAquant 5-plex system (Qiagen, Hilden, GE) in the Biochemistry and Molecular Biology laboratory at UFFS, using the SYBR Green detection system in a 20 µL total reaction. These 20 µL had the following composition: 2x qPCRBIO SyGreen Mix (PCR Biosystems Inc, London, UK), 0.8 µM of M1, 0.8 µM of M2, 4 µL of the DNA sample to be researched, and nuclease-free water to volume. The reactions followed the parameters of 3 minutes at 95°C; 40 cycles at 95°C for 5 seconds and 62°C for 30 seconds. Through serial dilution of control DNA extracted from the ATCC strain, the standard curve for analysis was generated, and a melting curve analysis was performed to verify the efficiency of the primers.

The independent variables considered were sociodemographic (age, self-reported skin color, education, marital status, employment status), behavioral (smoking habits, alcohol consumption, sexual activity, number of partners in recent years, and condom use), and clinical (time since last Pap smear, current clinical symptoms: leukorrhea, odor, pain, dyspareunia,

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pruritus). The outcome evaluated was positivity for *M. hominis*, measured using the qPCR technique.

The sociodemographic and clinical data obtained through the questionnaire, together with the laboratory results, were entered into the Epidata version 3.1 program (freely available). The PSPP statistical analysis program (freely available) was used to analyze the frequency distribution (prevalence of the outcome and proportions of independent variables). The distribution of frequencies of positivity for *M. hominis* in relation to the independent variables was verified using the chi-square test or Fisher's exact test, with a significance level of 5%.

RESULTS

The final sample consisted of 182 women, with a mean age of 43.1±10.9 years, White (64.8%), with a predominance of >5 years of schooling (53.3%), who had paid employment (59.3%). This categorization can be seen in Table 1. Additionally, 16.5% reported smoking, 26.9% reported drinking alcohol, 92.3% were sexually active, with 93.5% of these reporting a partner in the last year. Condom use was denied by 75.2% of participants, and 81.2% had undergone a Pap smear less than 36 months prior, as recommended by the Ministry of Health[9]. Regarding clinical complaints, the most frequent were pain and dyspareunia, with 36.3% and 34.6%, respectively.

Regarding laboratory analyses, 7.7% (n=14) of samples tested positive for *M. hominis*.

The relationship between sociodemographic, behavioral, and clinical variables and the detection of *M. hominis* is described in Table 2. The presence of this bacterium was not related to most variables ($p>0.05$). In contrast, *M. hominis* infection was significantly higher in women who had their last Pap smear more than three years ago ($p<0.05$). The presence of *M. hominis* was not related to the clinical complaints reported by patients in the current examination ($p>0.05$) (Table 3).

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DISCUSSION

The pathogenicity of genital mycoplasmas remains controversial in the literature; however, these microorganisms are often associated with other pathological conditions, such as bacterial vaginosis. In addition, epidemiological data indicate their relationship with infertility, as well as other gynecological-obstetric and neonatal problems[5]. Thus, studies of prevalence and relationship with clinical and epidemiological factors are important for a better understanding of the pathophysiology involved in vaginal infection by *M. hominis*.

The results of the present study showed that the prevalence of *M. hominis* in samples from women who underwent Pap smears was similar to other previous data, such as in the study by Leli *et al.*[10], who observed an overall prevalence of approximately 8 to 10%, while Moridi *et al.*[11] found a prevalence of 8.8% in a recent systematic review. In agreement, national studies report prevalences between 8 and 11%[12,13]. However, some specific conditions, considering different populations, sexual habits, age, pregnancy, and diagnostic methods used, may result in higher prevalences, from about 30% to 50%[14-16].

Regarding the age group, there was no relationship between the presence of *M. hominis* and age. Similarly, Al-Marsi et al.[17] also found no correlation between the presence of this microorganism and patients in different age groups, suggesting that there may be no predisposing age group and that all women of reproductive age may be affected by this pathogen.

It is known that condom use during sexual intercourse prevents the transmission of STIs, and although *M. hominis* is not classified as an STI, this habit reduces vaginal microbiota dysbiosis, which may protect women from this infection. In addition, a study of infertile couples indicated possible transmission of Mollicutes between marital partners[17]. However, there was no significant relationship between the factors, as reported by Plummer et al.[14], which may be related to the low number of condom users in the populations analyzed in both studies and the fact that most of the study population reported having had one partner in the last year.

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About previous cytopathological examinations, a significant relationship was observed between women who had their last examination more than three years ago and a higher prevalence of *M. hominis*, compared to those who had their last cytopathological examination less than three years ago. This data may indicate that women with greater access to women's health services, such as prevention and health promotion, following the Pap smear screening protocol, may have a lower degree of dysbiosis and presence of other microorganisms. However, to date, there is no literature that has researched this relationship. Corroborating this hypothesis, Bell et al. analyzed patients with and without STIs and described that more than a quarter of positive women had not been examined and tested at their first consultation and, thus, argue that the use of Pap smear samples may allow for increased screening in this population[18]. Additionally, in clinical practice, co-infections are often underestimated, and since screening for cytopathic changes compatible with the presence of Human Papillomavirus (HPV) is the main objective of the Pap smear, incorporating additional tests with the same biological sample to detect other microorganisms can mainly benefit HPV-positive patients. For example, Hernández-Rosas et al.[19] found co-infections and significant associations between genotypes and HPV pathogens, such as Mycoplasma spp., which frequently coexisted with HPV infection ($p<0.05$), highlighting the importance of screening within the deadlines established by the Ministry of Health to explore clinical implications for gynecological health.

While this study found no significant relationship between clinical complaints of leukorrhea and odor and positivity for *M. hominis*, other authors have reported that positivity for this bacterium increased the ratio of signs and symptoms of abnormal vaginal discharge and vaginal odor, particularly when associated with a concomitant diagnosis of bacterial vaginosis[14]. In this context, although no significant relationship was found, it was observed that most women who tested positive for *M. hominis* complained of leukorrhea, pain, odor, and dyspareunia.

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However, the role of the microorganism as a direct agent of such symptoms still needs to be further investigated, as does its role in vaginal dysbiosis[20].

Regarding the limitations of this study, it should be noted that the outpatient clinic where the patients were studied is a secondary healthcare facility and not a SUS entry point, which may restrict the population analyzed. In addition, the study had a cross-sectional design, so it was not possible to establish a causal relationship. Finally, this study contributes to the literature by presenting the overall prevalence of *M. hominis*, using the gold standard method, in a region that lacks such studies. Although routine screening of asymptomatic men and women for *M. hominis* is not recommended due to growing antibiotic resistance[6], the relationship between this bacterium and clinical and sociodemographic characteristics contributes to the current discussion of the role of this microorganism in the vaginal microbiota. In a recent review, the authors summarize the existence of symbiotic relationships between *M. hominis* and *Trichomonas vaginalis* and between dsRNA viruses, which characterize a complex pathogenic consortium that, in a context of vaginal dysbiosis, can lead to serious sequelae, including gestational complications and the acquisition and transmission of viral infections such as human immunodeficiency virus (HIV)[21].

It can be concluded that the prevalence of *M. hominis* in Cervicovaginal samples from women treated by the SUS in northern Rio Grande do Sul, Brazil is similar to that reported in the literature, occurring more frequently in women of reproductive age who have not had a preventive Pap smear in the last 3 years. Therefore, the female population with these characteristics should be considered for genital health monitoring in order to promote the identification of possible pathogens that may participate in microbiota dysbiosis, and new studies should be conducted to understand the interaction of this microorganism with the vaginal microbiota, elucidating its pathophysiological process as a host.

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Table 1: Sociodemographic, behavioral, and clinical characteristics of a sample of women treated by the Brazilian Unified Health System (SUS) who underwent cytopathological examination of the cervix in Passo Fundo, RS (n=182).

Variables	n	%
Age (full years)		
19-44	90	49.5
45-64	92	50.5
Self-reported skin color		
White	118	64.8
Not white	64	35.2
Education (complete years of study) (n=165)		
≤4 years	77	46.7
5-8 years	68	41.2
≥9 years	20	12.1
Marital status		
With partner	160	87.9
Without a partner	22	12.1
Employed	108	59.3
Smoking habit		
Yes	30	16.5
Non-smoker/former smoker	152	83.5
Alcohol consumption	49	26.9
Sexually active	168	92.3
Number of partners in the last 12 months (n=168)		
0	3	1.8
1	157	93.5
≥2	8	4.8
Condom use (n=165)		
Yes, always	25	15.2
Yes, sometimes	16	9.7
No	124	75.2
Last Pap smear (months) (n = 176)		
1-36	144	81.2
>36	32	18.2
Complaints in the current exam		
Leukorrhea	37	20.3
Odor	34	18.7
Pain	66	36.3
Dyspareunia	63	34.6
Itching	31	17.0
Positive for *Mycoplasma hominis*	14	7.7

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Table 2: Sociodemographic, behavioral, and clinical characteristics related to *Mycoplasma hominis* positivity in samples from women treated by the Brazilian Unified Health System (SUS) who underwent cytopathological examination of the cervix in Passo Fundo, RS (n=182).

	Mycoplasma hominis		
	Positive	Negative	
Variables	**n (%)**	**n (%)**	**p***
Age (full years)			0.374
19-44	8 (8.9)	82 (91.1)	
45-64	6 (6.5)	86 (93.5)	
Self-reported skin color			0.367
White	8 (6.8)	109 (93.2)	
Not white	6 (9.4)	58 (90.6)	
Education (complete years of study) (n=165)			0.300
≤4 years	4 (5.2)	73 (94.8)	
5-8 years	8 (11.8)	60 (88.2)	
≥9 years	1 (5.0)	19 (95.0)	
Marital status			0.474
With partner	13 (8.1)	147 (91.9)	
Without a partner	1 (4.5)	21 (95.5)	
Employed	6 (5.6)	102 (94.4)	0.153
Smoking habit	7 (11.3)	55 (88.7)	0.585
Yes	2 (6.7)	28 (93.3)	
Non-smoker/former smoker	12 (7.9)	140 (92.1)	
Alcohol consumption	2 (4.1)	47 (95.9)	0.219
Sexually active	13 (7.7)	155 (92.3)	0.707
Number of partners in the last 12 months			0.323
0	1 (6.7)	14 (93.3)	
1	11 (7.0)	146 (93.0)	
≥2	2 (20.0)	8 (80.0)	
Condom use (n=165)	15 (10.9)	123 (89.1)	0.764
Yes, always	2 (8.0)	23 (92.0)	
Yes, sometimes	2 (12.5)	14 (87.5)	
No	9 (7.3)	152 (92.1)	
Last Pap smear (months) (n = 176)			
1-36	7 (4.8)	137 (95.1)	0.044*
>36	5 (15.6)	27 (84.4)	

* chi-square test or Fisher's exact test; $p<0.05$

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Table 3: Relationship between *Mycoplasma hominis* positivity and clinical complaints in a sample of women treated by the Brazilian Unified Health System (SUS) who underwent cervical cytopathological testing. Passo Fundo, RS (n=182).

	Symptoms		p*
qPCR detection of Mycoplasma hominis	**Yes n (%)**	**No n (%)**	
	Leukorrhea		0.318
Positive	04 (28.6)	10 (71.4)	
Negative	33 (19.9)	133 (80.1)	
	Pain		0.09
Positive	08 (57.1)	06 (42.9)	
Negative	58 (35.2)	107 (64.8)	
	Odor		0.100
Positive	05 (35.7)	09 (64.3)	
Negative	29 (17.6)	136 (82.4)	
	Dyspareunia		0.283
Positive	06 (46.2)	7 (53.8)	
Negative	57 (34.3)	109 (65.7)	
	Itching		0.550
Positive	02 (14.3)	12 (85.7)	
Negative	29 (17.6)	136 (82.4)	

* chi-square test or Fisher's exact test; $p<0.05$

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