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Trichomonas vaginalis and *Mycoplasma* co-infection among women received in a microbiology laboratory in Dakar

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Abstract: Introduction: *Trichomonas vaginalis* and *Mycoplasma* are common causes of sexually transmitted infections, but limited prevalence data are available in Senegal. This study aimed to determine the prevalence of *T. vaginalis* and genital mycoplasma infection among women in Dakar. **Materials and Methods**: A retrospective study was carried out between 2016 and 2021 among women referred to the microbiology laboratory of the Military Hospital of Ouakam for vaginal discharge. *Mycoplasma* was detected by a commercial Mycoplasma EIS Kit using the endocervical swab. A wet mount smear and Gram staining were performed to detect *T. vaginalis*. **Results**: We analyzed a group of 1889 women, ranging in age from 14 to 81, with a mean age of 32.5 years (+/− 8.3 years). Our findings showed that 18.5% (95% CI (16–20)) of the women were infected with *Mycoplasma hominis*, while 50.5% (95% CI (48–53)) had *Ureaplasma urealyticum*. *Trichomonas vaginitis* was found in 3.5% (95% CI (2.7–4.5)) of the women. Out of the 66 patients with trichomoniasis, 68.2% were also infected with *Mycoplasma hominis* and 36.4% with *U. urealyticum*. We also observed that 88% of the women with *M. homininis* infection had *U. urealyticum.* **Conclusion**: Our study revealed a significant co-infection rate between *Trichomonas vaginalis* and *Mycoplasma*. This highlights the need for systematic mycoplasma screening in patients with urogenital trichomoniasis.

Keywords: *Trichomonas vaginalis*; *Mycoplasma hominis*; *Ureaplasma urealitycum*; women; Dakar

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1. Introduction

Vulvovaginal symptoms are among the most common reasons for women attending a health facility. The symptoms of vaginal discharge that a woman may perceive as abnormal include vulval irritation and itching. More than 30 bacteria, viruses, and parasites are known to be sexually transmitted. The most frequently identified include *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, and *Trichomonas vaginalis*, with over 350 million infections yearly worldwide [1]. Urogenital trichomoniasis is the only parasitic STI recognized as the most common non-viral STI globally, with 250 million new cases annually [2]. The infection is usually asymptomatic in men, although it may be associated with urethral discharge or dysuria. However, clinical manifestations in women are more frequent, with various symptoms such as greenish-yellow leucorrhea, dysuria, and vaginal mucosa with hemorrhagic staining [3]. In addition, *T. vaginalis* infection can cause complications in pregnant women, such as the premature rupture of membranes, premature delivery with low birth weight, infertility, or cervical cancer [4]. The transmission of *T. vaginalis* is extremely heterogeneous and depends on several factors, including socioeconomic status, age, hygiene habits, sexual behavior, post-menstrual phase, and access to health care. In addition, association with other infectious agents of the urogenital tract, such as mycoplasma, is often reported [5,6]. Mycoplasmas (*M. hominis* and *Ureaplasma* spp.) are commensals of the lower urogenital tract. Colonization varies with age, race, socioeconomic status, sexual activity, and hormonal status, and increases during pregnancy [7]. *Ureaplasma* spp. can be found vaginally in 30% of women, while *M. hominis* is found in less than 10% and these infections can cause cervicitis, infertility, premature delivery, or spontaneous abortion in pregnant women [8]. These consequences justify preventing these infections, especially in women of childbearing age. In addition, studies have suggested a symbiotic relationship between *T. vaginalis* and *Mycoplasma hominis*, but the potential synergy between these two pathogens has yet to be evaluated under clinical conditions [9]. In Senegal, most patients with signs suggestive of STIs (urethral discharge and vaginal discharge syndromes) are often diagnosed presumptively using a syndromic approach. However, this approach may lack sensitivity and specificity in some settings and lead to the mismanagement of several conditions, including urogenital trichomoniasis and *Mycoplasma* infections [10]. In addition, biological confirmation of these infections remains poor in many health facilities due to a lack of appropriate laboratories [11]. This results in a lack of available data on the epidemiology of *Mycoplasma* spp. and *T. vaginalis* co-infection, particularly in at-risk populations such as women of childbearing age. In this context, we conducted this study at the Ouakam Military Hospital. The aim was to determine the characteristics of *T. vaginalis* and mycoplasma (*M. hominis* and *U. urealyticum*) co-infection among women referred to the microbiology laboratory.

2. Material and Methods

2.1. Study design

Over a period of six years, from January 2016 to December 2021, a retrospective descriptive study was carried out. The study included all women referred to the microbiology laboratory of the military hospital of Ouakam for vaginal discharge and a search for genital mycoplasma.

From each participant, two vaginal swabs were collected as the routine diagnostic procedure. To assess the prevalence of *T. vaginalis* infection, a wet mount smear was performed immediately as part of a routine diagnostic procedure for a motile parasite. The wet mount smear was examined using an optical microscope at $\times 40$ to detect *T. vaginalis* and assess biological modifications such as the presence of epithelial cells, white blood cells, and red blood cells. *T. vaginalis* infection was considered based on a positive result from wet mount microscopy of motile trichomonad. The magnitude of white cells within the vaginal discharge was classified as follows: (i) rare: 1–5 cells/high-power field;

(ii) moderate: 6–10 cells/high-power field; (iii) many: 11–20 cells/high-power field; and (iv) high: 21 cells and above/high-power field, as described elsewhere [8]. In addition, a Gram-stained smear was performed to characterize the vaginal flora using Nugent scoring [12]. *Mycoplasma* testing was performed using the Mycoplasma IES kit (*Autobio Diagnostics Co., Ltd.; Zhengzhou-China*) for urogenital mycoplasma according to the manufacturer's recommendations. The Mycoplasma kit is based on the reaction of cultivation and biochemistry. Urea can be decomposed by Urease for *U.urealyticum* and release NH3; arginine can be decomposed by arginase for *M. hominis* and release NH3. Then, NH3 causes an increased pH of the liquid medium. The corresponding color change of the indicator is used to judge the result. The separated cultivation, identification, and enumeration of *U. urealyticum* and *M. hominis* are completed simultaneously.

2.2. Statistical analysis

Statistical analyses were performed using R software (R- 4.3.2). Continuous variables were described as mean with standard deviation. Normally distributed variables were compared with a t-test. Categorical variables were presented as percent, and Fisher exact or chi-squared tests were used for proportional assessments. Univariate logistic regression analysis assessed the association between the related risk factors and positivity. Five age groups (years) were defined for analysis, i.e., <20, 20 to 30, 31 to 40, 41 to 50, and over 50. We accepted that a two-sided significance level was set at $p \leqslant 0.05$ for all statistical tests.

3. Results

3.1. Characteristics of the study population and distribution of *Trichomonas vaginalis* infection

Over the course of the study period, 1889 patients were referred to the laboratory for vaginal discharge and genital mycoplasma testing. The mean age was 32.5 years $\pm$ 8.3. Within the study period, 2020 recorded the highest number of patients, at 18.5% (n = 355), while the lowest was noted in 2016, at 12.4% (n = 235). Out of all age groups, the 20–30-year-olds were the most frequent, accounting for 41.7% (n = 788). Vaginal flora type III was the most common, accounting for 44.8% (n = 847), followed by types IV and II with 35.5% and 19.4%, respectively. The number of white blood cells per high-power field was at the rare level, at 82% (Table 1). Out of 1889 patients, the prevalence of *Trichomonas vaginalis* infection was 3.5% (95% CI [2.7–4.5]). The highest frequency of infection was noted in 2016 at 4.7% and the lowest in 2021 at 2%. The difference was not statistically significant (p = 0.5). The frequency of *T. vaginalis* infection was highest in the 41-to-50-year-old age group, at 5.3%, while those over 50 years of age were the least affected, with a frequency of 2.4%. The difference was not statistically significant (p = 0.4). *T. vaginalis* infection was statistically associated with moderate WBC levels ($p < 0.001$) and with vaginal flora types III and IV (p = 0.048). The other characteristics of the distribution of *T. vaginalis* infection are detailed in Table 1.

Table 1: Characteristics of the study population and *Trichomonas vaginalis* infection distribution.

	Characteristics of the Study Population	***Trichomonas vaginalis* infection, N = 66**		**Overall, N = 1889**
	% [CI 95%]	**% [CI 95%]**	***p*-Value [1]**	
Study period				
2016	12 [11–14]	4.7 [2.5–8.4]		235 (100%)
2017	17 [16–19]	4.0 [2.2–6.8]		329 (100%)
2018	15 [14–17]	2.8 [1.3–5.6]	0.5	289 (100%)
2019	18 [16–19]	3.9 [2.2–6.8]		331 (100%)
2020	19 [17–21]	3.9 [2.3–6.7]		355 (100%)
2021	19 [17–21]	2.0 [0.88–4.3]		350 (100%)
Age group (years)				
< 20	4.8 [3.9–5.9]	4.4 [1.4–11]		91 (100%)
20–30	42 [39–44]	3.2 [2.1–4.7]		788 (100%)
31–40	36 [34–39]	3.1 [2.0–4.7]	0.4	687 (100%)
41–50	15 [13–17]	5.3 [3.1–8.8]		281 (100%)
> 50	2.2 [1.6–3.0]	2.4 [0.12–14]		42 (100%)
Vaginal flora type				
I	0.3 [0.10–0.65]	20.0 [1.1–70]		5 (100%)
II	19 [18–21]	1.9 [0.84–4.1]	0.048	367 (100%)
III	45 [43–47]	3.4 [2.3–4.9]		847 (100%)
IV	35 [33–38]	4.3 [3.0–6.2]		670 (100%)
White blood cells/high power field				
Rare	82 [80–84]	2.3 [1.7–3.2]		1548 (100%)
Moderate	17 [15–19]	8.4 [5.7–12]	<0.001	320 (100%)
Many	1.1 [0.71–1.7]	14.3 [3.8–37]		21 (100%)

[1] Pearsons chi-squared test; Fishers exact test.

3.2. Distribution of *Ureaplasma urealyticum* and *Mycoplasma hominis* infections

The overall prevalence of *U. urealyticum* was 50.5% (95% CI [48–53]). Within the study period, the frequency was the highest in 2018, at 55.9% (n = 171), and the lowest in 2016, at 33.2% (n = 78). The difference was statistically significant ($p < 0.001$). According to age group, patients aged between 21 and 30 were more affected, at 52.4%, followed by those under 20 and the 31–40 age group, at 51.6% and 49.2%, respectively. The difference was not statistically significant ($p = 0.6$). *U. urealyticum* prevalence was mainly associated with moderate WBC levels at 56.9% ($p = 0.04$), and vaginal flora types III and IV at 58.7% and 50.1%, respectively ($p < 0.001$). The prevalence of *M. hominis* infection was 18.2% (95% CI [16–20]). During the study period, the highest rate was observed in 2018, at 29.4% ($n = 85$), while 2019 had the lowest frequency at 13.6%. The difference was statistically significant ($p < 0.001$). Patients under 20 were the most affected, at 23.1%, while those over 41 were the least affected. This difference was not statistically significant ($p = 0.7$). *M. hominis* infection was mainly associated with many WBC levels at 28.6% ($p = 0.02$), and flora vaginal type IV in 25.8% ($p < 0.001$). Other characteristics of *M. hominis* and *U. urealyticum* infections are presented in Table 2.

Table 2: Distribution of *Ureaplasma urealyticum* and *Mycoplasma hominis* infections.

	Overall, N = 1889	***Ureaplasma urealyticum*, Positive = 954**		***Mycoplasma hominis*, Positive = 343**	
		% [95% CI]	***p*-Value [1]**	**% [95% CI]**	***p*-Value [1]**
Study period					
2016	235	33.2 [27–40]	<0.001	15.7 [11–21]	*<0.001*
2017	329	47.7 [42–53]		17.3% [13–22]	
2018	289	59.2 [53–65]		29.4 [24–35]	
2019	331	55.9 [50–61]		13.6 [10–18]	
2020	355	51.3 [46–57]		19.7 [16–24]	
2021	350	51.7 [46–57]		14.0 [11–18]	
Age group (years)					
<20	91	51.6 [41–62]	0.6	23.1 [15–33]	*0.7*
20–30	788	52.4 [49–56]		18.3 [16–21]	
31–40	687	49.2 [45–53]		18.0 [15–21]	
41–50	281	48.8 [43–55]		16.7 [13–22]	
>50	42	45.2 [30–61]		16.7 [7.5–32]	
Vaginal flora type					
I	5	40.0 [7.3–83]	<0.001	0.0 [0.00 – 54]	*<0.001*
II	367	36.8 [32–42]		6.5 [4.3–9.7]	
III	847	50.1 [47–53]		17.2 [13–23]	
IV	670	58.7 [55–62]		25.8 [23–29]	
White blood cells/high power field					
Rare	1548	49.2 [47–52]	0.043	17.1 [15–19]	*0.022*
Moderate	320	56.9 [51–62]		22.8 [18–28]	
Many	21	47.6 [26–70]		28.6 [12–52]	

[1] Pearsons Chi-squared test, Fishers exact test.

3.3. Co-infection *T. vaginalis* and *Mycoplasma*

Among the 66 patients infected with *T. vaginalis*, co-infection with *U. urealyticum* and *M. hominis* was noted in 36.4% (n = 24) and 68.2% (n = 45), respectively. The difference was statistically significant ($p < 0.001$).

3.4. Co-infection between *U. urealyticum* and *M. hominis*

Of the 343 patients infected with *M. hominis* infection, 302 presented *U. urealyticum* infection simultaneously, resulting in an 88% co-infection rate. This difference was statistically significant (0.003).

4. Discussion

Our goal was to evaluate *Mycoplasma* spp. and *Trichomonas vaginalis* infections in women in Dakar. Patients aged 20–30 were the most represented, followed by those aged 31–40. One possible explanation is that hospital-based investigation of women presenting with STI clinical signs are likely to detect genital infections in women who are sexually active and of childbearing age.

Trichomoniasis, caused by *T. vaginalis*, is a widely prevalent sexually transmitted infection (STI) worldwide. However, its prevalence varies significantly in different countries [2,13]. Limited data are available in Senegal regarding the epidemiological profile of *T. vaginalis* infections. Our study found an overall frequency of 3.5%, consistent with previous research conducted in Dakar by Tine,

who reported a frequency rate of 3.07% [14]. In addition, patients aged between 41 and 50 had the highest percentage of impact at 5.3%, though the difference was not statistically significant. In contrast, Tine et al.'s study showed that women under 25 were the most affected [14]. Reports from Egypt, Iran, and the USA indicate that urogenital trichomoniasis is prevalent among women aged 25 to 45 [15–17]. Urogenital trichomoniasis is more prevalent in sexually active age groups, which includes this age group, making them more exposed and more susceptible to infection [8,18]. Our study found a lower incidence of *T. vaginalis* infection than that reported in other countries. For instance, a prevalence of 9.5% was recorded in Zimbabwe [19]. Additionally, in many cities in Nigeria, frequencies of 18.66%, 24.1%, and 10.99% were reported in Zaria, Zos, and Maiduguri, respectively [20–22]. Differences in disease exposure or diagnostic methods could cause differences in frequency. In our study, only fresh direct examination and Gram-staining techniques were used, while it has been found that combining direct examination with culture is more effective for diagnosing urogenital trichomoniasis [23]. Previous studies have shown a strong link between urogenital trichomoniasis and low education, smoking, and sexual behavior [8,24]. However, our research did not collect any data on these variables.

T. vaginalis infection can cause non-specific biological changes, including significant inflammatory and cytolytic actions induced by the parasites. The severity of these pathogenic actions varies depending on the host and the strains of *T. vaginalis* [25,26]. Our research found that *T. vaginalis* infection caused a notable alteration in the vaginal flora, specifically of types III and IV. These findings align with previous longitudinal studies that indicate a higher likelihood of acquiring *T. vaginalis* in individuals with bacterial vaginosis [27,28].

According to our research, 50.5% of symptomatic patients had *U. urealyticum*, and 18.2% had *M. hominis*. The prevalence of most genital mycoplasma remained stable throughout the study. We could not compare our findings to previous studies on the trend of genital mycoplasma prevalence in symptomatic patients in Senegal during this period. Previously in Dakar, Tine et al. reported in their research, conducted at the Centre Hospitalier National et Universitaire de Fann, a similar prevalence of 54.9% for *U. urealyticum*. In comparison, the frequency of *M. hominis* infections was much higher at 57.4% [14]. Furthermore, according to several studies conducted in South Korea, the prevalence of *U. urealyticum* in symptomatic patients was higher than that of *M. hominis*. According to Moon et al., the prevalence of *U. urealyticum* and *M. hominis* was 21.3% and 2.9%, respectively [29], whereas it was 65.6% and 11.8% according to Kweon et al. [30], and 48.8% and 25.3% according to Jang et al. [31]. Similar values have been reported in Poland [32] and China [33]. The prevalence rates of *M. hominis* infection of 48%, 42.8%, 80%, and 70% have been reported in Cameroon, Korea, South Africa, and Papua New Guinea, respectively [34–37].

Our research indicates that *M. hominis* and *U. urealyticum* infections are more frequent in women under 40 years. However, the differences between age groups were not significant enough to draw a statistical conclusion. Tine et al. found a similar trend in their study, with the highest prevalence of STIs in patients under 45 [14]. One possible reason is that people within this age range tend to be sexually active and in their reproductive years, which may increase their susceptibility to such infections [8,18]. Furthermore, *Mycoplasma* caused significant biological changes, such as increased white blood cells observed on fresh examination. In addition, a significant association of these infections with type III or IV vaginal flora was noted. Indeed, *Mycoplasma* infections of the female urogenital tract are commonly associated with bacterial vaginosis [38,39], pelvic inflammatory disease, and cervicitis, which may result in increased white blood cell secretion [40].

As a dynamic environment, the female urogenital tract presents with a resident microflora of various species. The coexistence of other sexually transmitted microorganisms is quite common. It is due to several factors, such as a common route of transmission, the sexual behavior of the host, and the resident flora [41]. However, little attention has been paid to the presence of vaginal co-infections and their clinical and diagnostic implications. A symbiotic association between *T. vaginalis* and *M. hominis*, two microorganisms infecting the vaginal tract, has been demonstrated [37,42].

In our research, out of the 66 patients who had *T. vaginalis* infection, 45 patients had a co-infection with *M. hominis*, which is 68.2%. Additionally, it was observed that 88% of the cases had an association with *U. urealyticum*. The symbiotic relationship between *T. vaginalis* and *Mycoplasma* has been described in several studies [9,42]. It has been established that *T. vaginalis* can act as a niche and vector for transmitting *M. hominis* [43]. In addition, Mycoplasmas harbored by *T. vaginalis* have the privilege of evading the host immune response and enhancing the virulence of *T. vaginalis* [44]. This clinically significant symbiosis between these two obligate human microorganisms suggests that the routine screening of patients with *T. vaginalis* infection for mycoplasma is a better approach to optimize STI treatment practices [45]. In many cases, co-infection with *Trichomonas* and *Mycoplasma* may reduce susceptibility to antimicrobial agents, complicating the infection's eradication [37,43].

While this study provided relevant evidence on the association between *T. vaginalis* and mycoplasma, it has some limitations.

Firstly, laboratory tests included antimicrobial susceptibility testing; no follow-up assessment was performed after treatment. Further investigations would provide a better understanding of the effect of co-infection on treatment response in routine practice [46,47]. Secondly, the detection of *T. vaginalis* was based on fresh microscopic examination and staining as part of standard routine practice, and no additional investigations such as culture or PCR were performed. This may have led to an underestimation of its prevalence, as it is well established that culture in combination with direct microscopy is more sensitive to detection [48].

5. Conclusions

This study reveals that a higher percentage of the population was infected with *U. urealyticum*, while less than a quarter was infected with *M. hominis*. While the prevalence of *T. vaginalis* infection was low, an important co-infection with *Mycoplasma* spp. was observed. This emphasizes the importance of screening for mycoplasma in patients with urogenital trichomoniasis.

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Ethical Statement: This study was a hospital-based research conducted in normal conditions under the Declaration of Helsinki. Ethical permission was obtained from the hospital authorities. Information collected during the study was analyzed using the participant's identification code to ensure confidentiality.

Data Availability: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare that they have no competing interests.

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