Journal of Bacteriology & Parasitology

OPEN ACCESS Freely available online

Research Article

Mycoplasma genitalium in Senegalese Patients Attending a Private Laboratory in Dakar

Diop-Ndiaye H[1]*, Jaber R[2], Macondo E[2], Diagne R[3], Ndiaye AJS[2], Diakhaby EB[4], Sow-Ndoye A[1], Camara M[1], Ba-Diallo A[1], Dieng A[1], Siby T[2], Boye CSB[1], Toure-Kane C[2]

[1]Department of Bacteriology and Virology, Dakar Cheikh Anta Diop University, Dakar, Senegal;[2]Laboratory BIO 24, Dakar, Senegal;[3]Department of Health Sciences, University of Thies, Thies, Senegal;[4]Department of Bacteriology and Virology, CHNU Dalal Jam, Dakar, Senegal

ABSTRACT

Introduction: The contribution of M. *genitalium* in genital infections in Senegal is poorly understood due to a limited access to molecular biology platforms. The aim of this study is to document the place of M. *genitalium* infection in men and women attending a private laboratory and to document its association with other STI pathogens.

Materials and methods: M. *genitalium* detection was performed in genital secretions using RealLine *Chlamydia trachomatis/ Mycoplasma genitalium* (Biosynex, France) at BIO24 biomedical laboratory in Dakar (Senegal). In parallel, other genital pathogens including *N. gonorrhoeae* and common *Mycoplasma* species were detected through culture and microscopic analysis.

Results: From March 2016 to November 2017, genital secretions from 3550 patients were analyzed. The mean age was 32 years (range: 16 to 71) with a sex-ratio of 0.13. Overall, M. *genitalium*, C. *trachomatis*, *N. gonorrhoeae* and *U. urealyticum* were detected at a frequency of 1.7%, 2.82%, 0.5% and 11.86%, respectively. In women, C. *albicans*, bacterial vaginosis, *T. vaginalis* and M. *hominis* were found at a frequency of 19.6%, at 22%, 0.8% and 0.9%, respectively. M. *genitalium* infection was significantly more prevalent in men than women and more frequently associated with C. *trachomatis* than *N. gonorrhoeae*. One third of M. *genitalium* infected women presented also bacterial vaginosis signs and a high pH value (>4.5) of genital secretions was observed in all infected women.

Conclusion: M. *genitalium* appeared as a second most common STI pathogen identified in patients attending a private laboratory, indicating the need to include its routine detection for STI suffering patients also in public health sector.

Keywords: M. *genitalium*; Genital infections; Private laboratory; Senegal

INTRODUCTION

Mycoplasma genitalium is a sexually transmitted pathogen that has been poorly understood because of a slow and fastidious culture. Highlighted in the early 1990s with the advent of molecular biology [1], it is now well established that it is an emerging, cosmopolitan pathogen responsible for genital infections affecting both man and woman. For instance, M. *genitalium* is the common cause of non-gonococcal urethritis (UNG) in men after *Chlamydia trachomatis* [2-5]. In women, a significant association has been described in low genital tract infections such as cervicitis and urethritis and M. *genitalium* is to date the only *Mycoplasma* responsible for cervicitis [6-9].

M. *genitalium* is also responsible for upper genital tract infections like salpingitis or endometritis and certain studies showed M. *genitalium* implicated in severe complications such as ectopic pregnancies and tubal sterility [3,6,10]. It would also be responsible for prematurity [11].

However, this routine etiologic diagnosis is very little carried out in resource-limited countries such as Senegal due to the weakness of technical platforms and the need of molecular technics for M. *genitalium* diagnosis. In Senegal, only few private laboratories perform the detection of M. *genitalium* from clinical specimens and only few epidemiological data are currently available regarding the prevalence of this agent, which would be more prevalent in resource-limited countries [12].

Correspondence to: Halimatou Diop-Ndiaye, Department of Bacteriology and Virology, Dakar Cheikh Anta Diop University, Dakar, Senegal, Tel: 00221776500828; E-mail: drhalimatou@gmail.com

Received: October 18, 2019; **Accepted:** November 4, 2019; **Published:** November 11, 2019

Citation: Diop-Ndiaye H, Jaber R, Macondo E, Diagne R, Ndiaye AJS, Diakhaby EB, et al. (2019) *Mycoplasma genitalium* in Senegalese Patients Attending A Private Laboratory in Dakar. J Bacteriol Parasitol. 10:363. DOI: 10.35248/2155-9597.19.10.363.

The aim of this study is to document the place of *M. genitalium* infection in men and women attending a private laboratory for genital infection diagnosis and, to document its association with other STI pathogens.

MATERIALS AND METHODS

Study design

From March 2016 to November 2017, testing for *M. genitalium* was carried out on a cross sectional study in genital secretions for both men and women at BIO24 biomedical private laboratory in Dakar (Senegal) for each request of *C. trachomatis* detection.

Molecular testing

These 2 pathogens were identified using a multiplex real time PCR (Amplix Biosynex, France) from cervical swabs collected in women genital tract and from men urine samples. DNA extraction was performed using the Nucleic acid extractor platform ExiPrepTM16DX and real time PCR by using the RealLine *Chlamydia trachomatis/Mycoplasma genitalium* (Biosynex, France) on Amplix platform according to manufacturer instructions.

Culture and microscopic analysis

In parallel, other pathogens were detected through culture or microscopic analysis. *N. gonorrhoeae* diagnosis was performed by culture on a Thayer-Martin modified media with antibiotics (Biomerieux, France) and incubation is carried out under a CO_2 atmosphere at 37°C for 24-48 hours. *Candida* detection used vaginal swabs seeded on chromogenic yeast agar and incubates at 30°C for 24-48 hours. Diagnosis of *Mycoplasma hominis* and *Ureaplasma urealyticum* infection was performed using both liquid *Mycoplasma* IST2 media (Biomerieux, France) and *Mycoplasma* A7 media (Biomerieux, France). Trichomoniasis was diagnosed by detection of motile trichomonads on wet mount microscopy and disruption of the vaginal microbiota was characterized by Nugent score (0-3=normal microbiota (reference group), 4-6=intermediate microbiota disruption, and 7-10=Bacterial vaginosis).

Data analysis

Data were recorded in an excel file used as a support for results analysis. The infection rates were expressed as percentages (%) with their 95% confidence intervals. Statistical analysis included comparison of the frequencies were carried out by Chi-square test and a "p value" less than 0.05 was considered significant.

RESULTS

Patients

From March 2016 to November 2017, 3550 analysis requests for *C. trachomatis* and/or *M. genitalium* addressed to BIO24 and were performed accordingly to manufacturer instructions. Patients were aged from 16 to 71 years (mean=32 years old) and the sex-ratio was 0.13 (428 Men versus 3122 women).

STI and other genital pathogens detected

The STI and other genital pathogens detected in the studied population were presented in Table 1. Overall, *M. genitalium* and *C. trachomatis* were detected in 60 cases (1.7% (95% CI=[1.3-2.1]) and 100 cases (2.82% (95% CI=[2.3-3.4]), respectively. *N. gonorrhoeae* infection was detected in 19 patients giving a proportion of 0.5% (95% CI=[0.3-0.7]). In women in particular, fungal infections with *C. albicans* were found at a frequency of 19.6%, bacterial vaginosis at 22% and *T. vaginalis* at 0.8%. *U. urealyticum* infection was present in 4.2% of cases in men and 12.9% of cases in women. *M. hominis* was found in 0.9% of the study population, exclusively in women.

Table 1: Sexual transmitted pathogen and other agents detected in the genital tract.

	Total n (%) N=3550	95% CI
C. trachomatis	100 (2.82%)	2.3-3.4
M. genitalium	60 (1.69%)	1.3-2.1
N. gonorrhoeae	19 (0.53)	0.3-0.8
T. vaginalis	30 (0.84)	0.5-1.1
C. albicans	613(19.63%)*	18.3-21
G. vaginalis ± *Mobilincus*	688 (22%)*	20.5-23.5
U. urealyticum	421 (11.86%)	10.8-12.9
M. hominis	35 (0.98%)*	0.7-1.3

*Testing performed only in women

Factors associated to *M. genitalium* infection

According to sex distribution, as shown in Table 2, *M. genitalium* infection were significantly more prevalent in male than women as well as other STI pathogens except *U. urealyticum* which was significantly more frequent in women. Moreover, *M. genitalium* infection in women appears to be significantly less common than *C. trachomatis* infection; furthermore these 2 pathogens were significantly more frequent than *N. gonorrhoeae* infection in both men and women (Table 2).

Table 2: Pathogens detected according to sex.

	Men n (%) N=428	Women n (%) N=3122	p value
C. trachomatis	37 (8.64%)	63 (2.01%)	<0.01
M. genitalium	30 (7%)	30 (0.96%)	<0.01
N. gonorrhoeae	14 (3.27%)	5 (0.16%)	<0.01
U. urealyticum	18 (4.2%)	403 (12.9%)	<0.01

Table 3 highlights *M. genitalium* association with other pathogens. Regarding dual infection by both *M. genitalium* and *C. trachomatis*, 2.8% (12/428) of men were concerned whereas only 0.5% (16/3122) of women. Comparatively, the proportion of dual infection by both *M genitalium* and *N. gonorrheae* was very low with 0.7% (3/428) and 0.03% (1/3122) in men and women, respectively.

Table 3: Association of *M. genitalium* with other STI and genital pathogens.

	Men Mg pos		Women Mg pos		
	% (n/N)	OR [95% CI] ; p value	% (n/N)	OR [95% CI]; p value	p value (men vs. women)
C. Trachomatis	2.8% (12/428)	9.9 [4.3-22]; <0.01*	0.5% (16/3122)	74 [34.1-160]; <0.01*	<0.01*
N.gonorhoeae	0.7% (3/428)	3.9 [1-14]; 0.04*	0.03% (1/3122)	26.6 [2-245]; <0.01*	<0.01*
C. albicans	na		0.16% (5/3122)	0.8 [0.3-2.1]; 0.6	
G. vaginalis +/- Mobilincus	na		0,3% (10/3122)	1.8 [0.8-3.8]; 0.13	
U. urealyticum	0,2% (1/428)	0.7 [0.09-2]; 0.06	0.5% (7/3122)	2 [0.8-4.8]; 0.08	0.39

Therefore, *M. genitalium* appeared to be significantly more

frequently associated with *C. trachomatis* than *N. gonorrhoeae* (p<0.01). However, the risk of being co-infected is highest for women for both *C. trachomatis* (OR=74; p<0.01 vs. OR=9.9; p<0.01) and *N. gonorrhoeae* (OR=26.6; p<0.01 vs. OR=3.9; p<0.04). Moreover, one third of *M. genitalium* infected women was also infected with bacterial vaginosis pathogens, and a high pH value (>4.5) of genital secretions was observed in all infected women even in absence of bacterial vaginosis; two-third (66.7%) had abundant leucorrhea and 66.7% presented also inflammation with leucocytes>5 cells per field. Regarding symptoms in women, abundant leucorrhoea was the most common symptom for both pathogens while Pelvic pain was more common in *M. genitalium*-infected women (14.3%) than in *C. trachomatis*-infected women (2.1%) (Figure 1).

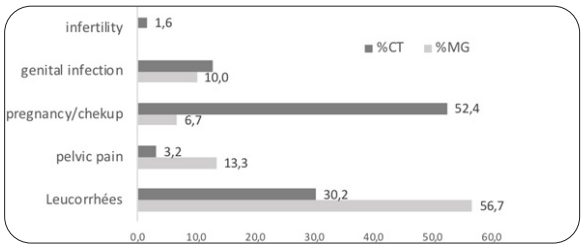

Figure 1: Distribution of symptoms/reason of consultation reported in women infected with *M. genitalium* and/or *C. trachomatis.*

Urethritis and micturition burns were the most common symptoms in men for both *M. genitalium* and *C. trachomatis* infection whereas infertility was noted in 5.4% of men infected with *C. trachomatis*. One case of prostatitis and one case of epididymitis were reported for patients infected by *M. genitalium* (Figure 2).

DISCUSSION

M. genitalium is an emerging STI pathogen; its role in genital infections has been ignored in most developing countries and still probably underestimated, due to a suboptimal diagnosis related to a lack of knowledge of the pathogen and lack of molecular biology platforms on health facilities and clinical laboratories. In Senegal, *M. genitalium* diagnosis is not realized in routine laboratory testing and only few private structures like BIO24 performed its detection using molecular tools. To our knowledge this is the first description of *M. genitalium* infection in the country. On a period of 19 months, a Mutiplex PCR was performed for the detection of both *M. genitalium* and *C. trachomatis* from a large number of cervical swabs and men's urine specimens.

With a prevalence of 1.69%, *M genitalium* appeared to be the 2nd most common STI pathogen detected after *C. trachomatis* as in different studies [13-15]. This prevalence was lowest than those reported in many developing countries where *M genitalium* was found at rates ranging from 3.2% to 5.2% [12]. This low prevalence includes patients without symptoms (one third of men and about

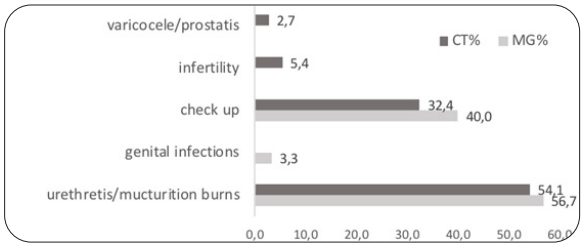

Figure 2: Distribution of symptoms/reason of consultation reported in men infected with *M. genitalium* and/or *C. trachomatis.*

40% of women detected positive for *M. genitalium*) and demonstrates that this pathogen could be identified in asymptomatic persons in a non-negligible proportion [16]. This prevalence was more related to those reported in high-income countries (HIC) like in study realized among 5628 patients consulting for STI [14], or among 1652 patients from general population [17], (or among adults (16-44 years) during the third national survey [18] with 1.9% in Netherland, 1.7% in Denmark and 1.2% in England, respectively.

However, in a multicenter study realized in France including 2652 urogenital tract specimens collected all around the country, the picture was different with 3.4% [16]. In fact, it is well established that M genitalium prevalence could varied among different risk groups; the highest rates were found in STI treatment centers [16,19], key populations such as Men having sex with men (MSM) or female sex workers [20-22], or HIV infected patients [23-25]. According to sex, M genitalium was found significantly more prevalent in men than women (7% vs. 0.96%; p<0.01). This is in line with studies in general population or community based in other africans countries but the prevalence found in women was slightly lower than those reported with 3.2% in Tanzania [26], 2.7% at Madagascar [12], as well as in a recent study in adult women in Chad [27].

In the other hand, men appear very vulnerable to M genitalium with prevalence (7%) similar to what was found in MSM [12] or in a specific population of men with urethral discharge [2,28]. This difference could be explained by the relatively small number of samples analyzed comparing to women, the bias linked to the frequentation of clinics by men only when they have symptoms, and finally related to the specificity of *M. genitalium* for urethritis in men. Clinical symptoms for *M. genitalium* positive patients were similar to those noted by Pereyre et al. in France with urethral discharge and vaginal discharge accounted for the majority of the signs observed. However, in this French study, unlike our study, a high percentage (70%) of *M. genitalium* positive patients were found with no associated symptoms [16].

Regarding the other factors associated with *M. genitalium* infection in women, it appears that vaginal pH was greater than 4.5 (ranging from 4.6 to 6.7) in all *M. genitalium* infected women which corroborate Huppert et al. results who demonstrated that pH>4.5 was a predictive sign of *M. genitalium* infection (odds ratio 4.4, p<0.05). He showed that among women without bacterial vaginosis, and not infected with TV, 25% of those with a vaginal pH>4.5 were infected with M. genitalium, compared to 9% for those with a pH ≤ 4.5 (p=0.02) [29].

Regarding the microscopic signs of the inflammatory reaction (number of leukocytes/ microscopic field), 66.7% of women positive for *M. genitalium* had an important inflammation with more than 4.5 leucocytes/microscopic field. These results agreed with Anagrius et al. study, which show a strong association between *M. genitalium* infection and microscopic signs of urethritis and/ or cervicitis [30]. *M. genitalium* appears to be also associated to bacterial vaginosis that could enhance patient susceptibility to infection as described par Lokken [31].

CONCLUSION

This pioneering study focusing on *M. genitalium* detection in both men and women in a private laboratory in Senegal, showed a prevalence that place it as a second most common STI pathogen identified in both symptomatic and asymptomatic patients.

In women, *M genitalium* infection was often associated to bacterial vaginosis and could be suspected in case of large inflammation reaction associated to pH value greater than 5 whereas the proportions in men highlighted the need of its detection especially in men suffered for urethritis. These results showed also the risk of co-infection with others STI pathogen that could impact at long term fertility and should be considered for a better uptake of cases. Further studies in public health sector as well as advocacy for clinician's awareness in order to increase demand of *M. genitalium* testing and for decision-makers to better consider this agent in STI programs are needed.

REFERENCES

1. Palmer HM, Gilroy CB, Furr PM, Taylor-Robinson D. Development and evaluation of the polymerase chain reaction to detect *Mycoplasma genitalium*. FEMS Microbiol Lett. 1991; 61(2):199-203.
2. Pépin J, Sobéla F, Deslandes S, Alary M, Wegner K, Khonde N, et al. Etiology of urethral discharge in West Africa: The role of *Mycoplasma genitalium* and *Trichomonas vaginalis*. Bull World Health Organ. 2001; 79(2): 118-126.
3. Cazanave C, Manhart LE, Bébéar C. *Mycoplasma genitalium*, an emerging sexually transmitted pathogen. Med Mal Infect. 2012;42(9):381-392.
4. Plantamura J, Bigaillon C, Bousquet A, Delaune D, Larreche S, Bugier S, et al. *Mycoplasma genitalium*: A mycoplasma still underestimated. Ann Biol Clin. 2017;75(2):209-214.
5. Libois A, Hallin M, Crucitti T, Delforge M, De Wit S. Prevalence of *Mycoplasma genitalium* in men with urethritis in a large public hospital in Brussels, Belgium: An observational, cross-sectional study. PLoS One. 2018;13(4):1-6.
6. Manhart LE, Critchlow CW, Holmes KK, Dutro SM, Eschenbach DA, Stevens CE, et al. Mucopurulent cervicitis and *Mycoplasma genitalium*. J Infect Dis. 2003; 187(4): 650-657.
7. Pepin J, Labbe AC, Khonde N, Deslandes S, Alary M, Dzokoto A, et al. *Mycoplasma genitalium*: An organism commonly associated with cervicitis among west African sex workers. Sex Transm Infect. 2005;81(1):67–72.
8. Moi H, Reinton N, Moghaddam A. *Mycoplasma genitalium* in women with lower genital tract inflammation. Sex Transm Infect. 2009;85(1):10-14.
9. Oakeshott P, Aghaizu A, Hay P, Reid F, Kerry S, Atherton H, et al. Is *Mycoplasma genitalium* in women the "New Chlamydia"? A community-based prospective cohort study. Clin Infect Dis. 2010;51(10):1160-1166.
10. Tsevat DG, Wiesenfeld HC, Parks C, Peipert JF. Sexually transmitted diseases and infertility. Am J Obstet Gynecol. 2017;216(1):1-9.
11. Hitti J, Garcia P, Totten P, Paul K, Astete S, Holmes KK. Correlates of cervical *Mycoplasma genitalium* and risk of preterm birth among Peruvian women. Sex Transm Dis. 2010; 37(2): 81-85.
12. Baumann L, Cina M, Egli-Gany D, Goutaki M, Halbeisen FS, Lohrer GR, et al. Prevalence of *Mycoplasma genitalium* in different population groups: Systematic review and meta-analysis. Sex Transm Infect. 2018; 94(4): 255-262.
13. Svenstrup HF, Dave SS, Carder C, Grant P, Morris-Jones S, Kidd M, et al. A cross-sectional study of *Mycoplasma genitalium* infection and correlates in women undergoing population-based screening or clinic-based testing for Chlamydia infection in London. BMJ Open. 2014;4(2):1-8.
14. Jenniskens ML, Veerbeek JH, Deurloo KL, van Hannen EJ, Thijsen SF. Routine testing of *Mycoplasma genitalium* and *Trichomonas vaginalis*. Infect Dis. 2017;49(6):461-465.
15. Ferré VM, Ekouevi DK, Gbeasor-Komlanvi FA, Collin G, Le Hingrat Q, Tchounga B, et al. Prevalence of human papillomavirus, human immunodeficiency virus and other sexually transmitted infections among female sex workers in Togo: a national cross-sectional survey. Clin Microbiol Infect. 2019;25(12):1-5.
16. Pereyre S, Nadalié CL, Bébéar C, investigator group, *Mycoplasma genitalium* and *Trichomonas vaginalis* in France: A point prevalence study in people screened for sexually transmitted diseases. Clin Microbiol Infect. 2017;23(2):3-6.
17. Andersen B, Sokolowski I, Østergaard L, Møller JK, Olesen F, Jensen JS. *Mycoplasma genitalium*: Prevalence and behavioural risk factors in the general population. Sex Transm Infect. 2007;83(3):237–241.
18. Sonnenberg P, Ison CA, Clifton S, Field N, Tanton C, Soldan K, et al. Epidemiology of *Mycoplasma genitalium* in British men and women aged 16–44 years: Evidence from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). Int J Epidemiol. 2015;44(6):1982–1994.
19. Mobley VL, Hobbs MM, Lau K, Weinbaum BS, Getman DK, Seña AC. *Mycoplasma genitalium* infection in women attending a sexually transmitted infection clinic: Diagnostic specimen type, coinfections, and predictors. Sex Transm Dis. 2012;39(9):706-709.
20. Edlund M, Blaxhult A, Bratt G. The spread of *Mycoplasma genitalium* among men who have sex with men. Int J STD and AIDS. 2012;23(6):455-456.
21. Vandepitte J, Muller E, Bukenya J, Nakubulwa S, Kyakuwa N, Buvé A, et al. Prevalence and correlates of *Mycoplasma genitalium* infection among female sex workers in Kampala, Uganda. J Infect Dis. 2012;205(2):289-296.
22. Deborde M, Pereyre S, Puges M, Bébéar C, Desclaux A, Hessamfar M, et al. High prevalence of Mycoplasma genitalium infection and macrolide resistance in patients enrolled in HIV pre-exposure prophylaxis program. Med Mal Infect. 2019;49(5):347-349.
23. Leutscher P, Jensen JS, Hoffmann S, Berthelsen L, Ramarakoto CE, Ramaniraka V, et al. Sexually transmitted infections in rural Madagascar at an early stage of the HIV epidemic: A 6-month community-based follow-up study. Sex Transm Dis. 2005;32(3):150-155.
24. Gatski M, Martin DH, Theall K, Amedee A, Clark RA, Dumestre J, et al. *Mycoplasma genitalium* infection among HIV-positive women: Prevalence, risk factors and association with vaginal shedding. Int J STD and AIDS. 2011;22(3):155-159.
25. Cazanave C, Lawson-Ayayi S, Hessamfar M, Neau D, Dupon M, Morlat P, et al. Prevalence of *Mycoplasma genitalium* among HIV-infected women, Agence Nationale de Recherches sur le SIDA et les hépatites virales CO3 Aquitaine Cohort, France. Sex Transm Dis. 2013;40(8):653-654.
26. Kapiga SH, Sam NE, Mlay J, Aboud S, Ballard RC, Shao JF, et al. The epidemiology of HIV-1 infection in northern tanzania: results from a community-based study. AIDS Care. 2006;18(4):379–387.
27. Nodjikouambaye ZA, Compain F, Sadjoli D, Mboumba Bouassa RS, Péré H, Veyer D, et al. Accuracy of Curable Sexually Transmitted Infections and Genital *Mycoplasmas* Screening by Multiplex Real-Time PCR Using a Self-Collected Veil among Adult Women in Sub-Saharan Africa. Infect Dis Obstet Gynecol. 2019;2019(1):12-15.
28. Ekiel A, Aptekorz M, Klucinski P, Smolec D, Wiechula B, Jozwiak J, et al. Prevalence of Urogenital *Mycoplasmas* Among Men with NGU in Upper Silesia, Poland. Preliminary Study. Pol J Microbiol. 2016;65(1):93-95.
29. Huppert JS, Bates JR, Weber AF, Quinn N, Gaydos CA. Abnormal vaginal pH and *Mycoplasma genitalium* infection. J Pediatr Adolesc Gynecol. 2013;26(1):36-39.
30. Anagrius C, Loré B, Jensen JS. *Mycoplasma genitalium*: Prevalence, clinical significance, and transmission. Sex Transm Infect. 2005;81(6):458-462.
31. Lokken EM, Balkus JE, Kiarie J, Hughes JP, Jaoko W, Totten PA, et al. Association of Recent Bacterial Vaginosis With Acquisition of *Mycoplasma genitalium*. Am J Epidemiol. 2017;186(2):194-201.