

# Sexually transmitted pathogens, coinfections and risk factors in patients attending obstetrics and gynecology clinics in Jalisco, Mexico

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Sexually transmitted pathogens, coinfections and risk factors in patients attending obstetrics and gynecology clinics in Jalisco, Mexico.  
Salud Publica Mex 2016;58:437-445.  
<http://dx.doi.org/10.21149/spm.v58i4.8024>

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## Abstract

**Objective.** To determine the frequency of nine sexually transmitted pathogens, coinfections and risk factors in patients attending obstetrics and gynecology clinics in Jalisco, Mexico. **Materials and methods.** Samples from 662 patients attending obstetrics and gynecology clinics were analyzed. *Treponema pallidum*, HIV, and HCV were detected by serology. HPV was detected by Polymerase Chain Reaction (PCR), and its genotype was determined by Restriction Fragment Length Polymorphism (RFLP). *Trichomonas vaginalis*, HSV-1, HSV-2, *Mycoplasma genitalium*, *Neisseria gonorrhoeae* and *T. pallidum* were detected by multiplex PCR. **Results.** By serology, HIV frequency was 6.8%, *T. pallidum* was 2.26%, and HCV was 0.15%. By PCR, HPV frequency was 13.9% (more frequent genotype was 16, 33.7%), followed by *T. vaginalis* (14.2%), HSV-1 (8.5%), *M. genitalium* (2.41%), *N. gonorrhoeae* (2.11%), HSV-2 (1.8%), and *T. pallidum* (1.05%). Patients

## Resumen

**Objetivo.** Determinar la frecuencia de nueve patógenos de transmisión sexual, coinfecciones y factores de riesgo en pacientes que acudieron a una consulta de ginecología y obstetricia en Jalisco, México. **Material y métodos.** Se analizaron muestras de 662 pacientes que asistieron a la consulta de ginecología y obstetricia. Se detectaron *Treponema pallidum*, VIH y VHC mediante serología. Se detectó VPH por Reacción de Cadena de Polimerasa (PCR) y sus genotipos se detectaron por Polimorfismos de Longitud de Fragmentos de Restricción (RFLP). Se detectaron *Trichomonas vaginalis*, VHS-1, VHS-2, *Mycoplasma genitalium*, *Neisseria gonorrhoeae* y *T. pallidum* por PCR múltiple. **Resultados.** Por serología, la frecuencia de VIH fue 6.8%, de *T. pallidum* fue 2.26% y de VHC fue 0.15%. Por PCR, la frecuencia más alta fue de VPH (13.9%, el genotipo más frecuente fue el 16, 33.7%), seguida de *T. vaginalis* (14.2%), VHS-1 (8.5%), *M. genitalium* (2.41%), *N. gonorrhoeae*

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Received on: November 17, 2015 • Accepted on: May 2, 2016

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infected with *T. vaginalis* were more likely to have multiple coinfections ( $p = 0.01$ ). **Conclusion.** The frequency of HPV, HSV-1, HSV-2, *M. genitalium* and *T. vaginalis* was lower than that reported. However, a high frequency of HIV, *T. pallidum*, and *N. gonorrhoeae* was detected.

**Keywords:** sexually transmitted disease; co-infections; pregnancy; gynecology; Mexico

(2.11%), VHS-2 (1.8%) y *T. pallidum* (1.05%). Los pacientes infectados con *T. vaginalis* presentaron más probabilidades de tener múltiples coinfecciones ( $p = 0.01$ ). **Conclusiones.** La frecuencia de infección por VPH, VHS-1, VHS-2, *M. genitalium* y *T. vaginalis* fue menor a lo reportado. Sin embargo, se detectó una alta frecuencia de VIH, *T. pallidum*, y *N. gonorrhoeae*.

**Palabras clave:** infecciones de transmisión sexual; coinfección; embarazo; ginecología; México

Sexually transmitted infections (STIs) are caused by more than 30 pathogens.<sup>1</sup> Each year, an estimated of 500 million individuals are infected with one of the four curable STIs. Some of these infections can have serious consequences beyond the immediate effects of the disease itself, such as a transmission of infection from mother to child during pregnancy or childbirth, and chronic illness. Furthermore, other STIs cause significant health problems to the population.<sup>2</sup>

In Mexico, STIs are one of the top five diseases on demand for medical care and are among the top ten causes of general morbidity in the group of 15-44 years old individuals, with an average of 220 000 cases annually.<sup>3</sup>

Although there are some STI studies in Mexico,<sup>4,5</sup> the frequency of STIs is not well known. There are few studies on the detection of these microorganisms by PCR, correlated coinfections, and risk factors involved.

The aim of this study was to determine the frequency of nine sexually transmitted pathogens, coinfections and risk factors in patients attending obstetrics and gynecology clinics in Jalisco, Mexico.

## Materials and methods

### Ethics statement

This study was revised and approved by the Ethics Committee of Hospital Civil de Guadalajara "Fray Antonio Alcalde" (Approval no. 062/13). Written informed consent was obtained from patients or from caretakers, or guardians on behalf of the minors enrolled in this study.

### Study population

Six hundred and sixty-two patients attending for the first time the obstetrics and gynecology clinics at the Hospital Civil de Guadalajara "Fray Antonio Alcalde", from September 2013 to August 2014 were enrolled in this study. Sociodemographic, behavioral and biological data associated with genital infections were collected.

### Sample collection, microbiological culture, and DNA extraction

For each patient, two endocervical cotton swabs transported in Stuart's medium, and serum samples were collected.

One cotton swab was used for microbiological culture and the second was used for DNA extraction. The culture was performed for identification of bacteria and yeast as recommended by the American Society for Microbiology.<sup>6</sup>

For DNA extraction, a cotton swab was resuspended in 200  $\mu$ L of 100 mM Tris-HCl. Following vortexing, 150  $\mu$ g of lysozyme was added, and the mixture was incubated at 37°C overnight, and then, genomic DNA was extracted with a standard phenol-chloroform-isoamyl alcohol protocol.

### Serological assays

IgG, IgM anti-*T. pallidum* antibodies, HIV antibodies and p24 antigen were detected in serum samples by chemoluminescence using the ARCHITECT instrument (Abbott, Germany) according to manufacturer's instructions.<sup>7</sup> Qualitative detection of HCV antibodies in serum samples was performed using gold colloid immunochromatography with the Advanced Quality Rapid Anti-HCV Test, Acitrack (Mexico City), according to manufacturer's instructions.

### VPH detection and genotyping

HPV detection was performed by PCR amplifying a highly conserved region of the L1 gene as previously described.<sup>8</sup> Positive samples were genotyped by RFLP as described.<sup>9</sup>

### Multiplex PCR for detection of pathogens

Detection of *T. vaginalis*, HSV-1, HSV-2, *M. genitalium*, *N. gonorrhoeae*, and *T. pallidum* was performed using

a multiplex PCR.<sup>10</sup> The final volume (25 uL) of PCR reaction consisted of 2.5 uL of 10X PCR-buffer, 100 ng of DNA, 3 mM MgCl<sub>2</sub>, 200 uM of each dNTP, and 1 U of Taq polymerase (Bioline, London, UK).

### Statistical analysis

The qualitative variables are summarized by calculating absolute frequency expressed in percentages. Two by two tables were used to assess the possible association between a risk factor "exposure" and a result "infection". Multivariate logistic regression models were used to estimate the odds ratio (OR) and 95% confidence intervals (95% CI) for the association between risk factors and prevalence of STIs.

The variables studied regarding the risk factors were divided into three groups: sociodemographic data (age, occupation [housewife, employee, merchant, professional, student], marital status [single, divorced, widow, separated, married, living with a partner]); behavioral data (alcohol consumption, smoking, age of first sexual activity [ $< 18$  years,  $\geq 18$  years], number of sexual partners [1, 2-4,  $\geq 5$ ], contraceptive methods [hormonal, condom, intrauterine device, bilateral tubal occlusion, none]); and biological factors (pregnant during the sampling, previous pregnancies, complications of previous pregnancy infections, bleeding, the risk of spontaneous abortion, premature birth, ectopic pregnancy, previous abortions, pelvic inflammatory disease).

## Results

### Study population

From 30 000 female patients estimated to visit the obstetrics and gynecology department, 662 patients were enrolled in this study. Only patients who agreed to participate and were attending the clinic for the first time in the study period were included. The mean age of the study population was 31 years (14 to 78 years). The largest proportion of patients was aged 20–29 years (38.5%,  $n= 255$ ). The majority of patients were housewives 69.5% ( $n= 460$ ). At the time of sampling, 43.3% ( $n= 287$ ) of patients were pregnant; most of the patients (93.8%  $n= 621$ ) had been pregnant at some point in their lives either before or during the sampling.

### Microbiological culture

In 53% ( $n= 336$ ) of patients there was no normal microbiota. Twenty-eight species non-associated to an STI were detected in the microbiological culture: *Enterococcus faecalis* (33.9%,  $n= 225$ ); followed by *Candida*

*albicans* (23.4%,  $n= 155$ ) and *Escherichia coli* (19%,  $n= 126$ ); *Acinetobacter baumannii*, *Citrobacter freundii*, *Citrobacter diversus*, *Elizabethkingia meningoseptica*, *Enterobacter aerogenes*, *Flavimonas oryzihabitans*, *Klebsiella oxytoca*, *Morganella morganii*, *Providencia rettgeri*, *Staphylococcus saprophyticus* and *Streptococcus pyogenes* (1% each). *N. gonorrhoeae* not detected.

### Detection of pathogens by PCR and by serology

From the study population, 47.7% had, at least, one microorganism associated with an STI; 2.26% ( $n= 15$ ) had IgG/IgM antibodies against *T. pallidum* (7 of them were pregnant) and 1.05% ( $n= 7$ ) was positive by the multiplex-PCR for *T. pallidum* (two of them were pregnant). *N. gonorrhoeae* was detected in 2.11% ( $n= 14$ ) patients by the multiplex PCR (none was positive for culture).

HPV was detected in 13.9% ( $n= 92$ ) of patients. According to the results of genotyping by RFLP, the HPV genotype 16 was the most frequently detected (33.7%,  $n= 31$ ), followed by genotype 31 (15.2%,  $n= 14$ ), 45 (14.1%,  $n= 13$ ), 66 (12%,  $n= 11$ ) and 58 (10.9%,  $n= 10$ ). Genotypes detected are shown in table I.

HIV was detected in 6.8% ( $n= 45$ ) of the patients by ELISA, and all were confirmed by Western-blot. Only one patient (0.15%) was positive for HCV.

**Table I**  
**FREQUENCY OF HPV GENOTYPE DETECTED**  
**IN THE STUDIED POPULATION**

Risk	Genotype	N	%
High	16	31	33.7
	18, 51, 73	1	1.1
	31	14	15.2
	33	9	9.8
	35, 39, 53, 82	2	2.2
	45	13	14.1
	52	8	8.7
	58	10	10.9
	59	6	6.5
	66	11	12.0
Low	6	28	30.4
	11, 43, 61, 81	2	2.2
	13	7	7.6
	30, 42, 54, 72, 74, 84, 91	1	1.1
	40, 69	3	3.3
	62, 64	16	17.4
	70	7	7.6
Indeterminate	26	1	1.1

Of the population studied, 8.5% (n= 56) was positive for HSV-1 by amplifying the glycoprotein G (US4) gene; and 1.8% (n= 12) was positive for HSV-2 by amplifying the glycoprotein D (US6) gene. Furthermore, 14.2 % (n= 94) were positive for *T. vaginalis* by PCR. *M. genitalium* was detected in 2.41% (n= 14) of the study population.

### Association of sexually transmitted pathogens with sociodemographic, behavioral, and biological factors and coinfections

Patients in the age range of 14-19 years were less frequently HIV-infected and, in contrast, patients in the age range 20-29 years or divorced /widowed /separated were more likely to be HIV-infected.

Patients with  $\geq 5$  sexual partners were more likely to be HIV-infected. The same observation was detected for HPV-infected patients (table II).

The infection of HSV-2 correlated with bilateral tubal occlusion as a contraceptive method ( $p= 0.005$ ) and the presence of pelvic inflammatory disease as a complication of previous pregnancies ( $p=0.032$ ).

Sixteen coinfections were detected as statistically significant: HIV-infected patients were more likely to be coinfecting with HPV ( $p=0.01$ ) and patients infected with HSV-1 were more likely to be coinfecting with *T. pallidum* ( $p= 0.02$ ), *M. genitalium*, *T. vaginalis* and *N. gonorrhoeae* ( $p= 0.01$  for all); unlike patients infected with HSV-2, which were more likely to be coinfecting only with *M. genitalium* ( $p= 0.01$ ).

Finally, patients infected with *T. vaginalis* were more likely to have multiple coinfections: HPV ( $p= 0.01$ ), HIV ( $p= 0.02$ ), *T. pallidum* ( $p= 0.01$ ), *M. genitalium* ( $p= 0.02$ ) and HSV-1 ( $p= 0.01$ ) (table II).

## Discussion

Most studies regarding STIs in Mexico have been limited to the assessment of individual pathogens and populations with clinical symptoms of the disease or high-risk behaviors with limited methodologies.<sup>5,11-13</sup> This study was performed in women attending gynecology and obstetrics clinics for routine testing and involves nine pathogens detected by molecular, serological and microbiological methods. Thus, contributing to increase the information available about STIs in Mexico.

The overall frequency of STI observed in the study population was 47.4%, which is similar to the frequency found in a study in Korea. The study included 799 healthy women. In the Korean study, 49.2% of the population had one or more STI.<sup>14</sup> However, compared

to other studies, the frequency reported in our study was higher than previously reported; for example, in an earlier study of a Paraguayan population that included 181 sexually active women, the frequency of STI was 41.4%.<sup>15</sup> Furthermore, a study of 734 Brazilian women detected a frequency of 19.6%.<sup>16</sup> A report that included 201 Mexican women showed a frequency of 57.7% of STI;<sup>17</sup> this data is superior to ours.

In Mexico, studies regarding the detection of *T. pallidum* have focused primarily on risk groups of infection. The study by Conde-González *et al.* detected a prevalence of *T. pallidum* antibodies of 2.66% in women, which is similar to our result (2.26%).<sup>18</sup> The study of Conde-González *et al.* was performed in the general population. The differences in populations studied may explain the discrepancies in results.

Early detection of syphilis during pregnancy is essential for the health of the mother and fetus, allowing the prompt instauration of treatment. In our study, 2.4% (n=7) pregnant patients had IgG/IgM antibodies, and 0.7% (n=2) was positive by PCR. In 2014, the prevalence of syphilis in pregnant women in Mexico was 0.38%. This percentage was obtained by serology.<sup>19</sup> The frequency observed in pregnant patients in our population is higher than previously reported in Mexico.

Of the seven (1%) patients positive by for *T. pallidum* by PCR, three were also positive by serology; this difference could be a result of the window period of infection, which lasts approximately three weeks. All positive patients were given medical treatment and close monitoring. No cases of congenital syphilis were detected.

The WHO estimates a prevalence of 0.8% of gonorrhea among women in America,<sup>20</sup> comparing our data with these reports, the frequency of 2.11% detected in our patients is high. However, it is necessary to take into account that the estimation of the WHO may be based on culture techniques, which are less sensitive than the sensitivity reported for PCR (sensitivity and specificity reported by 100%).<sup>21</sup> In fact, we did not detect *N. gonorrhoeae* by culture.

The frequency of HIV reported in our population was 6.8%; this rate is very high compared with frequency reported in world population and our region. The WHO estimated an overall prevalence of 0.8% in 2013 in the adult population in Latin America and this data seems to indicate a stabilization of the epidemic.<sup>22</sup>

HIV-positive patients were aware of their infected status and attended to receive supporting and medical care; this may be a bias in our study. We hypothesize that this fact may explain the higher frequency observed for *Treponema pallidum* and gonorrhoeae than in previous reports.

**Table II**  
**ASSOCIATIONS OF SEXUALLY TRANSMITTED PATHOGENS WITH SOCIODEMOGRAPHIC, BEHAVIORAL,**  
**AND BIOLOGICAL CHARACTERISTICS OF THE STUDY POPULATION AND COINFECTIONS**

Total positive population Characteristics	[n (%), n= 662]	T. pallidum positive by serology			HIV positive by serology			HPV positive		
		[n (%), n= 15]	P	Adjusted OR (95% CI)	[n (%), n= 45]	P	Adjusted OR (95% CI)	[n (%), n= 92]	P	Adjusted OR (95% CI)
<b>Sociodemographic data</b>										
<b>Age group (years)</b>										
14 – 19	93 (14.05)	2 (13.33)	0.93	0.94 (0.20, 4.23)	0 (0)			12 (13.04)	0.76	0.91 (0.47, 1.73)
20 – 29	255 (38.52)	5 (33.33)	0.67	0.79 (0.26, 2.35)	6 (13.33)	0	0.22 (0.09, 0.54)	32 (34.78)	0.42	0.83 (0.52, 1.31)
30 – 39	186 (28.10)	4 (26.67)	0.9	0.92 (0.29, 2.95)	23 (51.11)	0	2.91 (1.58, 5.36)	32 (34.78)	0.12	1.44 (0.90, 2.29)
≥ 40	129 (19.49)	4 (26.67)	0.46	1.53 (0.48, 4.89)	16 (35.56)	0	2.48 (1.30, 4.73)	16 (17.39)	0.61	0.86 (0.48, 1.53)
<b>Occupation</b>										
Housewife	460 (69.49)	9 (60.00)	0.41	0.64 (0.22, 1.84)	30 (66.67)	0.65	0.86 (0.45, 1.64)	66 (71.74)	0.63	1.13 (0.69, 1.83)
Employee/merchant	106 (16.01)	4 (26.67)	0.24	1.96 (0.61, 6.29)	14 (31.11)	0	2.61 (1.33, 5.09)	17 (18.48)	0.45	1.24 (0.70, 2.20)
Professional	63 (9.52)	1 (6.67)	0.7	0.67 (0.08, 5.21)	0 (0)	0.02		5 (5.43)	0.15	0.51 (0.19, 1.30)
Student	33 (4.98)	1 (6.67)	7.62	1.37 (0.17, 10.76)	1 (2.22)	0.37	0.41 (0.05, 3.11)	4 (4.35)	0.76	0.85 (0.29, 2.47)
<b>Marital status</b>										
Single	140 (21.15)	0 (0)	0.24		17 (37.78)	0.01	2.43 (1.29, 4.59)	25 (27.17)	0.12	1.47 (0.89, 2.44)
Divorced/widowed/separated	22 (3.32)	5 (33.33)	0.46		7 (15.56)	0	7.39 (2.84, 19.21)	4 (4.35)	0.55	1.39 (0.46, 4.21)
Married	238 (35.95)	5 (33.33)	0.83	0.88 (0.30, 2.63)	7 (15.56)	0	0.30 (0.13, 0.70)	27 (29.35)	0.15	0.71 (0.43, 1.14)
Living with partner	262 (39.58)	5 (33.33)	0.61	0.75 (0.25, 2.24)	14 (31.11)	0.22	0.67 (0.35, 1.28)	36 (39.13)	0.92	0.98 (0.62, 1.53)
<b>Behavioral data</b>										
<b>Age at first intercourse (years)</b>										
< 16 years	140 (21.15)	3 (20)	0.91	0.93 (0.25, 3.34)	14 (31.11)	0.09	1.76 (0.90, 3.40)	25 (27.17)	0.12	1.47 (0.89, 2.44)
≥ 16 years	522 (78.85)	12 (80)	0.91	1.07 (0.29, 3.86)	31 (68.89)	0.09	0.65 (0.29, 1.10)	67 (72.83)	0.12	0.68 (0.04, 1.12)
<b>No. of previous sex partners</b>										
0-1	318 (48.04)	9 (60)	0.34	1.64 (0.57, 4.66)	11 (24.44)	0.001	0.32 (0.16, 0.65)	36 (39.13)	0.06	0.66 (0.41, 1.02)
2 – 4	279 (42.15)	6 (40)	0.86	0.91 (0.32, 2.59)	23 (51.11)	0.2	1.47 (0.80, 2.70)	40 (43.48)	0.76	1.07 (0.68, 1.66)
≥ 5	65 (9.82)	0 (0)	0.19		11 (24.44)	0.001	3.37 (1.61, 7.03)	16 (17.39)	0.009	2.23 (1.21, 4.13)
<b>Contraceptive methods</b>										
Hormonal	102 (15.41)	0 (0)	0.09		4 (8.89)	0.21	0.51 (0.18, 1.47)	13 (14.13)	0.71	0.89 (0.47, 1.66)
Condom	249 (37.61)	7 (46.67)	0.46	1.46 (0.52, 4.08)	4 (8.89)	0.32	1.35 (0.73, 2.49)	39 (42.39)	0.3	1.26 (0.80, 1.97)
IUD	67 (10.12)	1 (6.67)	0.65	0.629 (0.08, 4.85)	20 (44.44)	0.77	0.85 (0.29, 2.47)	13 (14.13)	0.16	1.57 (0.82, 3.01)
Bilateral tubal occlusion	40 (6.04)	0 (0)	0.32		4 (8.89)	0.4	1.57 (0.53, 4.63)	4 (4.35)	0.46	0.67 (0.23, 1.94)
None	204 (30.82)	7 (46.67)	0.17	1.99 (0.71, 5.58)	13 (28.89)	0.77	0.90 (0.46, 1.76)	23 (25)	0.19	0.72 (0.43, 1.18)
Alcohol use	145 (21.90)	1 (6.67)	0.14	0.25 (0.03, 1.91)	11 (24.44)	0.66	1.16 (0.57, 2.36)	23 (25)	0.43	1.24 (0.73, 2.04)
Current smoker	200 (30.21)	3 (20.00)	0.38	0.57 (0.15, 2.04)	14 (0.02)	0.89	1.04 (0.54, 2.01)	33 (1.62)	0.2	
<b>Biological factors</b>										
Pregnant at sampling	287 (43.35)	7 (46.67)	0.79	1.14 (0.41, 3.20)	1 (2.22)	0	0.02 (0.004, 0.19)	39 (42.39)	0.84	0.96 (0.61, 1.49)
Previous pregnancies	621 (93.81)	13 (86.67)	0.26	0.42 (0.09, 1.96)	44 (97.78)	0.24	3.13 (0.42, 23.31)	88 (95.65)	0.39	1.57 (0.54, 4.51)
<b>Complications of previous pregnancy</b>										
Infections	386 (58.31)	7 (46.67)	0.35	0.61 (0.22, 1.72)	19 (42.22)	0.02	0.49 (0.27, 0.91)	5 (5.43)	0.7	0.92 (0.58, 1.43)
Bleeding	69 (10.42)	0 (0)	0.18		4 (8.89)	0.72	0.82 (0.28, 2.38)	5 (5.43)	0.09	0.45 (0.17, 1.61)
Risk of spontaneous abortion	116 (17.52)	1 (6.67)	0.99		8 (17.78)	0.96	1.01 (0.42, 2.23)	22 (23.91)	0.08	1.59 (0.93, 2.69)
Premature birth	50 (7.55)	0 (0)	0.26	0.33 (0.04, 2.53)	1 (2.22)	0.16	0.26 (0.03, 1.95)	9 (9.78)	0.383	1.39 (0.65, 2.98)
Ectopic pregnancy	9 (1.36)	0 (0)	0.64		0 (0)	0.41		0 (0)	0.22	
Previous abortions	163 (24.62)	3 (20)	0.99		12 (26.67)	0.02		25 (27.17)	0.35	
Pelvic inflammatory disease	295 (44.56)	1 (6.67)	0.86	1.09 (0.39, 3.03)	21 (46.67)	0.76	1.09 (0.59, 2.00)	41 (44.57)	0.99	1 (0.64, 1.55)
<b>Coinfections</b>										
HPV (PCR)	92 (13.90)	3 (20)	0.48		11 (24.44)	0.034	2.14 (1.04, 4.39)			
HIV (Serology)	45 (6.80)	1 (6.67)	0.98	0.97 (0.12, 7.61)				11 (11.96)	4.48	2.14 (1.04, 4.39)
T. pallidum (Serology)	15 (2.27)		0.01		1 (2.22)	0.98	0.97 (0.12, 7.61)	3 (3.26)	0.48	1.56 (0.43, 5.66)
T. pallidum (PCR)	7 (1.06)	3 (20)	0	40 (8.09, 199.48)	1 (2.22)	0.42	2.31 (0.27, 19.65)	3 (3.26)	0.02	4.77 (1.05, 21.66)
HSV-2 (PCR)	12 (1.81)	1 (6.67)	0.15	4.13 (0.49, 34.21)	0 (0)	0.345		2 (2.17)	0.78	1.24 (0.26, 5.77)
M. genitalium (PCR)	16 (2.42)	0 (0)	0.53		2 (4.44)	0.35	2 (0.44, 9.10)	1 (1.09)	0.37	0.41 (0.05, 3.11)
T. vaginalis (PCR)	94 (14.20)	4 (26.67)	0.162	2.25 (0.70, 7.22)	3 (6.67)	0.13	0.41 (0.12, 1.36)	25 (27.17)	0	2.7 (1.60, 4.57)
N. gonorrhoeae (PCR)	14 (2.11)	0 (0)	0.56		2 (4.44)	0.26	2.34 (0.50, 10.81)	2 (2.17)	0.96	1.03 (0.22, 4.69)
HSV-1 (PCR)	56 (8.46)	1 (6.67)	0.8	0.76 (0.99, 5.95)	2 (4.44)	0.31	0.48 (0.11, 2.05)	10 (10.87)	0.37	1.38 (0.67, 2.86)

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Characteristics	Total positive population			T. vaginalis positive by PCR			HVS-1 positive by PCR			HVS-2 positive by PCR		
	[n (%), n= 662]	[n (%), n= 94]	p	Adjusted OR (95% CI)	[n (%), n= 56]	p	Adjusted OR (95% CI)	[n (%), n= 12]	p	Adjusted OR (95% CI)		
<b>Sociodemographic data</b>												
<b>Age group (years)</b>												
14 – 19	93 (14.05)	14 (14.89)	0.79	1.08 (0.58, 2.00)	9 (16.07)	0.64	1.19 (0.56, 2.51)	0 (0)	0.15			
20 – 29	255 (38.52)	40 (42.55)	0.38	1.21 (0.78, 1.89)	22 (39.29)	0.9	1.03 (0.59, 1.81)	3 (25)	0.33	0.52 (0.14, 1.96)		
30 – 39	186 (28.10)	25 (26.60)	0.72	0.91 (0.56, 1.49)	17 (30.36)	0.69	1.12 (0.62, 2.04)	5 (41.67)	0.29	1.85 (0.58, 5.90)		
≥ 40	129 (19.49)	15 (15.96)	0.8	0.76 (0.42, 1.37)	8 (14.29)	0.31	0.67 (0.31, 1.46)	4 (33.33)	0.21	2.12 (0.62, 7.15)		
<b>Occupation</b>												
Housewife	460 (69.49)	65 (69.15)	0.91	0.97 (0.60, 1.56)	43 (76.79)	0.22	1.48 (0.78, 2.83)	8 (66.67)	0.82	0.87 (0.25, 2.92)		
Employee/merchant	106 (16.01)	18 (19.15)	0.34	1.3 (0.74, 2.29)	4 (7.14)	0.06	0.38 (0.13, 1.08)	4 (33.33)	0.09	2.71 (0.80, 9.19)		
Professional	63 (9.52)	6(6.38)	0.26	0.61 (0.25, 1.46)	5 (8.93)	0.87	0.92 (0.35, 2.41)	0 (0)	0.25			
Student	33 (4.98)	5 (5.32)	0.87	1.08 (0.40, 2.88)	4 (7.14)	0.43	1.53 (0.51, 4.52)	0 (0)	0.42			
<b>Marital status</b>												
Single	140 (21.15)	18 (19.15)	0.6	0.86 (0.49, 1.50)	9 (16.07)	0.33	0.69 (0.33, 1.45)	2 (16.67)	0.7	0.74 (0.16, 3.42)		
Divorced/widowed/separated	22 (3.32)	2 (2.13)	0.48	0.59 (0.13, 2.59)	2 (3.57)	0.91	1.08 (0.24, 4.76)	1 (8.33)	0.32	2.72 (0.33, 22.07)		
Married	238 (35.95)	30 (31.91)	3.79	0.811 (0.50, 1.29)	22 (39.29)	0.58	1.16 (0.66, 2.04)	7 (58.33)	0.1	2.53 (0.79, 8.09)		
Living with partner	262 (39.58)	44 (46.81)	0.12	1.41 (0.91, 2.19)	23 (41.07)	0.81	1.07 (0.61, 1.86)	2 (16.67)	0.1	0.3 (0.07, 1.38)		
<b>Behavioral data</b>												
<b>Age at first intercourse (years)</b>												
< 16 years	140 (21.15)	17 (18.09)	0.43	0.79 (0.45, 1.40)	11 (19.64)	0.77	0.90 (0.45, 1.79)	2 (16.67)	0.7	0.74 (0.16, 3.42)		
≥ 16 years	522 (78.85)	77 (81.91)	0.43	1.25 (0.71, 2.19)	45 (80.36)	0.77	1.10 (0.556, 2.220)	10 (83.33)	0.7	1.34 (0.29, 6.22)		
<b>No. of previous sex partners</b>												
0-1	318 (48.04)	43 (45.74)	0.63	0.89 (0.58, 1.39)	30 (53.57)	0.38	1.27 (0.73, 2.20)	5 (41.67)	0.65	0.76 (0.242, 2.44)		
2 – 4	279 (42.15)	41 (43.62)	0.755	1.07 (0.69, 1.66)	20 (35.71)	0.3	0.74 (0.42, 1.31)	6 (50)	0.57	1.38 (0.44, 4.32)		
≥ 5	65 (9.82)	10 (10.64)	0.73	1.1 (0.54, 2.26)	6 (10.71)	0.81	1.11 (0.45, 2.70)	1 (8.33)	0.86	0.83 (0.10, 6.55)		
<b>Contraceptive methods</b>												
Hormonal	102 (15.41)	20 (21.28)	0.08	1.6 (0.92, 2.76)	9 (16.07)	0.88	1.05 (0.50, 2.22)	3 (25)	0.35	1.85 (0.49, 6.97)		
Condom	249 (37.61)	33 (35.11)	0.58	0.88 (0.55, 1.39)	25 (44.64)	0.25	1.37 (0.79, 2.38)	5 (41.67)	0.77	1.18 (0.37, 3.78)		
IUD	67 (10.12)	13 (13.83)	0.19	1.52 (0.79, 2.92)	6 (10.71)	0.87	1.07 (0.44, 2.60)	0 (0)	0.24			
Bilateral tubal occlusion	40 (6.04)	4 (4.26)	0.43	0.65 (0.22, 1.89)	2 (3.57)	0.41	0.55 (0.13, 2.35)	3 (25)	0.005	5.52 (1.43, 21.26)		
None	204 (30.82)	24 (25.53)	0.23	0.73 (0.45, 1.21)	14 (25)	0.32	0.73 (0.38, 1.36)	1 (8.33)	0.08	0.2 (0.02, 1.56)		
<b>Alcohol use</b>												
Current smoker	145 (21.90)	25 (26.60)	0.235	1.35 (0.820, 2.23)	9 (16.07)	0.27	0.66 (0.31, 1.38)	1 (8.33)	0.25	0.31 (0.04, 2.49)		
<b>Biological factors</b>												
Pregnant at sampling	287 (43.35)	51 (54.26)	0.02	1.66 (1.07, 2.58)	28 (50)	0.29	1.34 (0.77, 2.31)	3 (25)	0.19	0.43 (0.11, 1.60)		
Previous pregnancies	621 (93.81)	87 (92.55)	0.63	0.81 (0.35, 1.89)	52 (92.86)	0.79	0.87 (0.29, 2.53)	12 (100)	0.36			
<b>Complications of previous pregnancy</b>												
Infections	386 (58.31)	54 (57.45)	0.85	0.96 (0.61, 1.49)	33 (58.93)	0.92	1.02 (0.59, 1.74)	6 (50)	0.55	0.71 (0.22, 2.22)		
Bleeding	69 (10.42)	5 (5.32)	0.08	0.44 (0.17, 1.13)	6 (10.71)	0.94	1.03 (0.42, 2.50)	0 (0)	0.23			
Risk of spontaneous abortion	116 (17.52)	14 (14.89)	0.46	0.8 (0.43, 1.46)	7 (12.50)	0.3	0.65 (0.28, 1.47)	3 (25)	0.49	1.58 (0.42, 5.94)		
Premature birth	50 (7.55)	7 (7.45)	0.96	0.982 (0.428, 2.25)	8 (14.29)	0.04	2.23 (0.1, 5.03)	1 (8.33)	0.91	1.11 (0.14, 8.81)		
Ectopic pregnancy	9 (1.36)	2 (2.13)	0.48	1.74 (0.35, 8.51)	2 (3.57)	0.13	3.16 (0.64, 15.63)	0 (0)	0.68			
Previous abortions	163 (24.62)	18 (19.15)	0.66		11 (19.64)	0.78		5 (41.67)	0.86			
Pelvic inflammatory disease	295 (44.56)	44 (46.81)	0.63	1.11 (0.71, 1.72)	28 (50)	0.39	1.27 (0.73, 2.19)	9 (75)	0.032	3.81 (1.02, 14.23)		
<b>Coinfections</b>												
HPV (PCR)	92 (13.90)	25 (26.60)	0	2.7 (1.60, 4.57)	10 (17.86)	0.37	(0.67, 2.86)	2 (16.7)	0.78	1.24 (0.26, 5.77)		
HIV (Serology)	45 (6.80)	3 (3.19)	0.13	0.41 (0.12, 1.36)	2 (3.57)	0.31	0.48 (0.11, 2.05)	0 (0)	0.34			
<i>T. pallidum</i> (Serology)	15 (2.27)	4 (4.26)	0.16	2.25 (0.70, 7.22)	1 (1.79)	0.8	0.76 (0.09, 5.95)	1 (8.3)	0.15	4.13 (0.49, 34.21)		
<i>T. pallidum</i> (PCR)	7 (1.06)	4 (4.26)	0	8.37 (1.84, 38.01)	2 (3.57)	0.05	4.45 (0.84, 23.49)	0 (0)	0.71			
HSV-2 (PCR)	12 (1.81)	2 (2.13)	0.8	1.21 (0.26, 5.62)	1 (1.79)	0.98	0.98 (0.12, 7.76)					
<i>M. genitalium</i> (PCR)	16 (2.42)	5 (5.32)	0.04	2.84 (0.96, 8.38)	4 (7.14)	0.01	3.8 (1.18, 12.22)	2 (16.7)	0.001	9.08 (1.82, 45.35)		
<i>T. vaginalis</i> (PCR)	94 (14.20)				35 (62.50)	0	15.42 (8.44, 28.26)	2 (16.7)	0.8	1.21 (0.26, 5.62)		
<i>N. gonorrhoeae</i> (PCR)	14 (2.11)	0 (0)	0.12		4 (7.14)	0.006	4.58 (1.39, 15.12)	1 (8.3)	0.13	4.45 (0.53, 37.09)		
HSV-1 (PCR)	56 (8.46)	35 (37.23)	0	15.45 (8.44, 28.26)				1 (8.3)	0.98	0.98 (0.12, 7.76)		

(continues...)

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Total positive population Characteristics	[n (%), n= 662]	M. genitalium positive by PCR			N. gonorrhoeae positive by PCR			T. pallidum positive by PCR		
		[n (%), n= 16]	P	Adjusted OR (95% CI)	[n (%), n= 14]	P	Adjusted OR (95% CI)	[n (%), n= 7]	P	Adjusted OR (95% CI)
<b>Sociodemographic data</b>										
Age group (years)										
14 – 19	93 (14.05)	0 (0)	0.1		1 (7.14)	0.45	0.46 (0.06, 3.59)	0 (0)	0.28	
20 – 29	255 (38.52)	7 (43.75)	0.66	1.24 (0.45, 3.39)	6 (42.86)	0.73	1.2 (0.41, 3.50)	2 (28.57)	0.58	0.63 (0.12, 3.30)
30 – 39	186 (28.10)	4 (25)	0.78	0.85 (0.271, 2.66)	1 (7.14)	0.07	0.19 (0.02, 1.48)	3 (42.86)	0.38	1.93 (0.42, 8.72)
≥ 40	129 (19.49)	5 (31.25)	0.22	1.93 (0.65, 5.66)	6 (42.86)	0.02	3.23 (1.10, 9.49)	2 (28.57)	0.53	1.67 (0.32, 8.75)
<b>Occupation</b>										
Housewife	460 (69.49)	14 (87.50)	0.11	3.11 (0.70, 13.84)	11 (78.57)	0.46	1.61 (0.44, 5.84)	3 (42.86)	0.12	0.32 (0.07, 1.45)
Employee/merchant	106 (16.01)	2 (12.50)	0.7	0.75 (0.16, 3.36)	2 (14.29)	0.87	0.88 (0.19, 3.99)	3 (42.86)	0.04	4.06 (0.89, 18.43)
Professional	63 (9.52)	0 (0)	0.18		1 (7.14)	0.76	0.72 (0.09, 5.65)	1 (14.29)	0.66	1.59 (0.18, 13.45)
Student	33 (4.98)	0 (0)	0.35		0 (0)	0.38		0 (0)	0.54	
<b>Marital status</b>										
Single	140 (21.15)	1 (6.25)	0.14	0.24 (0.03, 1.85)	5 (35.71)	0.17	2.11 (0.69, 6.40)	3 (42.86)	0.15	2.83 (0.62, 12.82)
Divorced/widowed/separated	22 (3.32)	0 (0)	0.45		0 (0)	0.48		0 (0)	0.62	
Married	238 (35.95)	6 (37.50)	0.89	1.07 (0.38, 2.98)	4 (28.57)	0.56	0.70 (0.22, 2.28)	4 (57.14)	0.24	2.39 (0.53, 10.81)
Living with partner	262 (39.58)	9 (56.25)	0.16	1.99 (0.73, 5.43)	5 (35.71)	0.76	0.84 (0.28, 2.55)	0 (0)	0.03	
<b>Behavioral data</b>										
Age at first intercourse (years)										
< 16 years	140 (21.15)	4 (25)	0.7	1.25 (0.39, 3.93)	5 (35.71)	0.17	2.11 (0.696, 6.40)	1 (14.29)	0.65	0.619 (0.074, 5.18)
≥ 16 years	522 (78.85)	12 (75)	0.7	0.8 (0.254, 2.52)	9 (64.29)	0.17	0.47 (0.15, 1.43)	6 (85.71)	0.65	1.61 (0.19, 13.53)
<b>No. of previous sex partners</b>										
0-1	318 (48.04)	6 (37.50)	0.39	0.64 (0.23, 1.78)	5 (35.71)	0.35	0.59 (0.19, 1.79)	3 (42.86)	0.78	0.81 (0.18, 3.64)
2 – 4	279 (42.15)	6 (37.50)	0.7	0.82 (0.29, 2.28)	7 (50)	0.54	1.38 (0.47, 3.98)	2 (28.57)	0.46	0.54 (0.10, 2.83)
≥ 5	65 (9.82)	4 (25)	0.03	3.19 (1.00, 10.21)	2 (14.29)	0.57	1.54 (0.33, 7.07)	2 (28.57)	0.09	3.75 (0.71, 19.77)
<b>Contraceptive methods</b>										
Hormonal	102 (15.41)	1 (6.25)	0.3	0.36 (0.04, 2.75)	1 (7.14)	0.38	0.41 (0.05, 3.22)	1 (14.29)	0.93	0.91 (0.10, 7.67)
Condom	249 (37.61)	4 (25.00)	0.29	0.546 (0.17, 1.71)	7 (50)	0.33	1.67 (0.58, 4.84)	0 (0)	0.03	
IUD	67 (10.12)	3 (18.75)	0.24	2.09 (0.58, 7.56)	1 (7.14)	0.7	0.67 (0.08, 5.26)	2 (28.57)	0.1	3.63 (0.69, 19.09)
Bilateral tubal occlusion	40 (6.04)	2 (12.50)	0.27	2.28 (0.50, 10.42)	2 (14.29)	0.19	2.67 (0.57, 12.38)	0 (0)	0.5	
None	204 (30.82)	6 (37.50)	0.55	1.35 (0.48, 3.78)	3 (21.43)	0.44	0.60 (0.16, 2.19)	4 (57.14)	0.12	3.03 (0.67, 13.67)
Alcohol use	145 (21.90)	4 (25)	0.54	1.19 (0.37, 3.75)	4 (28.57)	0.54	1.43 (0.44, 4.65)	1 (14.29)	0.62	0.59 (0.07, 4.95)
Current smoker	200 (30.21)	5 (31.25)	0.29	1.05 (0.36, 3.06)	6 (42.86)	0.29	1.75 (0.60, 5.12)	2 (28.57)	0.924	0.92 (1.78, 4.89)
<b>Biological factors</b>										
Pregnant at sampling	287 (43.35)	7 (43.75)	0.25	1.01 (0.37, 2.76)	4 (28.57)	0.25	0.51 (0.16, 1.66)	6 (85.71)	4.28	0.51 (0.10, 2.69)
Previous pregnancies	621 (93.81)	16 (100)	0.9		13 (92.86)	0.9	0.87 (0.11, 6.87)	2 (28.57)	0.38	0.40 (0.04, 3.40)
<b>Complications of previous pregnancy</b>										
Infections	386 (58.31)	8 (50)	0.31	0.70 (0.26, 1.91)	10 (71.43)	0.31	1.8 (0.56, 5.82)	0 (0)	0.1	0.28 (0.05, 1.46)
Bleeding	69 (10.42)	1 (6.25)	0.17	0.56 (0.07, 4.35)	3 (21.43)	0.17	2.4 (0.65, 8.84)	0 (0)	0.36	
Risk of spontaneous abortion	116 (17.52)	0 (0)	0.74	1.58 (0.50, 5.01)	2 (14.29)	0.74	0.78 (0.17, 3.53)	0 (0)	0.22	
Premature birth	50 (7.55)	0 (0)	0.28		0 (0.00)	0.28		0 (0)	0.44	
Ectopic pregnancy	9 (1.36)	0 (0)	0.65		0 (0)	0.65		0 (0)	0.76	
Previous abortions	163 (24.62)	2 (12.50)	0.99		3 (21.43)	0.99		2 (28.57)	0.99	
Pelvic inflammatory disease	295 (44.56)	8 (50)	0.67	1.25 (0.46, 3.37)	7 (50)	0.67	1.25 (0.43, 3.60)	3 (42.86)	0.92	0.93 (0.20, 4.19)
<b>Coinfections</b>										
HPV (PCR)	92 (13.90)	1 (6.25)	0.96		2 (14.3)	0.96	1.03 (0.22, 4.69)	3 (42.9)	0.02	4.77 (1.05, 21.66)
HIV (Serology)	45 (6.80)	2 (12.5)	0.26	0.40 (0.05, 3.11)	2 (14.3)	0.26	2.34 (0.50, 10.81)	1 (14.3)	0.42	2.31 (0.27, 19.65)
T. pallidum (Serology)	15 (2.27)	0 (0)	0.56		0 (0)	0.56		3 (42.9)	0	40.18 (8.09, 199.48)
T. pallidum (PCR)	7 (1.06)	1 (6.25)	0.02	7.11 (0.80, 62.78)	1 (7.1)	0.02	8.23 (0.92, 73.32)	0		
HSV-2 (PCR)	12 (1.81)	2 (12.5)	0.13	9.08 (1.82, 43.35)	2 (7.1)	0.13	4.45 (0.53, 37.09)	0	0.71	
M. genitalium (PCR)	16 (2.42)		0		3 (21.4)	0	13.32 (3.31, 53.46)	1 (14.3)	0.04	7.11 (0.80, 62.78)
T. vaginalis (PCR)	94 (14.20)	5 (31.25)	0.12	2.84 (0.96, 8.38)	0 (0)	0.12		4 (57.1)	0.001	8.37 (1.84, 38.01)
N. gonorrhoeae (PCR)	14 (2.11)	3 (18.75)		13.32 (3.31, 53.46)				1 (14.3)	0.02	8.23 (0.92, 73.32)
HSV-1 (PCR)	56 (8.46)	4 (25)	0.006	3.8 (1.18, 12.22)	4 (28.6)	0.006	4.58 (1.39, 15.12)	2 (28.6)	0.06	4.45 (0.84, 23.49)

*M. genitalium* is detected more often in people with risk factors for the infection, reaching percentages from 20 to 40% among sex workers.<sup>23</sup> The prevalence of *M. genitalium* infections reported in the general population varies from 1 to 10% in most populations,<sup>24</sup> but significant differences are reported worldwide. A frequency of 0.5% has been reported for Mexico.<sup>17</sup> These differences underline the relevance of studying the frequency of this opportunistic pathogen in different populations.

Two Mexican studies showed frequencies of 33.8<sup>25</sup> and 18.4%<sup>17</sup> for HPV. Both reports showed higher frequencies than ours. A third Mexican report showed a frequency of 9.1%.<sup>26</sup> All reports used different methodologies and studied different populations. These factors may explain the different frequencies observed.

The genotype most frequently detected in our study was type 16, which is the most common genotype found in healthy women worldwide<sup>27</sup> and the WHO reports that is one of the most frequent genotypes.

In our study, the 18 genotype was detected in only one patient, representing the 1.1% of the HPV-positive population; the mean prevalence of the 18 genotype was previously reported for the Mexican population.<sup>17</sup> This data is relevant because genotype 18 is associated with more aggressive behavior and is detected mainly in high-grade lesions.<sup>28</sup>

Our study showed the prevalence of HSV-1/HSV-2 detected by PCR; the studies reported at present of the prevalence of these viruses are mainly by serological tests. The differences in methodologies make it difficult to make a comparison of frequencies of this virus. As expected, we detected lower frequencies than observed by serology. Contrary to expected it is interesting that there is a higher percentage of HSV-1 than HSV-2. Further studies need to understand the basis of this switch.

In this study, patients with five or more sexual partners were more likely to be HIV or HPV infected. Several studies have consistently shown that the risk of HPV and HIV infection increase with the number of sexual partners.<sup>29-31</sup> Our results are in agreement with these studies.

The differences between the frequencies reported of STIs among countries could represent genuine differences in patterns of sexual behavior and efforts to control these infections, but may also be the result of variations in study design, diagnostic methods, and participation rates. Reliable data on the frequency of these infections are scarce and often more about the age-specific region as well as its association with different risk factors; the diagnosis of STIs is not routine, so the prevalence of STIs is not known in depth in our population. This study will contribute to increase the knowledge about the frequency of STIs in Mexico.

## Acknowledgements

The authors would like to thank the study participants and our research team Acevedo-Duarte L, González-Guzmán M, Martínez-Macías SK, Jiménez-Gutiérrez C, and Carbajal-Rimoldi A for their contributions to this research.

*Declaration of conflict of interests.* The authors declare that they have no conflict of interests.

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