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Research article

Prevalence of coinfections in a cross-sectional cohort of women screened for multiple pathogens in Peru

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ABSTRACT

Objective: To determine the prevalence and risk factors of sexually transmitted infections (STIs) including *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma genitalium* among asymptomatic women with human papillomavirus (HPV) infection.
Methods: A cross-sectional study was performed in 842 asymptomatic women from Cajamarca, Peru. The pathogens were detected using polymerase chain reaction (PCR) and the results were analyzed according to the HPV status: high-risk HPV, low-risk HPV and negative for HPV. Demographical and gyneco-obstetric data was analyzed to identify risk factors.
Results: We found that 23.99% (202/842) women were positive for HPV, of whom 79.21% (160/202) were infected with a high-risk genotype. Co-infections were evaluated and 14.38% (23/160) were positive for *Ureaplasma urealyticum*, 9.38% (15/160) for *Chlamydia trachomatis* and 1.25% (2/160) for *Mycoplasma genitalium.* We found a significant association between HPV genotype and the number of children, partners, and history of sexual abuse. The co-infection between high-risk HPV and *Chlamydia trachomatis* was associated with number of abortions, number of sexual partners and no use of condom. Finally, co-infection between high-risk HPV and *Ureaplasma urealyticum* was associated with no use of condom and history of STIs.
Conclusion: HPV infection continues to be a highly relevant problem in Peru, particularly due to the high prevalence of high-risk genotypes. In addition, we report high rates of co-infections with other STIs, such as *U. urealyticum* and *C. trachomatis*. We highlight the importance of active surveillance to promptly diagnose these infections, since they may lead to persistent HPV infections.

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1. Introduction

The World Health Organization (WHO) released their report on global sexually transmitted infection (STI) surveillance in 2018, which estimates that more than 1 million people between the ages of 15 and 49 acquire a STI every day [1]. More than 376 million new cases of STIs are reported annually, being the most frequent: chlamydia, gonorrhea, trichomoniasis and syphilis [1,2].

STIs are an important public health problem in Peru, as shown by a recent national population-based survey. The three most important sexually transmitted pathogens in women were: herpes simplex virus 2, *Chlamydia trachomatis* and *Trichomonas vaginalis*, with a prevalence of 13.6%, 6.5% and 4.9% respectively [3]. Among these, *C. trachomatis* infection causes a great impact in terms of morbidity, which is responsible for causing pelvic inflammatory disease (PID), infertility and pregnancy-related complications such as chorioamnionitis, preterm labor and ectopic pregnancy [4]. Nonetheless, recent trends, show that *C. trachomatis* infections have been declining since the beginning of the decade in Peru [5].

Recently, pathogens such as *Mycoplasma genitalium* and *Ureaplasma urealyticum* have emerged as important causes of STIs [6]. These gram-negative bacteria belong to the *Mollicutes* class, which are part of the normal microbiota of the genitourinary tract in healthy women, however they may cause opportunistic infections [6,7]. The alteration of the microbiota and the increase in the population of these bacteria have been associated with cases of cervicitis, pyelonephritis, PID, endometrial alterations, infertility, low birth weight, spontaneous abortion, among other pathologies [8–10].

Cervical cancer is the 4th most frequent cancer in women worldwide and causes more than 280 000 deaths in women every year [11]. In Peru, it is still the second most common cancer, with more than 4270 new cases every year [12]. Infection by human papillomavirus (HPV) is one of the most important causes of cervical cancer, with more than 70% of the cases attributed to this infection [13].

It has been established that co-infection between HPV and other STIs may increase the risk of progression to cervical cancer [14]. The epidemiology of *C. trachomatis, U. urealyticum* and *M. genitalium* in women with HPV infection has not been previously studied in Peru. Therefore, the objective of this study was to determine the prevalence of *C. trachomatis, U. urealyticum* and *M. genitalium* in asymptomatic women with HPV infection, also to evaluate the risk factors of co-infections.

2. Materials and methods

2.1. Patients and study design

A cross-sectional prospective study was carried out in asymptomatic women (absence of symptoms of HPV infection) attending the cervical cancer screening program in the "Hospital Regional Docente de Cajamarca" during the years 2017–2019. Sexually active women who were 18 years and older were included. Exclusion criteria were: pregnancy, abnormal gynecological bleeding, history of hysterectomy and HPV related diseases (cervical cancer, genital warts or other cutaneous manifestations) and sexual activity in the last 2 days.

2.2. Sample collection

A cytobrush was used to collect a sample from the ectocervix and endocervix junctional zone, the sample was later immersed in universal transport medium (UTM). All the samples were stored at 4 °C until they were transported to the Molecular Biology Laboratory of the "Universidad Peruana de Ciencias Aplicadas-Instituto de Investigacion Nutricional". Finally, all the samples were aliquoted and stored in sterile tubes at a temperature of −80 °C until later processed.

2.3. DNA extraction

A volume of 200 μL of endocervical sample was used to obtain bacterial DNA. We used the commercial High Pure Template Preparation kit (Roche Applied Science, Penzberg Germany), according to the manufacturer's instructions. The extracted DNA was eluted in 50 μL of DNase-free water and immediately stored at −20 °C until it was processed for the polymerase chain reaction (PCR) technique.

Polymerase chain reaction (PCR) for the detection of C*hlamydia trachomatis, Ureaplasma urealyticum* and *Mycoplasma genitalium.*

We used specific primers for the detection of each pathogen. For the diagnosis of HPV, we used primers described by Lurchachaiwong et al. [15]. For the amplification of genetic material of *C. trachomatis, U. urealyticum* and *M. genitalium* we used primers previously described by Gupta et al. [16], Vancutsem et al. [17] and Jensen et al. [18] (Table S1).

For each microorganism analyzed a reaction mixture was prepared to a final volume of 50 μL. The final mix contained a total of 25 μL of the prepared enzyme (Taq polymerase, 2.5 mM Mg Cl2; 15 mM Tris/HCl PH 8.3, 50 mM KCl, 200 μM of each deoxynucleotide) (Kappa Biosystem), 20 mol of each primer (Macrogen-Korea), water and 5 μL of DNA. The thermocycler conditions were established as follows: pre-denaturation for 5 min at 95 °C, followed by 55 cycles of denaturation for 1 min at 95 °C, annealing for 1 min at 55 °C, elongation for 45 s at 72 °C, with a final elongation of 10 min at 72 °C. A positive controls were used for the PCR identification of each pathogen.

Amplification was performed using a Veriti gradient thermal cycler (Applied Biosystem, California, USA). The presence and size of the amplified product was analyzed in 2.5% agarose electrophoresis (FMC, Rockland, ME), the gel was previously stained with 3 μg/mL of ethidium bromide and immersed in a tris acetate-EDTA buffer. The bands were visualized using a KODAK LOGIC 1500 Gel

Table 1
Demographic characteristics and sexually transmitted infections in women from Cajamarca, Peru.

Variable	Total Samples (n = 842)	High-risk HPV (n = 160)	Low - risk HPV (n = 42)	*p-value*	*M. genitalium* positive + High-risk HPV (n = 2)		*U. urealyticum* positive + High-risk HPV (n = 23)		*C. trachomatis* positive + High-risk HPV (n = 15)		*U. urealyticum* positive + Low-risk HPV (n = 6)		*C.trachomatis* positive + Low-risk HPV (n = 7)	
					n (%)	p-value[a]	n (%)	p-value[a]	n (%)	p-value[a]	n (%)	p-value[a]	n (%)	p-value[a]
Age (years): n (%)														
18–24	66 (7.8)	13 (8.1)	6 (14.3)	0.002	0	1.00	2 (8.7)	0.650	1 (6.7)	0.830	1 (16.7)	0.170	1 (14.3)	0.680
25–34	250 (29.7)	67 (41.9)	13 (31)		1 (50.0)		9 (39.1)		6 (40.0)		4 (66.7)		1 (14.3)	
35–44	299 (35.5)	52 (32.5)	15 (35.7)		1 (50.0)		6 (26.1)		4 (26.7)		1 (16.7)		4 (57.1)	
≥45	227 (27.0)	28 (17.5)	8 (19.1)		0		6 (26.1)		4 (26.7)		0		1 (14.3)	
Marital status: n (%)														
Single	209 (24.8)	80 (50.0)	15 (35.7)	<0.001	1 (50.0)	1.00	13 (56.5)	0.070	6 (40.0)	0.42	2 (33.3)	0.730	2 (28.6)	0.640
Cohabiting	416 (49.4)	53 (33.1)	19 (45.2)		1 (50.0)		9 (39.1)		5 (33.3)		2 (33.3)		3 (42.9)	
Married	197 (23.4)	25 (15.6)	8 (19.1)		0		1 (4.4)		4 (26.7)		2 (33.3)		2 (28.6)	
Others	20 (2.4)	2 (1.3)	0		0		0		0		0		0	

High-risk HPV: includes high-risk and probably oncogenic genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68; 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97.
Low-risk HPV: include low-risk and other genotypes not related to malignancy: 6, 11, 14, 40, 42, 43, 44, 54, 55, 71, 74, 80, 81, 90, 91, 101.
[a] Pearson's Chi Square Test/Fisher's Exact Test.

Table 2
Risk factors for co-infection between sexually transmitted pathogens and HPV.

Risk factors	Total cases (n = 842)	High-risk HPV (n = 160)	Low-risk HPV (n = 42)	*p-value*	High-risk HPV						Low-risk HPV			
					MG (n = 2)	*p-value*	UU (n = 23)	*p-value*	CT (n = 15)	*p-value*	UU (n = 6)	*p-value*	CT (n = 7)	*p-value*
Age of first intercourse														
≤16	295 (35.0)	56 (35.0)	11 (26.2)	0.44*	2 (100)	0.17	5 (21.7)	0.35*	8 (53.3)	0.06*	3 (50.0)	0.52	4 (57.2)	0.51
17 a 20	384 (45.6)	68 (42.5)	20 (47.6)		0		12 (52.2)		7 (46.7)		2 (33.3)		1 (14.3)	
≥21	163 (19.4)	36 (22.5)	11 (26.2)		0		6 (26.1)		0		1 (16.7)		2 (28.6)	
Number of children														
0	86 (10.2)	20 (12.5)	**30 (31.0)**	<0.001*	0	0.08	4 (17.4)	0.14	1 (6.7)	0.96	1 (16.7)	0.87	1 (14.3)	0.75
1	182 (21.6)	51 (31.9)	**10 (28.8)**		0		4 (17.4)		5 (33.3)		2 (33.3)		2 (28.6)	
2	243 (28.9)	50 (31.3)	**10 (28.8)**		0		11 (47.8)		5 (33.3)		2 (33.3)		2 (28.6)	
3	193 (22.9)	**24 (15.0)**	**7 (16.7)**		1 (50)		**1 (4.4)**		3 (20.0)		1 (16.7)		2 (28.6)	
4	78 (9.3)	**6 (3.8)**	**1 (2.4)**		1 (50)		1 (4.4)		0		0		0	
5 or most	60 (7.1)	9 (5.6)	**1 (2.4)**		0		2 (8.7)		1 (6.7)		0		0	
Number of abortions														
0	593 (70.4)	115(71.9)	29 (69.1)	0.83*	2 (100)	1.00	17 (73.9)	0.86	7 (46.7)	0.02	4 (66.7)	1.00	4 (57.2)	0.42
1	190 (22.6)	33 (20.6)	11 (26.2)		0		4 (17.4)		**4 (26.7)**		2 (33.3)		2 (28.6)	
2	47 (5.6)	8 (5)	2 (4.8)		0		1 (4.4)		3 (20.0)		0		1 (14.3)	
3 or most	12 (1.4)	4 (2.50)	0		0		1 (4.4)		1 (6.7)		0		0	
Number of sexual partners														
1	431 (51.2)	63 (39.4)	17 (40.5)	0.001*	1 (50.0)	0.70	9 (39.1)	0.99*	4 (26.7)	0.008*	4 (66.7)	0.25	4 (57.1)	0.60
2	269 (32.0)	**61 (38.1)**	12 (28.6)		0		9 (39.1)		**11 (73.3)**		0		1 (14.3)	
3 or most	142 (16.9)	**36 (22.5)**	**13 (31.0)**		1 (50.0)		5 (21.8)		0		2 (33.3)		2 (28.6)	
Intercourse during the last 6 months														
Yes	736 (87.4)	**140 (87.5)**	36 (85.7)	0.94*	2 (100)	1.00	20 (87.0)	1.00	13 (86.7)	1.00	6 (100.0)	0.57*	6 (85.7)	1.00
No	106 (12.6)	20 (12.5)	6 (14.3)		0		3 (13.0)		2 (13.3)		0		1 (14.3)	
Use of condom														
Yes	231 (27.4)	54 (33.8)	13 (31.0)	0.10*	0	0.55	**12 (52.2)**	0.04*	**10 (66.7)**	0.005*	1 (16.7)	0.65	2 (28.6)	1.00
No	611 (72.6)	106 (66.3)	29 (69.1)		2 (100)		11 (47.8)		5 (33.3)		5 (83.3)		5 (71.4)	
Use of sexual toys														
Yes	10 (1.2)	3 (1.9)	1 (2.4)	0.47*	0	1.00	0	1.00	0	1.00	**1 (16.7)**	0.14	0	1.00
No	832 (98.8)	157 (98.1)	41 (97.6)		2 (100)		23 (100)		15 (100)		5 (83.3)		7 (100)	
Extramarital intercourse														
Yes	40 (4.8)	**13 (8.12)**	1 (2.4)	0.07*	0	1.00	0	0.22	2 (13.3)	0.35	0	1.00	0	1.00
No	802 (95.3)	147 (91.8)	41 (97.6)		2 (100)		23 (100)		13 (86.7)		6 (100)		7 (100)	
History of STI														
Yes	114 (13.5)	25 (15.6)	7 (16.7)	0.54*	0	1.00	**8 (34.8)**	0.01*	2 (13.3)	1.00	1 (16.7)	1.00	1 (14.3)	1.00
No	728 (86.5)	135 (84.4)	35 (83.3)		2 (100)		15 (65.2)		13 (86.7)		5 (83.3)		6 (85.7)	
History of sexual abuse														
Yes	51 (6.1)	**16 (10)**	1 (2.4)	0.05*	0	1.00	3 (13.1)	0.7	**3 (20.0)**	0.18	0	1.00	1 (14.3)	0.17
No	791 (93.9)	144 (90)	41 (97.6)		2 (100)		20 (87.0)		12 (80.0)		6 (100)		6 (85.7)	

Data is shown as n (%). MG, *Mycoplasma genitalium*; UU, *Ureaplasma urealyticum*; CT, *Chlamydia trachomatis*.
P-value: Fisher's Exact Test or Pearson's Chi Square Test (if more than 20% of cells with expected counts of less than 5) is marked with "*".
Univariate and multivariable analysis using polychotomous logistical model, significant p-values are highlighted in bold (*p-value* < 0.05).

Table 3
Odds ratio with 95% confidence intervals (CIs) from multinomial logistic regression.

Risk factors	High-risk HPV (n = 160)	Low-risk HPV (n = 42)	High-risk HPV, OR (95% CI)			Low-risk HPV, RRR (95% CI)	
			M. genitalium (n = 2)	*U. urealyticum* (n = 23)	*C. trachomatis* (n = 15)	*U. urealyticum* (n = 6)	*C. trachomatis* (n = 7)
Age of first intercourse, reference: "≤ 16"							
17 a 20	0.9 (0.63–1.39)	1.4 (0.66–2.98)	ND	1.8 (0.63–5.22)	0.7 (0.24–1.85)	0.5 (0.09–3.13)	0.2 (0.02–1.75)
≥21	1.3 (0.79–2.03)	2.0 (0.83–4.67)	ND	2.3 (0.68–7.54)	ND	0.6 (0.06–6.04)	0.9 (0.17–5.16)
Number of children, reference: "0"							
1	1.1 (0.61–2.05)	**0.3 (0.14–0.82)**[a;b]	ND	0.5 (0.12–2.08)	2.5 (0.29–22.00)	0.8 (0.08–9.50)	0.8 (0.08–9.50)
2	0.7 (0.40–1.32)	**0.2 (0.09–0.54)**[a];[b]	ND	0.9 (0.29–3.05)	1.7 (0.20–14.90)	0.6 (0.06–7.01)	0.6 (0.06–7.01)
3	**0.4 (0.20–0.77)**[a;b]	**0.2 (0.07–0.46)**[a;b]	ND	**0.1 (0.01–0.89)**[a;b]	1.2 (0.12–11.46)	0.4 (0.02–6.36)	0.8 (0.07–8.79)
4	**0.2 (0.08–0.60)**[a;b]	**0.1 (0.01–0.45)**[a;b]	ND	0.2 (0.02–2.10)	ND	ND	
5 or most	0.5 (0.20–1.15)	0.1 (0.01–0.65)[a]	ND	0.6 (0.11–3.67)	1.3 (0.08–21.19)	ND	
Number of abortions, reference: "0"							
1	0.9 (0.57–1.36)	1.2 (0.57–2.39)	ND	0.7 (0.24–2.16)	1.7 (0.50–6.02)	1.6 (0.29–8.67)	1.6 (0.29–8.67)
2	0.8 (0.38–1.86)	0.8 (0.19–3.65)	ND	0.7 (0.09–5.56)	5.3 (1.31–21.12)[a;b]	ND	3.1 (0.34–28.63)
3 or most	2.0 (0.58–6.59)	ND	ND	3.5 (0.42–29.71)	8.5 (0.94–77.71)	ND	ND
Number of sexual partners, reference: "1"							
2	**1.7 (1.17–2.57)**[a]	1.3 (0.59–2.70)	ND	1.8 (0.69–4.53)	**4.9 (1.53–15.47)**[a;b]	ND	0.4 (0.04–3.62)
3 or most	**2.2 (1.35–3.45)**[a;b]	**2.9 (1.35–6.16)**[a]	3.5 (0.22–55.98)	1.9 (0.63–5.88)	ND	1.6 (0.29–8.86)	1.6 (0.29–8.86)
Intercourse during the last 6 months							
yes vs. no	**1.0 (0.59–1.69)**[a]	0.9 (0.35–2.10)	ND	1.0 (0.28–3.31)	0.9 (0.21–4.23)	ND	0.9 (0.10–7.19)
Use of condom							
yes vs. no	1.5 (1.02–2.15)	1.3 (0.66–2.56)	ND	**3.1 (1.35–7.18)**[a;b]	**3.7 (1.92–16.92)**[a;b]	0.5 (0.06–4.60)	1.1 (0.21–5.54)
Use of sexual toys							
yes vs. no	2.0 (0.50–8.16)	2.6 (0.30–21.91)	ND	ND	ND	**17.6 (1.86–165.9)**[a;b]	ND
Extramarital intercourse							
yes vs. no	2.0 (1.05–4.16)[a]	0.6 (0.08–4.35)	ND	ND	3.7 (0.80–17.37)	ND	ND
History of STI							
yes vs. no	1.3 (0.78–2.05)	1.4 (0.59–3.17)	ND	**3.6 (1.46–8.62)**[a;b]	1.0 (0.23–4.62)	1.3 (0.15–11.19)	1.1 (0.13–9.05)
History of sexual abuse							
yes vs. no	2.0 (1.06–3.69)[a]	0.4 (0.06–3.26)	ND	2.8 (0.79–9.78)	**4.6 (1.25–17.13)**[a;b]	ND	2.5 (0.30–21.17)

ND: No data for this group/category; OR, Odds ratio; CI, confidence interval.

[a] Univariate analysis using polychotomous logistical model, significant p-values are highlighted in bold (*p-value* < 0.05).

[b] Multivariable analysis using polychotomous logistical model, significant p-values are highlighted in bold (*p-value* < 0.05).

Documentation System (Eastman Kodak Company, Rochester, New York, USA). The amplified products were recovered from the gel, purified (SpinPrepTM Gel DNA Kit, San Diego, USA) and sent for commercial sequencing service (Macrogen, Seoul, Korea) to confirm the results.

2.4. Statistical analysis

All the data and information were compiled in a database using the Excel 2015 software Microsoft Corporation-US). Then, it was exported to the Stata VS program. 15 (STATA Corp. College Station, Tx) to perform the statistical analysis. For the analysis purpose, HPV genotypes were classified as two groups: "High-risk HPV" and "Low-risk HPV". The included "High-risk HPV" group included high-risk and probably oncogenic genotypes. The "Low-risk HPV" included low-risk and other genotypes not related to malignancy. The HPV classification was based according to Bouvard et al. [19] (Table S2). For the univariate analysis, absolute frequencies and percentages were calculated. For the bivariate analysis, the Pearson's Chi square test and the Fisher's exact test were used.

In addition, the univariate and multivariate analyses were carried out to analyze the risk factors between sexually transmitted pathogens (*M. genitalium, U. urealyticum and C. trachomatis*) and HPV (High or low risk), for which the multinominal logistic regression was used. The dependent variable in the multinomial analysis had three categories: cases negatives (was considered as the reference group), coinfection HPV/sexually transmitted pathogens, and mono-infection HPV. The multivariate analysis included variables with a p-value less than 0.2 in the univariate analysis. *P-values* less than 0.05 were considered statistically significant.

2.5. Ethical considerations

This study was approved by the ethics committees of the Cajamarca Regional Teaching Hospital and the *Universidad Peruana de Ciencias Aplicadas* (UPC) (Document Nº FSC-CEI/356-06-21). All samples were obtained at the Specialized Preventive Oncology Service of the Cajamarca Regional Teaching Hospital, as part of the cervical cancer screening program. All samples were analyzed after signing the informed consent of each of the patients. All procedures were performed under international ethical guidelines for human health research by the Council for International Organizations of Medical Sciences (CIOMS) and the World Health Organization (WHO).

3. Results

A total of 842 samples were analyzed by PCR for *C. trachomatis, U. urealyticum*, and *M. genitalium*, and PCR followed by automated sequencing for HPV. The studied population was analyzed according to age group. We observed that 35.51% (299/842) of the cases were between the age range of 35 and 44 years, 29.6% (250/842) of the cases in the range of 25 to 34 years and 7.84% (66/842) were under 25 years. Regarding marital status, most of the women were cohabiting with 49.4% (416/842), followed by 24.82% (209/842) of single women and 23.40% (197/842) of married women (Table 1).

Of the total samples, 23.99% (202/842) were positive for HPV, of which 79.21% (160/202) corresponded to high-risk genotypes and 20.79% (42/202) to low-risk genotypes. A statistically significant association was found between high-risk and low-risk HPV genotypes in reference to the patient's age range ($p = 0.002$), as well as marital status ($p < 0.001$). No significant differences were found in terms of age and marital status in patients with high-risk or low-risk HPV who present co-infections with other sexually transmitted pathogens (Table 1).

In the high-risk HPV group, a total of 1.25% (2/160) had coinfection with *M. genitalium,* 14.38% (23/160) with *U. urealyticum*, and 9.38% (15/160) with *C. trachomatis*. In the samples categorized as low-risk HPV, 14.29% (6/42) had coinfection with *U. urealyticum*, 16.67% (7/42) with *C. trachomatis*, and no coinfections with *M. genitalium* were found.

Table 2 shows the analysis of the risk factors for co-infection between sexually transmitted pathogens and HPV. We found statistically significant differences between the HPV genotype and the number of children ($p < 0.001$), the number of sexual partners ($p = 0.001$) and the history of sexual abuse ($p = 0.05$).

In women who had co-infection between high-risk HPV and *U. urealyticum* significant differences were found in the use of condom, with 47.83% of the women reporting not using condom consistently ($p = 0.04$). Likewise, in the same group of patients, we found an association between a history of STIs ($p = 0.01$) and co-infection between high-risk HPV and *U. urealyticum,* with 34.78% of cases reporting history of STIs.

In patients with co-infection between high-risk HPV with *C. trachomatis*, significant difference was observed in the number of abortions ($p = 0.02$). A total of 46.67% of the cases had no history of abortions, 26.67% had a history of an abortion, and 20% had a history of two abortions. In addition, number of sexual partners was associated with this co-infection ($p = 0.008$), in which 73.33% had two sexual partners and 26.67% had only one sexual partner. Finally, an association was found in the use of condoms ($p = 0.005$), between high-risk HPV and *C. trachomatis*, where 66.67% of the cases used condoms and 33.33% did not use them. No association of risk factors was observed between low-risk HPV and co-infection with STIs, as shown in Table 2.

Table 3 shows the univariate and multivariate analysis of risk factors between sexually transmitted pathogens considering the negative test as the reference category. In the univariate analysis, patients co-infected with high-risk HPV/*U. urealyticum* were more likely to present a history of sexually transmitted infections ($p < 0.05$). Multivariate analysis showed that cases with high-risk HPV/*C. trachomatis* the major risk factor was had two abortions, with a OR of 5.3 times more than the negatives cases. History of sexual abuse ($OR = 4.6$, $p < 0.05$), have had 2 abortions ($OR = 5.3$, $p < 0.05$) and have had two sexual partners ($OR = 4.9$, $p < 0.05$) were also risk factors. The coinfection of high-risk HPV/*U. urealyticum* presented 3.6 times higher odds of history of sexually transmitted

infections and 3.1 times higher odds of use of condom compared to cases negatives. The use of sexual toys was a risk factor for the coinfection of low-risk HPV/*U. urealyticum* with OR 17.6 times than cases negatives.

The prevalence of *C. trachomatis*, *U. urealyticum* and *M. genitalium* in HPV negative population was *M. genitalium* (0.16%, 1/640), *U. urealyticum* (15%, 96/640) and *C. trachomatis* (7.66%, 49/640).

4. Discussion

HPV infection is considered the main cause of cervical cancer and is considered the fourth most common cancer among women worldwide and the second most common cancer among women in Peru [11]. In this context, the STI rate in Latin America is higher compared to other regions [1]. The impact of co-infections of sexually transmitted pathogens and HPV infection has not been fully elucidated [20]. For example, some studies suggest that coinfections are common and can lead to persistent HPV infections, facilitating progression to neoplasia [14,21,22]. In addition to the above, it has also been described that the prevalence of STIs is higher among patients with suspected cervical cancer [23]. Among the different STIs, *C. trachomatis* has been reported to be one of the most prevalent pathogens in HPV patients [24]. However, co-detection of other pathogens such as *Mycoplasma hominis* and *Ureaplasma urealyticum* has also been reported in multiple settings [22,25,26].

In this study we report that 23.9% of asymptomatic women were positive for HPV in our population, being 79.21% high-risk genotypes and 20.79% low-risk genotypes. These findings are consistent with other studies previously carried out in Cajamarca. For example, in 2018 Ponce-Benavente et al. found that 30.48% samples were positive for HPV, of which 63.6% were high-risk genotypes, 23.1% were of probable oncogenic risk, and 7.4% were low risk [27]. Another study carried out in Cajamarca also found that the most frequent genotypes were high risk, with HPV-52, HPV-31 and HPV-16 being the most prevalent [28]. Likewise, a study from Brazil found a prevalence of 33.8% of HPV, where 59.1% were high risk, 16.9% probable oncogenic risk and 24% low risk [29]. These studies showed a higher prevalence of HPV than our study, however, high-risk genotypes were predominant across all studies.

We report a prevalence of 8.43% cases of *C. trachomatis* in the whole study population. Among HPV positive women, we found that 10.89% had co-infection with this pathogen, with greater cases among high-risk genotypes. Previous studies have also reported similar prevalence of co-infections between these pathogens. Similar to our study, Martinelli et al. found that 7% of HPV patients also had *C. trachomatis* [30]. On the other hand, Bellaminutti et al. reported that 17% of HPV patients were co-infected with *C. trachomatis* [24]. Finally, a study performed in Brazil showed a prevalence of co-infection between these microorganisms of 12.5% [31]. It is important to screen women with HPV for the presence of *C. trachomatis*, as these infections may be associated [21] and also lead to persistent infection, with progression to cervical neoplasia [24,29]. Particularly, young women with more than one sexual partner should be evaluated, as they are prone to present with co-infections according to our results.

The relationship between HPV infection with pathogens like *U. urealyticum* and *M. genitalium* is less clear. These microorganisms may be present as part of the normal microbiota, however, an increase in the population of these bacteria has also been reported in disease states [7,8]. We report that *U. urealyticum* was the most frequent co-infection, being detected in 14.38% of HPV positive patients. Risk factors for this co-infection were previous history of STIs and lack of use of condom. Likewise, Parthenis et al. [20] report that *Ureaplasma* spp. was the most frequently detected pathogen in Greek women, which was found in 18.2% of the study population. Another study performed by Camporiondo et al. found a significant association between these pathogens. They report that HPV positive women had 6 times more risk to present with *U. urealyticum* infection [32]. Another study performed on sex workers showed that 66.7% of women with HPV-16 and 46.2% of women with HPV-18 were co-infected with *U. urealyticum* [33]. However, this study may not be extrapolated to our setting, as the study populations are significantly different.

Finally, we found that only 3 samples were positive for *M. genitalium,* being 0.99% of the total HPV positive samples. These cases occurred in patients with high-risk HPV. Our results are similar to those reported in Brazil, in which only one sample was positive for this pathogen, which happened to be a co-infection with a high-risk genotype [29]. Also a Mexican study found a prevalence of *M. genitalium* of 0.5%, which represented only one sample that presented with low grade intraepithelial lesion [34].

In reference to the risk factors associated with the prevalence of co-infection in sexually transmitted infections, it has been reported that there is an independent association with alcohol consumption, having a sexual partner suffering from a sexually transmitted infection, having more than three sexual partners in life in addition to presenting more frequently among healthy women without cervical injuries and those under 18 years of age compared to older women. Our study found the number of children, the number of sexual partners, a history of sexual abuse and the inappropriate use or non-use of condoms as associated risk factors [35–37].

Regarding the clinical importance of simultaneous infection between HPV and other sexually transmitted bacteria, it has been reported that vaccination against HPV can also confer benefits in terms of prevention of co-infections, leading to a decreased risk of developing cervical cancer. Specifically, it is reported that the simultaneous presence of HPV/*Chlamydia trachomatis* (Ct) increases the risk of carcinogenesis induced by HPV, since frequent isolations of this bacterium have been observed in precancerous and cancerous lesions. Sexually transmitted infections caused by bacteria induce chronic inflammation followed by damage to epithelial cells of the cervix and increase susceptibility to HPV infection, which can lead to cell transformation [38–40].

5. Limitations

Our study has two important limitations. Firstly, information regarding Papanicolau smears and/or cervical cytology were not available. We could not evaluate the presence of co-infections and their association with cervical lesions. Also, we did not have information regarding status vaccination in the women evaluated, which could be an important factor that determines the prevalence of the different genotypes.

6. Conclusions

HPV infection continues to be a highly relevant problem in Peru, particularly due to the high prevalence of high-risk genotypes. In addition, we report high rates of co-infections with other STIs, such as *U. urealyticum* and *C. trachomatis*. We highlight the importance of active surveillance to promptly diagnose these infections, since they may lead to persistent HPV infections. Preventive measures such as vaccination, use of condom and timely screening for multiple STIs should be strengthen.

Author contribution statement

Juana del Valle: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Miguel Angel Aguilar-Luis: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Angela Cornejo-Tapia, Johanna Martins-Luna, Lorena Becerra-Goicochea, Luis Pinillos-Vilca: Performed the experiments.

Priscilla Pella-Saavedra, Fatima Ramos-Vallejos, Hugo Carrillo-Ng: Analyzed and interpreted the data; Wrote the paper.

Luis Pinillos-Vilca, Wilmer Silva-Caso: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at https://doi.org/10.6084/m9.figshare.19826917.

Declaration of interest's statement

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e14257.

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