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Human papillomavirus and coinfections with *Chlamydia trachomatis*, *Gardnerella vaginalis*, and *Trichomonas vaginalis* in self-collected samples from female sex workers in the Central-Western region of Brazil

Papilomavírus humano e coinfecção por Chlamydia trachomatis, Gardnerella vaginalis *e* Trichomonas vaginalis *em amostras autocoletadas de mulheres profissionais do sexo da região Centro-Oeste do Brasil*

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ABSTRACT

Introduction: Human papillomavirus (HPV) is intimately associated with cervical cancer, and the presence of coinfections, such as with *Chlamydia trachomatis*, *Gardnerella vaginalis* and *Trichomonas vaginalis*, may potentiate or facilitate HPV infection. Female sex workers are considered vulnerable to the acquisition of these infections due to exposure to risk factors. **Objective:** To determine HPV infection, viral types and coinfections in self-collected samples from female sex workers. **Methods:** Self-collected samples from female sex workers, of vaginal canal and uterine cervix, were subjected to HPV-deoxyribonucleic acid (DNA) detection, viral genotyping by type-specific polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and the detection of coinfection. **Results:** HPV-DNA was detected in 19.4% of the samples, and HPV 31, 6, and 53 were the most frequently detected types. There was a predominance of high-risk oncogenic HPV (HR-HPV) and a strong presence of simultaneous infections with multiple HPV types (84.6%). Coinfections with both HPV and *C. trachomatis*, and HPV and *G. vaginalis* were detected. The variables that were statistically associated with HPV infection and the presence of multiple infections were non-use of condoms and non-compliance with regular cervical cytology screening. **Conclusion:** The results highlight the importance of more comprehensive studies among vulnerable populations, aiming to establish measures to raise awareness about the risks of contracting sexually transmitted infections, as well as to support future studies for introducing HPV vaccines with wider coverage of viral types.

Key words: papillomavirus infections; coinfection; sex workers.

INTRODUCTION

Sexual contact is the main form of transmission of human papillomavirus (HPV), which causes one of the most common infections in the world. Female sex workers (FSW) constitute a vulnerable group for the development of cervical cancer precursor lesions, since they are constantly exposed to risk factors that facilitate sexually transmitted disease (STD) contagion. Several factors may influence the persistence of infection and the progression of HPV-associated lesions, such as coinfection with *Chlamydia trachomatis*, *Gardnerella vaginalis*, and *Trichomonas vaginalis*. The presence of these pathogens is associated with high-risk oncogenic HPV infection (HR-HPV), and may potentiate the infection by HPV and thus increase the risk of neoplastic progression[1, 2].

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Self-collection of cervicovaginal samples is a viable alternative for acquiring samples from hard-to-reach vulnerable populations, for example, FSW. The obtained samples have previously successfully been used for the identification of HPV-deoxyribonucleic acid (DNA)[3]. The objective of this study was to characterize HPV infection, identify the main HPV types, and evaluate coinfection with *C. trachomatis*, *G. vaginalis*, and *T. vaginalis* in cervicovaginal self-collected samples from FSW.

METHODS

Participants and study design

A cross-sectional descriptive study of FSW working in public places (squares, parks, gardens, streets, avenues, etc.) and private places (saunas, nightclubs, and brothels) was carried out in Campo Grande, Mato Grosso do Sul, Central-Western Brazil. Samples were self-collected by FSW between June and December, 2011, using an endocervical brush, under verbal and written guidance. The participants answered the questionnaire with socio-epidemiological information, and ethical approval was granted by the Research Ethics Committee (CAAE no. 873.060).

HPV-DNA detection

HPV-DNA was detected by polymerase chain reaction (PCR) with a pool of consensus primers PGMY09/11 (450 bp)[4]. As endogenous control, human β-globin (286 bp) was amplified with primers PC04 and GH20[5]. PCR products were separated on a 1.5% agarose gel by electrophoresis and visualized by ethidium bromide staining under ultraviolet (UV) light. Molecular weights were determined by comparison with a 100 bp DNA ladder.

Type-specific PCR (TS-PCR) and restriction fragment length polymorphism (RFLP)

HPV-DNA positive samples were genotyped by PCR using type-specific primers (TS-PCR) for the L1, E6, and E7 gene DNA sequences of HPV 6, 11, 16, 18, 31, 33, and 45[6-10]. PCR products were separated on a 1.5% agarose gel by electrophoresis and visualized by ethidium bromide staining under UV light, and their molecular weights were determined by comparison with a 100 bp and 50 bp DNA ladder. The same samples were then analyzed by RFLP. The samples containing the PGMY 09/11 PCR product were subjected to enzymatic digestion for 1 hour at 37°C. The enzymes used for the digestion reaction were *Pst* I, *Hae* III, *Dde* I, and *Rsa* I. The digestion pattern was analyzed following electrophoresis on a 3% agarose gel containing ethidium bromide, and interpreted using a previously described algorithm[11].

G. vaginalis, *C. trachomatis*, and *T. vaginalis* PCR

G. vaginalis was identified using modified primers, GV1 and GV2 (310 pb)[11]. *C. trachomatis* was identified using KL1 and KL2 primers (241 bp)[12]. *T. vaginalis* was identified as previously described, with TVK3 and TVK7 primers (300 bp)[13].

PCR products were separated by electrophoresis on a 1.5% agarose gel by ethidium bromide staining to visualize the DNA under UV light. Molecular weights were determined by comparison with a 100 bp DNA ladder.

Statistical analysis

Participants were grouped according to their age: up to 25 years, from 26 to 35, and over 36 years. For HPV infection type, participants were placed into two categories: multiple infections with up to two types of HPV, and with more than two types of HPV. Both the presence of multiple infections and the presence of HR-HPV were related to coinfections with *C. trachomatis*, *G. vaginalis*, and *T. vaginalis*.

Frequency tables were analyzed by Pearson's chi-squared test, with a 95% confidence interval (CI), and the contingency tables (2 × 2) were analyzed by Fisher's exact test. In both cases, results were considered to be statistically significant when $p \leq 0.05$. Significant variables in these tests were analyzed by multinomial logistic regression analysis to estimate odds ratio (OR). Statistical analysis was performed using SPSS 10.0 for Windows and BioEstat version 5.0 for Windows.

RESULTS

Seventy-nine FSW, with ages ranging from 18 to 42 years (mean of 26.7 years) participated in the study. Out of the 79 self-collected samples, 12 (15.2%) were negative for the product of amplification of the human β-globin gene, whereas the remaining 67 were positive for the amplicon. When analyzed by PGMY 09/11 PCR, 19.4% (13/67) of the samples were positive for HPV-DNA.

The variables that were statistically associated with HPV infection were non-use of condoms and non-compliance with regular cervical cytology screening ($p \leq 0.05$).

Amongst the FSW who were positive for HPV-DNA, the age group up to 25 years had the highest incidence of HPV, with 15.8% of participants positive for HPV-DNA (9/57), and a mean age of

22.7 years. Among the participants over 35 years of age, no HPV-DNA was detected.

All HPV-DNA positive samples (n = 13) had their HPV viral types identified (**Figure**). HR-HPV was found in 84.6% (11/13) of the positive samples, and HPV of low oncogenic risk (LR-HPV) was detected in 69.2% (9/13). A statistical association was found between the presence of HR-HPV and the non-use of condoms (χ^2 = 3.909, OR = 4.444, p = 0.048).

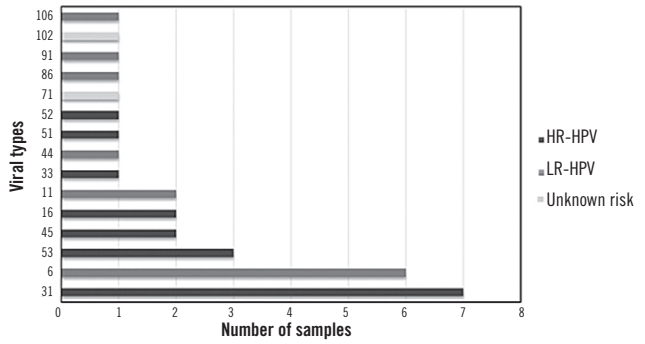

FIGURE – *Distribution of viral types identified by TS-PCR and RFLP in HPV positive self-collected samples from female sex workers*

TS-PCR: type-specific polymerase chain reaction; RFLP: restriction fragment length polymorphism; HPV: human papillomavirus; HR-HPV: high-risk oncogenic HPV; LR-HPV: low-risk oncogenic HPV.

Infections with multiple different strains of HPV were detected in 84.6% (11/13) of the HPV-DNA positive samples. Among these samples, the presence of up to two viral types was detected in 54.5% (6/11), and that of more than two types was found in 45.5% (5/11). In most of the samples, mixed infections with HR- and LR-HPV were observed. Among the samples with up to two viral types (n = 6), 50% (3/6) were positive for HR-HPV only, and 33.3% (2/6) had both HR- and LR-HPV. The presence of multiple different strains of HPV and the non-use of condoms were statistically correlated, as well as multiple different strains of HPV infection and non- compliance with regular cervical cytology screening ($p \leq 0.05$) (**Table 1**).

TABLE 1 – Condom use and oncotic cytology in relation to the presence of multiple-type HPV infections in female sex workers (n = 57[#])

Variables		MI						χ^2	p
		Neg and SI		MI[*]		MI[**]			
		n	%	*n*	%	*n*	%		
Condom use	Yes	40	70.2	3	5.3	1	1.8	8.057	0.017
	No	8	14	2	3.5	3	5.3		
Cervical cytology screening	Yes	41	74.5	2	3.6	3	5.5	8.189	0.024
	No	5	9.1	3	5.5	1	1.8		

[#]57 women answered the questions; [*]multiple infection by 2 types of HPV; [**]multiple infection by more than 2 types of HPV; HPV: HPV: human papillomavirus; Neg: negative; SI: simple infection; MI: multiple infection; χ^2: Pearson's chi-squared test; p: *Fisher's exact test.*

We observed that 71.6% (48/67), 25.4% (17/67), and 4.5% (3/67) of the positive samples for amplification of the human β-globin gene were positive for *G. vaginalis*, *C. trachomatis*, and *T. vaginalis*, respectively. Results regarding coinfection of HPV with *G. vaginalis* (76.9%), HPV and *C. trachomatis* (38.5%), are presented in **Table 2**.

TABLE 2 – Coinfection with HPV, *Chlamydia trachomatis*, *Gardnerella vaginalis* and *Trichomonas vaginalis* in self-collected samples of vaginal canal excoriation and uterine cervix from female sex workers (n = 67)

Microorganism	Samples		Total
	Negative HPV	Positive HPV	
G. vaginalis	79.6% (43/54)	76.9% (10/13)	79.1% (53/67)
C. trachomatis	22.2% (12/54)	38.5% (5/13)	25.4% (17/67)
T. vaginalis and *G. vaginalis*	5.6% (3/54)	0% (0/13)	4.5% (3/67)
C. trachomatis and *G. vaginalis*	18.5% (10/54)	30.8% (4/13)	20.9% (14/67)
C. trachomatis, *G.vaginalis* and *T. vaginalis*	1.8% (1/54)	0% (0/13)	1.5% (1/67)

HPV: human papillomavirus.

DISCUSSION

In this study involving FSW from the Central-Western region of Brazil, we detected HPV-DNA in 19.4% of self-collected vaginal canal and uterine cervix samples, with the most frequent type being HR-HPV 31 (53.8%), followed by LR-HPV 6 (46.1%) and HR-HPV 53 (23%). No HPV 18 was detected, what corroborates a previous study carried out in young university students in the same region of Brazil[14]. Soohoo *et al*. (2013)[15] reviewed existing literature and found that HR-HPV 16, 52, and 31 were the HPV types most frequently found in FSW. These authors argue that the variation in the prevalence of types between different regions may reflect regional characteristics or even the test applied for genotyping. However, all the studies analyzed by Soohoo *et al*. (2013)[15] conclude that HR-HPV are the most prevalent types.

The frequency of detection of HPV-DNA was higher in women aged up to 25 years, what coincides with the phase of more intense sexual activity[14]. Higher infection rates can also occur between ages 30 and 45, owing to reinfection or reactivation of a previous infection[15]. However, no HPV-DNA was detected in participants over 35 years old in this study, probably owing to the small sample size in this age group.

Viral load is known to be high in young adult women, possibly because of exposure to HPV while they are immunologically naive to the virus – only about 10% of these women remain infected later in life[16]. It is possible that participants over 30 years of age may have better innate and adaptive immune system reactions in relation to the virus, or observe sexual practices involving greater protection.

It is known that FSW are more vulnerable to HPV infection than the general population because of their massive exposure to several risk factors[17]. The prevalence of HPV infection among FSW may vary according to the geographic region, ranging from 2.3% to 100% infection rate, with an average of 42.7%[15]. In our study, we detected HPV-DNA in 19.4% of participants, which is similar to HPV prevalence in Singapore (14.4%)[18] and Mexico (11.8%)[19].

Studies conducted in Vietnam involving FSW also demonstrated geographic variations in the rate of HPV infection, since 49.5% of FSW in the north of the country were positive for HPV, whereas 85% of FSW were positive for HPV in the south[20, 21]. The results obtained in the present study may have been influenced by the approach to the participants and by the sample collection method, as the vaginal canal might have substances that interfere with DNA amplification by PCR.

In the present study, strain typing by TS-PCR and RFLP was carried out in all HPV positive samples, and the predominance of HR-HPV infections was clear, with HPV 31 and 53 as the most frequent types. The concomitant application of two genotyping methods increased the possibility of detection of infection with multiple types of HPV. In Belgium, a study comparing FSW and women in general, matched by age, revealed higher incidence of infection among the former, with HPV 31, 16, and 52 being the most prevalent types[22].

Soohoo *et al*. (2013)[15] observed that among studies that also contained multiple strain infection data, the mean prevalence was 20.4%, and multiple strain infections caused by two viral types were most frequently found. In the present study, 84.61% of the samples presented multiple-type infections, and in 54.5%, up to two viral types were found. In 45.5%, more than two viral types were detected. The proportion of multiple-type infections in our study was higher than that found in Vietnam (61.2%)[20] and China (23.6%)[23].

The prevalence of multiple-type infections and HR-HPV may vary according to several factors, such as age, sexual behavior, host immune response, methods of detection and genotyping. The distinction between HR- and LR-HPV and, especially, the identification of viral types are important prognostic indicators in clinical screening, since they may be used to identify patients with higher risk of developing cervical lesions[24].

To improve cervical cancer control programs, two components are essential: primary prevention by introducing HPV vaccines, and secondary prevention through the improvement of existing screening programs. For this purpose, reliable estimates of the disease prevalence and the predominant viral types in each region are required.

In Brazil, the quadrivalent anti-HPV vaccine for girls aged 9 to 13 years was included in the vaccination schedule since 2014. This vaccine provides protection against HPV 6, 11, 16 and 18. In the light of our study, it would seem that some of the most common viral types found among FSW in the Central-Western region of Brazil are not covered by the currently available vaccine. This could enable the spread of infection and the development of high-grade lesions. As a result, it is necessary to improve diagnostic screening methods to reach vulnerable populations, such as FSW, and to introduce new vaccines covering other HR-HPV, thus increasing protection in these communities.

In addition to HPV infection, FSW are also exposed to infection by other sexually transmitted pathogens such as *C. trachomatis* and *T. vaginalis*[25], whose infection may influence the acquisition and persistence of HPV.

C. trachomatis causes changes in epithelial cells that may favor HPV infection; it also causes changes in the immune response profile, decreasing the host's ability to eliminate viral infection and, therefore, facilitating viral persistence[26]. *T. vaginalis* has been associated with HR-HPV 16. According to Lazenby *et al*. (2014)[27], patients with *T. vaginalis* are 6.5 times more likely to have HPV 16 than those who are not infected.

In addition to these pathogens, *G. vaginalis*, commonly found in almost all women with bacterial vaginosis (BV)[28], has also been found to be associated with HPV infection. In women with normal microbiota, i.e. without BV, a frequency of *G. vaginalis* of 14.4%[29] was found in the vaginal microbiome. In our study, we detected a total frequency of *G. vaginalis* of 79.1% and in the HPV-DNA positive samples, the frequency was 76.9%. The high frequency of this bacterium in the studied population could be due to the presence of BV, since the study participants are exposed to risk factors that favor BV infection, which may affect the protective microbiota allowing anaerobic bacteria, including *G. vaginalis*, to proliferate, facilitating the entry of other pathogens[2].

In the present study, *C. trachomatis* was detected in 25.4% of the samples analyzed, whereas in the Northern region of Brazil, the rate of detection was 20.7% among FSW[12].

A study carried out among FSW in Guatemala has reported that the incidence of this infection was 6.2%[30]. Another study, carried out by Friedek *et al*. (2004)[31], observed that in patients presenting low-grade squamous intraepithelial lesions (LSIL), there was a statistical correlation between positivity for *C. trachomatis* and the presence of HPV, especially when the infection was by HR-HPV.

In HPV-positive patients with no cytological abnormalities, coinfection by both microorganisms has been observed, thus

suggesting a relationship between the pathogens[32]. It should be emphasized that the presence of the *C. trachomatis* bacterium in the cervical microenvironment associated with HPV can lead to more severe viral infections[1].

The frequency of *T. vaginalis* infection is also low among women of the general population, and in the study it was detected in 4.5% of the samples. In studies performed with FSW in China, the reported frequency is approximately 2.1%[23]. While the incidence of this infection may vary, this microorganism is not a part of the vaginal microbiota.

Among the three microorganisms tested, only *T. vaginalis* was not found among the HPV-DNA positive samples. Both HPV/*G. vaginalis* and HPV/*C. trachomatis* coinfection rates were high, 76.9% and 38.5%, respectively. The simultaneous presence of HPV, *G. vaginalis*, and *C. trachomatis* was detected in 30.8% of the analyzed samples. It is known that the presence of these pathogens coinfecting the patient may potentiate neoplastic progression[1, 2], demonstrating the importance of broader studies correlating the presence of these pathogens prior to cervical cancer.

A divergence regarding the use of condoms was also observed in this study: in general, 77.2% of participants reported using the device. However, HPV-DNA was detected in 13.6% of FSW who admitted using condoms, which demonstrates unprotected sex is still occurring despite the STD risk negotiating condom use is known to be a common practice among these women, increasing their vulnerability to sexually transmitted infections.

Besides the relationship between infection and non-use of condoms, statistically significant correlations were found between the presence of HPV-DNA and the lack of regular cervical cytology screening on a routine basis ($p < 0.05$). According to the participants' report, 83.6% of FSW undergo oncotic cytology. Considering that this exam is used by the public health service in Brazil for screening and prevention of cervical cancer, it is a positive factor to raise this group's awareness of the importance of undergoing this exam periodically.

Based on the results of the present study, the importance of the use of condoms and the frequency with which FSW undergo oncotic cytology examinations were highlighted. These represent protective factors, pointing out to awareness and the establishment of preventative measures in vulnerable populations. Our findings demonstrate the need of more comprehensive studies among vulnerable populations to adapt preventative measures, and to develop awareness about the need for anti-HPV vaccines with a wider coverage of viral types.

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RESUMO

Introdução: *O papilomavírus humano (HPV) está intimamente associado ao câncer cervical, e a presença de coinfecções, como por* Chlamydia trachomatis, Gardnerella vaginalis *e* Trichomonas vaginalis, *pode potencializar ou facilitar a infecção por HPV. As mulheres profissionais do sexo são consideradas vulneráveis à aquisição dessas infecções devido à exposição aos fatores de risco.* ***Objetivo:*** *Determinar a infecção por HPV, os tipos virais e as coinfecções em amostras autocoletadas de mulheres profissionais do sexo.* ***Métodos:*** *Amostras autocoletadas de mulheres profissionais do sexo, do canal vaginal e da cérvice uterina, foram submetidas a detecção do HPV-ácido desoxirribonucleico (DNA), genotipagem viral por reação em cadeia da polimerase (PCR) tipo específica e* restriction fragment length polymorphism *(RFLP) e detecção de coinfecção.* ***Resultados:*** *O HPV-DNA foi detectado em 19,4% das amostras, sendo os tipos HPV 31, 6 e 53 os mais frequentes. Houve predominância de HPV de alto risco (HR-HPV) e elevada presença de infecções múltiplas (84,6%). A presença de coinfecções foi observada tanto para HPV e* C. trachomatis *quanto para HPV e* G. vaginalis*. Observou-se também que mulheres profissionais do sexo que não fazem uso de preservativos e aquelas que não realizam o exame citológico rotineiramente estão predispostas à aquisição da infecção causada pelo HPV.* ***Conclusão:*** *Os resultados obtidos ressaltam a importância de estudos mais abrangentes entre as populações vulneráveis, objetivando estabelecer medidas para a conscientização sobre os riscos de aquisição das infecções sexualmente transmitidas, bem como auxiliar estudos futuros para introdução de vacinas contra o HPV com maior cobertura de tipos virais.*

Unitermos: *infecções por papilomavírus; coinfecção; profissionais do sexo.*

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