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#### Abstract

**Background:** Human papillomavirus (HPV) is the most common cause of cervical cancer worldwide. The aim of this study was to determine the prevalence and to characterize circulating high-risk HPV genotypes among female sex workers in Ouagadougou.

**Patients and Methods:** A total of 200 sex workers voluntarily agreed to take part in this study and swabs were taken from the endocervical canal for HPV detection. Real-time PCR was used for HPV genotype identification.

**Results:** Overall HPV carriage infection was 53%. One hundred six (106) sex workers were positive for at least one high-risk HPV genotype. Fourteen genotypes corresponding to 225 infections, were characterized: HPV 68 (14.6%), HPV 31 (12%), HPV 52 (9.3%), HPV 51 (8.9%), HPV 56 (7, 6%), HPV 66 (7.6%), HPV 58 (7.1%), HPV 35 (7.1%), HPV 39 (6.2%), HPV 18 (6.2%), HPV 45 (5.8%), HPV 59 (3.1%), HPV 16 (2.7%), HPV 33 (1.8%). Multiple infection (2 - 9 genotypes) was detected in 53.8% of infected sex workers. The number of sexual partners was statistically associated with carriage of HPV ( $p \le 0.01$ , OR = 2.000, 95% CI = 0.56 - 7.14), early intercourse was not.

**Conclusion:** The prevalence of HPV carriage in this study was high and associated with having a high number of multiple infections. Primary preventions such as sex education and vaccine use could significantly decrease the incidence of HPV infections.

Keywords: Sex-Workers; HPV; Genotypes; PCR

### Introduction

Human papillomavirus (HPV) infections are a real public health problem, not only because of their high prevalence, but especially because of the associated cancer risk [1,2]. Infection is acquired early at the onset of sexual activity and the prevalence would reach 40% within two years of first intercourse, according to some studies [3]. The natural cause of genital HPV infection is most often towards viral clearance [4]. However, in some women, the infection can become chronic and constitute cervical dysplasia which is a precursor of cancerous lesions [2].

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The risk of developing cervical cancer is about three times higher in women with ten different sexual partners compared with one partner [5]. Sex workers are also more at risk because of the multiplicity of their partners. Currently available prophylactic vaccines only target genotypes 6, 11, 16 and 18 [6]. The genotypes targeted by these vaccines are not sometimes the most found in Burkina Faso and some West African countries [7-12]. Therefore, this study was conducted in order to determine the prevalence of the carriage and characterize HPV genotypes among sex-workers in Ouagadougou.

### Methods

The study was conducted in Ouagadougou, the political capital of Burkina Faso in west Africa.

Biomolecular analyzes were carried out at the Laboratory of Molecular Biology and Genetics of the University Ouaga I Professor Joseph Ki-Zerbo and the Biomolecular Research Center Pietro Annigoni, CERBA/LABIOGENE.

### Period and study population

An initial screening awareness campaign was carried out during the month of June 2017.

A second phase consisted of a collection of samples over a period of 4 weeks, from July 24, 2017 to August 24, 2017.

The study population consisted of 200 sex workers who gave their free and informed consent to participate in this study.

The sample sizes (200) were obtained following a 4-week collection period, from July 24, 2017 to August 24, 2017 and sex workers were included in the study as soon as their consent was obtained.

### **Inclusion criteria**

All sex workers who consented freely after information on the study had been explained to them.

### **Criteria of non-inclusion**

All sex workers in the menstrual period or pregnant or having undergone total hysterectomy were not included in the study.

#### **Data collection**

Clinical data were collected using an individual collection card administered to each sex worker in the study population.

The samples were obtained after swabbing of the endocervix and the exocervix, performed by midwives. After highlighting the cervix using a disposable speculum, a simple inspection of the cervix was performed followed by the introduction of a sterile cotton-tipped swab at the area of pavimento-cylindrical junction by turning three times anti-clockwise.

The attribution of a code made it possible to respect the anonymity. The sample thus collected was introduced into a transport medium and stored at -20°C pending the extraction of the DNA.

After endocervical sampling, visual inspection with acetic acid (VIA) and visual inspection with lugol's iodine (VILI) were performed.

### **Extraction and amplification of HPV DNA**

Extraction of HPV viral DNA was done using the SACACE biotechnologies<sup>®</sup> DNA-Sorb-A kit following the protocol provided by the manufacturer.

The real-time amplification kit used was SACACE biotechnologies<sup>®</sup> "HPV Genotypes 14 Real-TM Quant" (reference V67-100FRT, lot 26G17K414) which makes it possible to detect fourteen HPV genotypes at high risk (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52.56, 58, 59, 66.68). The actual PCR was carried out using the SaCycler-96 Real Time PCR device in version 7.3 of SACACE biotechnologies<sup>®</sup>.

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The data was then processed and analyzed using SPSS software version 17.0 of the Microsoft EXCEL 2010 software and EndNote X7 software. Chi-square or Fisher tests were used to compare proportions and averages. The difference was statistically significant for p < 0.05.

### **Ethical considerations**

This study obtained authorization N ° 2017-1026/MS/RCEN/DRSC from the regional health director of the Center. Written informed consent was obtained from the all of sex workers enrolled in the study.

### Results

### Sociodemographic, behavioral and sexual characteristics

The age of participants ranged from 16 to 50 years with an average of  $27.3 \pm 0.4$  years. Females aged from 15 to 24 years and accounted for 33.5% of cases (67/200). The age at first intercourse varied between 10 and 22 years, with an average age of  $16.9 \pm 0.1$  years. The median age at first intercourse was 17 years old. In 10.0% of the cases, the first sexual intercourse occurred before the age of 15 (20/200). The number of sexual partners that female sex workers estimated to have in a year ranged from 300 to 600, with an average of  $416 \pm 5.3$ . In 23.5% of cases, female sex workers reported having had five hundred (500) sexual partners or more in one year (47/200).

Few female sex workers used male condom consistently during sex (25%) compared to those who used the male condom occasionally (75%). HIV seropositivity was found in 4.5% of female sex workers (9/200).

	Effective	Percentage %
Age (years old)		
15 - 19	12	6.0
20 - 24	55	27.5
25 - 29	67	33.5
30 - 34	44	22.0
35 - 39	13	6.5
40 - 45	6	3.0
45 and over	3	1.5
Condom use		
Occasionally	150	75.0
Always	50	25.0
Never	0	0
Frequency of intercourse		
< 10 times a week	55	27.5
10 - 20 times a week	122	61.0
20 - 30 times a week	23	11.5
Level of education		
Illiterate	50	25.0
Primary	41	20.5
Secondary	103	51.5
University	6	3.0
Age at first intercourse		
≥16 years old	158	79.0
<16 years old	42	21.0

Table 1: Sociodemographic behavioral and sexual characteristics.

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### Prevalence of HPV infection at high risk

Of the 200 sex workers, 106 were positive for at least one high-risk HPV genotype, a prevalence of 53% (95% CI [45.84 - 60.04]).

### Frequency of different high-risk HPV genotypes found

As shown in figure 1 HPV 68 accounted for 14.6% of recovered HPV. HPV 16 and 18 together, accounted for 8.9% of cases.



Figure 1: Frequency of detected HPV genotypes in relation to their total number.

#### Carriage of HPV by number of genotypes per sex worker: multiple or isolated infection

Among the 106 high-risk HPV-positive sex workers, the number of genotypes per sex worker ranged from 1 to 9 with an average of 4.57. In 53.8% of cases (57/106), there were multiple infections with two or more high-risk HPV genotypes. Nine (9) high risk genotypes were found in a female sex worker.

#### HPV carriage according to the factors associated with the infection

As shown in table 2 and 3 we studied the association of HPV infection with age, educational level, age at first sexual intercourse, number of sexual partners, recent change in sexual partner, the frequency of sexual intercourse, condom use, and the results of VIA and VILI. Of the 106 HPV-positive sex workers, only one had positive VIA/VILI and 9 were positive for VIA. Carriage of HPV was significantly associated with number of sexual partners (p <0.001), educational level (p = 0.019) and condom use (p < 0.001).

The prevalence of cervical dysplasia among female sex workers in our study by VIA was 17.5%. Table 3 shows that there is no statistically significant relationship between HPV carriage and the VIA/VILI test result.

### Discussion

Our study has known limitations and constraints related in part to the accessibility of the population of sex workers and often to their refusal to be examined. Indeed after the awareness period, few sex workers agreed to make the trip. We were often forced to travel to go to their site to take samples.

The mean age at first intercourse of  $16.9 \pm 0.1$  years. This result is in line with that found in the Rousseau, *et al.* in 2006 [10]. These data show that the age of onset of sexual intercourse would not have varied overall between 2006 and 2017.

Characteristics	HPV Negative n (%)	HPV Positive n (%)	P value
Age (years)			
15 - 19	4 (4.3)	8 (7.5)	
20 - 24	22 (23.4)	33 (31.1)	
25 - 29	36 (38.2)	31 (29.2)	
30 - 34	23 (24.4)	21 (19.9)	0.399
35 - 39	4 (4.3)	9 (8.5)	
40 - 44	4 (4.3)	2 (1.9)	
45 and over	1 (1.1)	2 (1.9)	
Level of education Illiterate	32 (34.1)	18 (16.9)	
Primary	22 (23.4)	19 (17.9)	0.019
Secondary	38 (40.4)	65 (61.4)	
University	2 (2.1)	4 (3.8)	
Age at first intercourse/years			
≥ 16	76 (80.9)	82 (77.3)	0.545
< 16	18 (19.1)	24 (22.7)	
Number of sexual partners/year			
300	24 (25.5)	5 (4.7)	
400	52 (55.3)	72 (67.9)	< 0.001
500	11 (11.7)	22 (20.8)	
600	7 (7.5)	7 (6.6)	
Recent change of sexual partner			
Two days before	2 (2.1)	2 (1.9)	0.990
The day before	82 (87.2)	93 (87.8)	
The same day	10 (10.7)	11 (10.3)	
Frequency of intercourse/per week			
< 10	32 (34.1)	23 (21.7)	0.149
10 - 20	52 (55.3)	70 (66)	
20 - 30	10 (10.6)	13 (12.3)	
Condom use			
Always	50 (53.1)	0 (0)	< 0.001
Occasionally	44 (46.9)	106 (100)	
Never	0 (0)	0 (0)	

Table 2: HPV carriage by factors associated with infection

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	HPV Negative n (%)	HPV Positive n (%)	P value
VIA			
Negative	83 (88.3)	82 (77.3)	
Positive	11 (11.7)	24 (22.7)	0.042
Total	94	106	
VILI			
Negative	94 (100)	105 (99)	
Positive	0 (0)	1 (1)	0.345
Total	94	106	

Table 3: HPV carriage based on VIA and VILI results.

The average number of sexual partners in a year per commercial sex worker was  $416 \pm 5.3$  in our study. Our result is slightly higher compare to the one of Latora., *et al.* in 2006 [14] who found an average of 400 sex partners per year. This could be explained by a multitude of reasons. In the one hand, evolution of morals tending to westernize with the impact of the media: young people are confronted and exposed to sexuality very early and most often without the supervision of a young person responsible adult. In addition, the age of men and women at the time of marriage tends to decline, that is, people get married later and later.

On the other hand, this can be explained by the fact that conducted our study was in Ouagadougou, a metropolis where the inhabitants are not as familiar with each other as in the small towns or in the rural areas.

In terms of condom use, we found 25% of female sex workers who still used condoms during sex. Our result is lower than the one of Rousseau., *et al.* of 2006 in Burkina Faso [10]. This difference may be due to the fact that in Rousseau., *et al.* the "occasional condom use" parameter was not addressed.

HIV infection was found in 4.5% of female sex workers. This value is lower than that of Rousseau., *et al.* in 2006 in Burkina Faso [10]. This discrepancy can be explained by the under-declaration of the serological status of sex workers for fear of severe discrimination of society and also by lack of confidence in the investigators during this study. Indeed, by confessing to be HIV positive, they would still run the risk of losing their customers in case it was known.

The prevalence of cervical dysplasia among female sex workers in our study by VIA was 17.5%. This prevalence is lower than that of Rousseau., *et al.* [10] found 24.9% cervical cytological abnormalities in a study of 379 high-risk women. These results also seem surprising to us in view of our study population. The multiplication of screening campaigns for precancerous lesions and the establishment of free medical procedures by the Ministry of Health could explain these results. Our population residing in the middle of Ouagadougou easily accesses health services and would be much more sensitive to their sexual health.

We found 53% of female sex workers who had at least one high-risk HPV genotype. This result is inferior to that obtained by other authors. Thus Marek., *et al.* [11], in a study of HPV infections among female sex workers in Hungary, found a prevalence of 55.9%. This gap could be explained by the prevalence of genital HPV infections that varies by region of the world [19].

In our study, the most common genotype was HPV 68 (14.6%), followed by HPV 31 (12%), HPV 52 (9.3%), 51 (8.9%), 56 and 66 (7.6%), 35 and 58 (7.1%) and 39 (6.2%). HPV 16 and 18, currently vaccinated, were found in 2.7% and 6.2% of cases, respectively. Our results differ from those in the literature. Thus, Marek., *et al.* [11] in Hungary found that female genotypes 31 was the most common, followed by 16, 66, 18, 51, 58 and 56. As for Zohoncon., *et al.* they found that in the general population of women in Ouagadougou, the most common genotypes were HPV 52 and 35, followed by HPV 31,18, 58, 56, 51, 59, 45 and 33; the least common genotypes were HPV

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16 and 39 [20]. Why do genotypes also diverge in two different but geographically similar populations? We think this could be due to the genotype of HPV itself. There may be genotypes that are more persistent than others or so cross-immunity between different genotypes may have played a major role in this situation, especially in a population of women as sexually exposed as ours.

We found a prevalence of 53.8% multiple infections in the population of high-risk HPV-positive sex workers. Our result is very high compared to that of Traoré., *et al.* in Bobo Dioulasso who found a prevalence of 15.2% among women in the general population therefore not belonging to a risk group such as ours [15]. The target group difference would justify this difference. Moreover, our result is roughly similar to that of Rousseau., *et al.* [10] who found a prevalence of 52.9% among female sex workers in Burkina Faso.

In our study, we found that the number of HPV genotypes ranged from two to nine in multiple infections. Rousseau., *et al.* found two to seven genotypes in their study among female sex workers in Burkina Faso [10]. Chatuverdi., *et al.* found two to eight HPV genotypes (high risk and low risk combined) in multiple infections [21].

There was a statistically significant association between the positive result of IVA and the carriage of HPV. In contrast, the positive result of IVL was not statistically associated with HPV carriage.

This result in our study could be explained by the IVA/IVL screening method which is an operator-dependent method and lacks specificity.

Age was not statistically associated with HPV carriage. Our result is different from that of Panatto., *et al.* who found a highly significant association between age and the positive or negative HPV outcome of subjects in their study [16]. This difference could be justified by the fact that in their study, these authors considered an age range of 16 to 26 years.

In our study, the number of sexual partners that sex workers could have had in one year was statistically associated with the carriage of HPV (p < 0.001). Our result corroborates that of the literature. Thus, Ramanakumar., *et al.* found that carriage of HPV was slightly higher in women with more than two sexual partners [17].

For genital HPV, consistent condom use only partially protects [22,23]. This is because HPV is not transmitted only during intercourse, but different sexual practices can transmit the virus through hands or soiled objects.

Similarly, the number of sexual partners was statistically associated with the number of HPV genotypes present at the cervical level. Our result is similar to that of Rousseau., *et al.* who found that the high number of sexual partners was a risk factor for multiple infections [18]. Also, Chatuverdi., *et al.* found that multiple infections appeared to be more common among those who had more than five sexual partners, compared to those who had only one [21].

The risk of genital infection with HPV increases the risk of HIV infection and vice versa. In Ghana, Obiri-Yeboah., *et al.* reported that the prevalence of HR-HPV genotypes was higher among HIV-1 seropositive women (65.6% vs. 30.2%, P < 0.0001), as was proportion with multiple HR-HPV infections (60.6% vs. 21.3%, P < 0.0001) [24].

### Conclusion

During their sex life, female sex workers are particularly at risk from HPV infections, whose chronic carriage leads to cervical cancer following the development of lesions. In our study, more than half of sex workers were infected with at least one high-risk HPV genotype. The most common genotypes found in our study were HPV 68, HPV 31, HPV 52, HPV 51 and HPV 56. Female sex workers are a high-risk group for sexually transmitted infections. Preventive measures should be put in earlier in place in order to manage this situation. In addition, there is a need of HPV mapping in the West African sub-region in particular, to guide research into new polyvalent vaccines taking into account other high-risk HPV genotypes that circulate there.

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### **Competing Interests**

Authors declared they have no conflicts of interest.

### **Authors' Contribution**

All authors contributed in the designed, analysis and preparation of the manuscript.

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