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Humans Papillomavirus (Hpv) Infections in Female Sex Workers in Cote D'ivoire

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ABSTRACT

Background: Human Papillomaviruses (HPV) are small virus non-enveloped double-stranded circular DNA. They infect epithelial cells (mucous membranes and skin). Many studies have indicated that having multiple sexual partners may lead to higher HPV transmission. Thus female sex workers (FSWs) may be at greater risk of infection compared to the general population. In female sex workers (FSWs), the risk of HPV infection and cervical cancer is especially high. The aim of this work is to determine the prevalence and the genotypes of Humans Papillomavirus (HPV) that circulate in female sex workers populations in Cote d'Ivoire. **Methods:** From December 2015 to May 2016, cervical samples from 350 female sex workers were tested for some HR-HPV. HPV DNA was amplified using PGMY09 /11 primers which generated 450 base pairs at the L1 region. The samples harboring HPV DNA were genotyped using the multiplex PCR with HPV 16, 18, 31, 33, 35, 45 and 51 primers. **Results:** The mean age of this population was 32.5 years. On 350 female sex workers HPV DNA was obtained in 51.5% of the population. A total of 168 (94.38%) specimens harboring HPV DNA were genotypes using multiplex PCR versus 5.61 %, which were not genotyped using HPV 16,18, 31, 33, 35, 45 and 51 by multiplex PCR. These 168 strains permit us to identify 204 strains of HPV on whom we have 88.69 % with single infection while 11.30 % with a multiple infection. Among the multiple infection 36.84 % had respectively double and triple HPV infection and 26.31 % had four HPV infections. HPV genotypes prevalence was the followed: HPV 16 (22.47%), HPV 18 (26.97%), HPV 35 (11.23%), HPV 31 and HPV 33 (7.86%) respectively and HPV 45 (7.30%). Any case of HPV genotype 51 was founded. **Conclusion:** The prevalence of HPV infection in female sex workers is high. The most genotypes which circulate in female sex workers are type 16 and type 18 which are the best known in the world. **Keywords:** HPV-Prevalence- Genotypes- female sex workers-Côte d'Ivoire

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Introduction

Human Papillomaviruses (HPV) are small virus non-enveloped double-stranded circular DNA. They infect epithelial cells (mucous membranes and skin). There are currently 120 genotypes of HPV that have been identified, including 40 with a specifically genital tropism (1). Papillomaviruses are classified into two groups according to their oncogenic potential: papillomaviruses with high oncogenic risk (Type 16 and 18) and low risk papillomaviruses (type 6 and 11). HPV at low risk are responsible for benign lesions such as genital warts, papillomas (2; 3; 4; 5) while HPV at high risk are responsible for precancerous lesions, cancers (cervix, vulva).

Today it is very well established by Harald Zur Hausen that infection with specific types of HPV can cause cervical cancer (6). Cote d'Ivoire has a population of 6.37 million women ages 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year 1346 women are diagnosed with cervical cancer and 866 die from the disease. Cervical cancer ranks as the 2nd most frequent cancer among women in Cote d'Ivoire and the 2nd most frequent cancer among women between 15 and 44 years of age. Data is not yet available on the HPV burden in the general population of Cote d'Ivoire. However, in Western Africa, the region Cote d'Ivoire belongs to, about 4.3% of women in the general population are estimated to harbour cervical HPV- 16/18 infection at a given time, and 56.7% of invasive cervical cancers are attributed to HPVs 16 or 18. Cancer of the cervix uteri is the 4th most common cancer among women worldwide, with an estimated 527,624 new cases and 265,672 deaths in 2012 (GLOBOCAN). The majority of cases are squamous cell carcinoma followed by adenocarcinomas (7).

About 1,346 new cervical cancer cases are diagnosed annually in Côte d'Ivoire (estimations for 2012). Cervical cancer ranks as the 2nd leading cause of female cancer in Côte

d'Ivoire. Cervical cancer is the 2th most common female cancer in women aged 15 to 44 years in Côte d'Ivoire. About 866 cervical cancer deaths occur annually in Côte d'Ivoire (estimations for 2012). Cervical cancer ranks as the 2nd leading cause of female cancer deaths in Côte d'Ivoire. Cervical cancer is the 2nd leading cause of cancer deaths in women aged 15 to 44 years in Côte d'Ivoire (7).

Studies have indicated that having multiple sexual partners may lead to higher HPV transmission. Thus female sex workers (FSWs) may be at greater risk of infection compared to the general population. In female sex workers (FSWs), the risk of HPV infection and cervical cancer is especially high. A previous studies have reported that female sex workers had more than twice the probability of having HPV infection than women from the general population and have a prevalence of abnormal pap smears (8,9). In addition, HPV can be transmitted from FSWs to the general population through clients thereby increasing the prevalence of the virus. In Cote d'Ivoire there is a lack of data on the prevalence of HPV in the population of sex workers.

The aim of this work is to determine the prevalence and the genotypes of Humans Papillomavirus (HPV) that circulate in female sex workers populations in Cote d'Ivoire.

Materiel and methods

Patient Recruitment: This study was carried out at the Red Ruban clinic at Yopougon and Clinique Confiance of Bietry Zone 4 in Abidjan where Men Who have sex with Men and sex workers are taken care and 9 quarters of Abidjan (Abobo, Attecoubé, Adjame, Cocody, Marcory, Koumassi, Port Bouet, Treichville and Yopougon) and the inside of Cote d'Ivoire (Abengourou, Bondoukou, Daloa, Gagnoa, Man and Guiglo) from December 2015 to May 2016.

Methods: After women's consent, they are administered a questionnaire assessing socio-demographic characteristics and sexual habits

(i.e. age, lifetime number of sexual partners, date of first sex, educational level, condoms use, sex habits, parity and number of pregnancies and place of living and tobacco use and alcohol drink).

Samples collection: The woman once in the gynecological position a non-lubricated speculum is placed to make visible the cervix with the aid of an ordinary light. Samples are taken in the cervix the vagina and stored in virus medium Transport (VTM). The samples were stored at -20° C and then at -80°C at the biobank of Pasteur Institute of Cote d'Ivoire before using for the PCR

PCR detection of HPV: The PCR detection of HPV was performed at Molecular Biology Platforms of Pasteur Institute of Côte d'Ivoire. PCR detection of HPV was performed according to the procedure of Ausubel and *al.* (10). This procedure is based on the chloroform phenol extraction method which consists in treating the cell lysate (obtained after enzymatic digestion with proteinase K) with a mixture of phenol / chloroform/isoamyl alcohol. Phenol is a deproteinizing agent in which the nucleic acids are not soluble. Chloroform is capable of causing protein denaturation. The anti-foaming activity of isoamyl alcohol will promote the separation of the deproteinized aqueous phase. Recovery of the genomic DNA is achieved following precipitation steps with ethanol and centrifugation steps

HPV DNA extraction protocol: 500 µL of endocervical cell samples were centrifuged at 14000 rpm for 15 minutes. The pellet (endocervical cells) obtained after centrifugation is subjected to lysis at 65 ° C. for 1 hour by adding 400 µL of cell lysis buffer (200 µL of Tris-HCl pH 8 (0.1 M), 0.8 ML of EDTA (0.5 M), 20 µL of SDS (10%), 1.5 µL of RNase (4 mg / mL) and 10 µL of proteinase K (10 mg / mL). After enzymatic digestion, the mixture was centrifuged at 14000 rpm for 5 minutes. Then 2 volumes of phenol / chloroform / isoamyl alcohol (25/24/1) are added to the lysate. The mixture thus obtained is stirred for 2 minutes in

a vortex and then centrifuged for 5 min at 12000 rpm. The DNA contained in the aqueous phase is supplemented with 2 volumes of absolute ethanol, 1/10 volume of 3 M NaCl and precipitated at -20 ° C. overnight and then centrifuged for 10 min at 14000 rpm. The pellet obtained was washed in 500 µL of 70% ethanol at 14000 rpm for 10 minutes. The precipitate containing the viral DNA is dried with DNA SpeedVac and taken up in 60 µL of elution buffer. The DNA extract is subsequently stored at -20 ° C for further use or at 4 ° C for immediate use.

HPV detection by PGMY11/09 PCR: Consensus primers (primarily targeting the HPV L1 region) were used to detect the viral presence. The PCR was carried out in a reaction medium of 50 µL containing 5 µL of 5X colored buffer, 5 µL of uncoloured 5 × buffer, 3 µL of MgCl₂ (25 mM), 0.5 µL of dNTPs (10 µM), 1 µL of each primer (10 µM), 0.4 µL of GoTaq polymerase and 10 µL of DNA. The DNA was amplified in a thermal cycler under the following conditions: Initial denaturation at 94 ° C, 5 minutes, Denaturation at 94°C, 30 seconds, Hybridization at 53°C, 30 seconds (35 cycles), Elongation at 72°C, 30 seconds, Final elongation at 72°C, 7 minutes. For each serial of cases tested, a negative control containing no matrix DNA was carried out in parallel in order to check the absence of any contamination of the reagents used. A positive control is also tested in parallel with each set of cases. The consensus primers used for HPV detection targeting the conserved L1 region and producing 450 bp amplicons (11, 12,13) are:

Sequence (5 '3') of the forward primer PGMY11
A: GCACAGGGACATAACAATGG

Sequence (5 '3') of the reverse primer PGMY09
F: CGTCCCAAAGGAAACTGATC

Separation of DNA fragments by agarose gel electrophoresis: The electrophoresis apparatus is formed of a Plexiglas plate placed horizontally on a flat support. A comb for forming the wells is aligned parallel to the top of the plate. The assembly is connected by

electrodes to a current generator. Agarose was prepared at a concentration of 1.5% with 1X TBE buffer. The cooled microwave-fused agarose solution was added with 10 μ L of BET. The solution was then poured onto the plate. After curing the gel, the comb was removed and the cured gel coated with $1 \times 15 \mu$ L TBE buffer of each DNA sample was deposited in each well. In parallel with the samples, a molecular weight marker is deposited in a well. The migration lasts 20 to 35 minutes and is carried out under a voltage of 110V.

HPV Genotyping (Tsakiogiannis et al) (14):

A method of HPV genotyping by designing seven type-specific primer sets that allow the amplification of strictly conserved regions of L1 gene in order to identify the HPV genotypes 16, 18, 45, 35, 33, 51, and 31 and using the human β -actin gene as internal control. The method was established by designing nine type-specific primer sets that target conserved regions of the L1 gene. In the present study, we developed a method of HPV genotyping by designing nine type-specific primer sets that allow the amplification of strictly conserved regions of L1 gene in order to identify the HPV genotypes 16, 18, 31, 33, 35, 45, and 51 and using the human β -actin gene as internal control.

In particular, two distinct primer mixtures were arranged, each containing three L1 type-specific primer sets (Table 1). Moreover, the primer set b-actinF/b-actinR, which allows the amplification of the human β -actin gene, was included in each multiplex PCR primer mixture as an internal control. Specifically, primer mixture I contained primers specific for HPV 16, 31 and 33; primer mixture II contained primers specific for HPV 18, 35, 45 and 51

Each multiplex PCR primer mixture contained 1 μ l of each L1 type-specific primer set and 1 μ mol of the primer pair b-actinF/b-actinR. Each multiplex PCR assay was performed in a final volume of 50 μ l, containing 40 μ l of the corresponding multiplex PCR primer mixture, 5 μ l Buffer A, 2 mM MgCl₂, 1.2 mM dNTPs and 0.5 U of thermostable DNA polymerase. PCR

cycling conditions were as follows: an initial denaturation step at 95 °C for 2 min and then 40 cycles of 95 °C for 30 s, 58 °C for 50 s and 72 °C for 10 s. The PCR reaction was ended with a 1-min incubation step at 72 °C.

Results

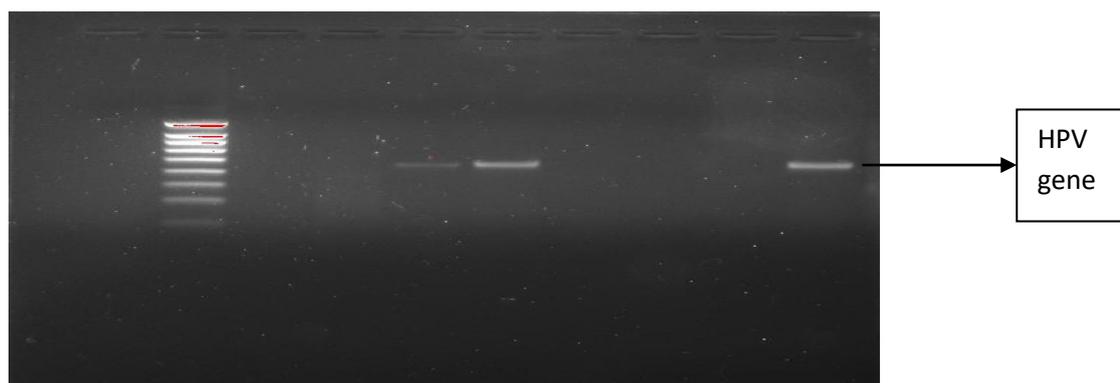
The study's population is about 350 sex workers from Abidjan and some cities of Cote d'Ivoire. The minimum age is 17 years and the maximum age is 56 years with an average age of 32.5 years. At the matrimonial level, the population consisted of 70.8 % single women, 0.57 % divorced women, 12.0 % married women, 16.0 % common-law women and 0.85 % widowed women. The majority of the population came from the city of Abidjan and in particular from the quarter of Port Bouet at 14.57% followed by Yopougon (12.0 %), Marcory (9.71 %), Koumassi (8.29 %), Adjamé (8.0 %), Attécoubé (5.71 %), Treichville and Abobo respectively at 5.43 % and Cocody (1.14.0 %). From the country we have Gagnoa at 8.86% followed by Man (7.14%), Guiglo (5.71 %), Bondoukou (2.0 %) and Daloa (1.14 %). The majority of the population was not educated (76.29%). They are all a sex workers and they all use condoms to work. Any of them used oral contraceptives. 96.28% of women had below 5 pregnancies and 3.72 % above 5 pregnancies. All of women had below 5 children.

182 (52%) women had consumed tobacco. 64 % of the women in this study had their sexual intercourse before the age of 18 years. 57.14% of women reported working in brothels, 25.43% in the bar and 17.43% on sidewalks. 86.28% of women have less than 10 sexual partners per week.

Of the 350 women who agreed to participate to this study 178 (51.5%) were positive for HPV DNA. A total of 168 (94.38%) specimens harboring HPV DNA were genotypes using multiplex PCR versus 5.61 %, which were not genotyped using HPV 16,18, 31, 33, 35, 45 and

Table 1: Sequences of HPV type-specific primer sets and the sizes of the respective amplicons (16)

Primers	Sequences	Tailles	References
HPV 16	R : 5'GCAATGTAGGTGTATCTCCA3' F : 5'GTGTGTAAGCAAGCAACAG3'	395 pb	Tsakogiannis(2015)
HPV 18	R : 5'CACGCACACGCTTGGCAGGT3' F : 5'AAGGATGCTGCACCGGCTGA3'	217 pb	Tsakogiannis(2015)
HPV 31	R : 5'GTAGTTGCAGGACAACTGAC3' F : 5'ATGGTGTGTACACAACACC3'	514 pb	Tsakogiannis(2015)
HPV 33	F : 5'ATGATAGATGATGTAACGCC3' R : 5'GCACACTCCATGCGTATCAG3'	455 pb	Tsakogiannis(2015)
HPV 35	F : 5'CCCGAGGCAACTGACCTATA3' R : 5'GGGGCACACTATTCCAAATG3'	230 pb	Tsakogiannis(2015)
HPV 45	2F :5'GCTACAGCTGTTATTACGCAG3' 2R : 5'GCAATTGTGCAGGTTTACA3'	141 pb	Fontaine (42)
HPV 51	2F : 5'CCTAAAACCTCAACGCGTGCT3' 2R : 5'TTGTTGTGCATTGCCATTTGC3'	266 pb	Fontaine (42)
Beta globine			Tsakogiannis(2015)
GH20	5' GAA GAG GCA AGG ACAGGTAC3'	268 pb	
PCO4	5' CAA CTT CAT CCA CGT TCA CC3'		

**Figure 1:** Agarose gel (1, 5%) showing PCR electrophoretic profile using PGMY09/11 oligonucleotide primers for HPV-L1 gene detection corresponding to ~450 pb

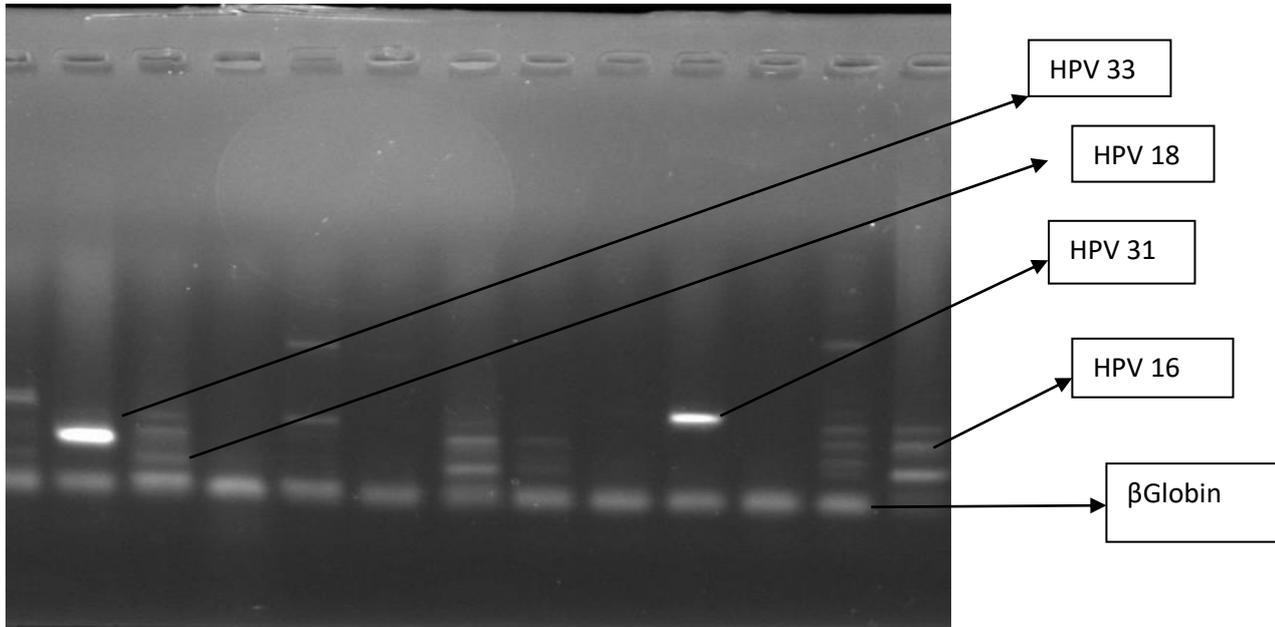


Figure 2: Agarose gel electrophoresis (1, 5%) of HPV Multiplex reaction-PCR reactions from twelve cervicovaginal samples.

Table 2: Distribution of HPV genotypes founded in this study

Genotypes	Frequency	Percent	Strains
16, 18, 35,45	3	1,69%	12
16, 18, 33,45	2	1,12%	8
16	40	22,47%	40
16, 18,45	3	1,69%	9
16,33,	1	0,56%	2
16, 35,33	4	2,25%	12
16,45	1	0,56%	2
18	48	26,97%	48
18,35	1	0,56%	2
18,45	2	1,12%	4
31	14	7,86%	14
31,45	1	0,56%	2
33	14	7,86%	14
33,45	1	0,56%	2
35	20	11,23%	20
45	13	7,30%	13
NG	10		
TOTAL	178	100,00%	204

Table 3: Distribution of HPV according to age class

Age	HPV +	HPV-	Total
[15,30[16 (4.57)	4 (1.14)	20 (5.43)
[30,45[83 (23.71)	71 (42.42)	54 (44.00)
[45,60[79 (22.57)	97 (12.5)	176 (50.28)
Total	178	172	350

Table 4: Characteristics of the study population and HPV + population in Côte d'Ivoire.

	Study population	HPV DNA+
Age		
Mean	32.51	33.29
Min	17	17
Max	60	60
Marital status		
Single	247 (70.57%)	129(72.47%)
Married	42 (12%)	19(10.67%)
widowed	03 (0.85%)	01(0.56%)
Cohabitant	57 (16.28%)	28(15.73%)
Divorcé	1 (0.28%)	1(0.58%)
Place of living		
Out of Abidjan	104 (29.71%)	45(25.28%)
Abidjan	246 (70.29)	133(74.72%)
Abobo	19(5.43%)	12(6.74%)
Attecoubé	20(5.71)	10(5.62%)
Adjame	28(8.00%)	10(5.62%)
Yopougon	42(12.00%)	20(11.24%)
Cocody	04(1.14%)	03(1.69%)
Koumassi	29(8.29%)	19(10.67%)
Marcory	34(9.71)	22(12.36%)
Treichville	19(5.43%)	08(4.49%)
Port Bouet	51(14.57%)	22(12.36%)
Formal education		
Non educated	267(76.29%)	138(77.53%)
Educated	83(23.71%)	40(22.47)
Primary	43(12.28%)	21(11.80)
Secondary	40(11.43%)	19(10.67%)
Gesity		

<5	337 (96.29%)	169(94.94%)
>5	13(3.71%)	9(5.06)
Partity		
<5	350(100%)	178(100%)
>5	00	00
Oral contraception		
Yes	55(15.76%)	31(17.42%)
No	294 (84.24%)	147(82.58)
Condom use		
No	00	00
Yes	350(100%)	178 (100%)
If Yes already	00	00
Sometimes	00	00
Rarely	00	00
Profession		
Sex workers	350 (100%)	178 (100%)
Number of sexual partenaires /week		
<10	302(86.28%)	154(86.52%)
>10	48(13.71%)	24(13.48%)
Age at first sexual intercourse		
<18	297(84.86%)	156(87.54%)
>18	53(15.16%)	22(12.36)
Place of work		
Home	142(40.57%)	90(50.56%)
Bars/night club	89 (24.43%)	42(23.59%)
Hotels	58(16.57%)	13(7.3%)
Streets	61(17.43%)	33(18.53%)
Tobacco use		
Yes	168 (48%)	100(56.18%)
No	182 (52%)	78(43.82%)
Alcohol drink		
Yes	190(54.28)	115(64.60%)
No	160(45.71%)	63(35.39%)

51 by multiplex PCR. These 168 strains permit us to identify 204 strains of HPV on whom we have 88.69 % with single infection while 11.30 % with a multiple infection. Among the multiple infection 36.84 % had respectively double and triple HPV infection and 26.31 % had four HPV infections. HPV genotypes prevalence was the followed: HPV 16 (22.47%), HPV 18 (26.97%), HPV 35 (11.23%), HPV 31 and HPV 33 (7.86%) respectively and HPV 45 (7.30%). Any case of HPV genotype 51 was founded.

Discussion

The aim of this work was to determine the presence of *Papillomavirus* DNA in the genital samples of Female sex workers in Cote d'Ivoire. This work permit us to found the rate of 51.05 % of women carrying HPV DNA . This rate seems high and could be explained by several factors. First, the study population consists mainly of sex workers whose number of partners varies from 2 to 17 per week with 15.14% having 7 partners. Numerous studies have shown that the frequency of ratios is a determining factor in the acquisition of Papillomavirus infections. In addition, some studies have identified an association between non-penetrating sexual behaviors and the detection of HPV in girls. Non-penetrative sexual practices, including handegenital contact and genital sex, have been identified as risk factors for HPV acquisition in heterosexual women (15, 16) and in homosexual women who Report having never had a vaginal sex to the penis [17]. Finally the use of condoms was shown to be protective against HPV infection in our study group, which is an issue that has not been well clarified. A meta-analysis of the effect of condom use on the prevention of HPV infection revealed that there was no consistent evidence that condom use reduces the risk of HPV infection (18). Conversely, the study by Tideman et al. (19) showed that a failure to use condoms was significantly associated with HPV infection. The prevalence of HPV obtained in this study seems close to those reported by many

studies in Africa, particularly in Kenya (20) Burkina Fasso (21,22), South Africa (23,24), Tunisia (25), North America specifically in Mexico (26, 27), in South America in Peru (28, 29). To these countries we must add those of Asia including South Korea (30, 31), Japan (32) and Cambodia (33). It should be noted that this prevalence of HPV obtained in female sex workers is much higher than that obtained in most European countries (34,35,36,37) and some Asian and Singaporean countries (38,35,36,37). and Bangladesh (39) but also far below some countries such as India (40) and Vietnam (41). These similarities or differences may be due to a variety of factors including environmental factors and population sensitization on HPV infections. This study revealed Papillomavirus genotypes encountered in FSW and whose order was HPV 18 (26.97%), 16 (22.47%), HPV HPV 35 (11.23%), HPV 31 and HPV 33 (7.86%) respectively. and HPV 45 (7.30%). Any case of HPV genotype 51 was founded. These different genotypes have also been highlighted by many worldwide in Africa (20,21,22,23,24), Asia (38,39,40,41), Europe (34,35,36,37).) and in America (26,27,28,29) but not in this order because for most studies it is first the type 16 followed by the type 18 that are the most encountered. Some studies have also found Type 51 in Burkina Faso, Kenya, South Africa (20,21,22,23,24) and Guatemala (8), which was not the case in this study . Regarding the type 45 that has not been identified in some studies as in Burkina Faso in South Africa and Mexico (21,22,27) while this one identified in this study

Conclusion

The prevalence of HPV infection in female sex workers is high about 50.51. The most genotypes which circulate in female sex workers are type 16 and type 18 which are the best known in the world. However, some high-risk oncogenic genotypes such as types 31, 33,35 and 45 have been found in significant amounts and should be included in vaccination.

Limitations

The main limitation of this study was the small number of women included thus limits the generalization of our data to the whole Ivoirians populations. Then, all positive samples were not confirmed cytologically. The DNA extraction was done by the method of phenol chloroform and this kind of methods deemed too long have been abandoned in favor of extraction kit. Despite these limitations, this study shows the necessity the need to continue HPV research in low- income countries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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