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Molecular Epidemiology of High- and Low-Risk Human Papillomavirus Genotypes among Female Students in Public Universities in Burkina Faso

Jamila Maria Yasmine Ouedraogo[1,2], Samiratou Ouedraogo[1,3,4], Stéphane Bénagabou[5], Benjamin Sombié[1], Prosper Bado[6], Estelle Ouedraogo[6], Théodora Mahoukèdè Zohoncon[6,7], Olga Mélanie Lompo[1,2,6]

[1]OR Tambo Africa Research Chair (ORTARChI) on Action against Cancer, Faculty of Health Sciences, University Joseph KI-ZERBO, Ouagadougou, Burkina Faso
[2]Morphology and Organogenesis Laboratory (LAMO), University Joseph KI-ZERBO, UFR/SDS, Ouagadougou, Burkina Faso
[3]National Observatory of Population Health, National Institute of Public Health, Ouagadougou, Burkina Faso
[4]Department of Global and Public Health, School of Population and Global Health, Montreal, Canada
[5]National Center for University Services (CENOU), University Joseph KI-ZERBO Ouagadougou, Burkina Faso
[6]Laboratory of Molecular Biology and Genetics (LABIOGENE), University Joseph KI-ZERBO, Ouagadougou, Burkina Faso
[7]Faculty of Health, University Saint Thomas d'Aquin (USTA), Ouagadougou, Burkina Faso
Email: jamilamaria02@gmail.com

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Abstract

Introduction: Chronic infection with human papillomavirus (HPV) is the primary cause of cervical cancer and represents a major public health concern in resource-limited countries. This study aimed to assess HPV prevalence and genotype distribution among female university students in Burkina Faso. **Methods:** A cross-sectional study was conducted between June and December 2024 across four major universities located in three cities: Ouagadougou (the capital), Bobo-Dioulasso, and Koudougou. Cervical samples were collected from sexually active female students aged 18 years and older who provided informed consent. HPV genotyping was performed using the HPV Direct Flow Chip kit, and data were analyzed using R software (version 4.4.2). **Results:** A total of 469 female students with a mean age of 23 ± 2.93 years were included. Overall HPV prevalence was 35.8%, with variations across study sites: Ouagadougou had the highest prevalence (45.1%), followed by Koudougou (29.9%) and Bobo-Dioulasso (24.6%). Among high-risk genotypes, HPV-16 was the most prevalent, followed by HPV-56 and HPV-52. The most common low-risk genotypes were HPV-62, HPV-81, and HPV-42. Multiple HPV infections were observed in more than half of the positive cases (55.35%).

Conclusion: The high prevalence of HPV infection highlights the urgent need to strengthen HPV surveillance and to tailor cervical cancer prevention strategies to the local context.

Keywords

Human Papillomavirus, Genotypes, Cervical Cancer, University Students, Burkina Faso

1. Introduction

Cervical cancer remains a significant public health issue, ranking as the fourth most common cancer among women worldwide, with approximately 604,000 new cases and 279,000 deaths estimated in 2024 according to the Global Cancer Observatory (GLOBOCAN) of the International Agency for Research on Cancer (IARC) [1]. The burden of cervical cancer is particularly high in low- and middle-income countries, where nearly 90% of deaths occur. Mortality rates are up to 18 times higher than developed countries, reflecting substantial disparities to access vaccination, screening, and treatment services [2]. Burkina Faso, for example, is heavily affected by this disease, data from GLOBOCAN 2022 showed that cervical cancer was the third deadliest cancer, with 775 deaths [3].

The etiological role of human papillomavirus (HPV) in causing cervical cancer is now well known [4]-[6]. HPV genotypes are classified according to their oncogenic potential into low-risk genotypes, which generally cause genital warts, and high-risk genotypes, which are strongly associated with the development of precancerous and cancerous cervical lesions [7]. Among genotypes implicated in cervical cancer, HPV-16 is the most frequently detected in invasive cervical carcinomas, followed by HPV-18. Together, these two genotypes account for approximately 70% of worldwide cases [8]. Other high-risk genotypes, including HPV-31, HPV-33, HPV-35, HPV-45, HPV-52, and HPV-58, also make a substantial contribution to the global burden of disease, collectively representing an additional 20% of cervical cancer cases [8]. However, the distribution of these genotypes is not worldwidly uniform and varies substantial across geographic regions and reflecting the characteristics of the studied populations.

To decrease the morbidity and mortality linked to persistent HPV infection and cervical cancer, HPV-specific vaccination is highly recommended [9]. Many effective vaccines that prevent HPV infection are currently available, targeting HPV-6, -11, -16, -18, -31, -33, -45, -52, and/or -58 [10]. In Burkina Faso, the HPV vaccine against HPV-6, -11, -16, and -18 was introduced into the national Expanded Program on Immunization on April 26, 2022, for girls aged 9 to 14 years [11]. However, there is a significant knowledge gap about the prevalence and distribution of HPV genotypes among young women, and evidence on whether the currently available vaccines adequately cover the strains most relevant in the

country. Yet, understanding HPV epidemiology in this group is crucial for tracking HPV persistence, clearance, and changes in infection patterns over time. Such data are also important in strengthening the evidence base needed to design more targeted, equitable, and effective cervical cancer prevention strategies for young women in Burkina Faso. We therefore conducted a study in 2024 with the main objective of determining the prevalence and genotypic diversity of HPV among female students at public universities in Burkina Faso.

2. Materials and Methods

2.1. Study and Period

We conducted a cross-sectional, descriptive, multicenter study between June and December 2024. It was part of an awareness and screening campaign for sexually transmitted infections (STIs), organized by the Centre national des œuvres universitaires (CENOU) in partnership with the Chair for Research and Action Against Cancer (Chair ReAAC). The aim of the campaign was to promote sexual and reproductive health in public universities in Burkina Faso by offering free screening for HIV, hepatitis B, hepatitis C, and, and papillomavirus (HPV) for eligible females.

2.2. Study Population and Recruitment Process

Our study population consisted of female students from four main public universities in Burkina Faso, namely Joseph Ki-Zerbo University (UJKZ) and Thomas Sankara University (UTS) in the capital city Ouagadougou, located in the central region; Norbert Zongo University (UNZ) in the city of Koudougou in the central-west, and Nazi Boni University (UNB) in the city of Bobo-Dioulasso in the south-west.

The sampling strategy was based on a convenience sample derived from the screening campaign conducted across these universities. A total of 649 female students participated in the screening campaign, including 306 in Ouagadougou, 186 in Koudougou, and 157 in Bobo-Dioulasso. Among these, 469 students met the eligibility criteria, provided written informed consent, and were ultimately included in the present analysis. All females tested provided their age and university of origin prior to sample collection.

Students eligible for HPV testing were female, aged 18 years or older, sexually active, and who had provided written informed consent. Exclusion criteria included women who were virgins, pregnant, or in the postpartum period; those who had engaged in unprotected sexual intercourse or performed vaginal douching within 24 hours prior to sample collection; and those who had undergone a total hysterectomy.

2.3. Sample Collection

Cervical sample collection was performed by trained healthcare personnel using a standardized procedure. A sterile speculum was gently inserted without prior antiseptic cleansing of the exocervix to avoid interference with downstream mo-

lecular analyses. A sterile swab was then inserted into the endocervical canal and rotated at the level of both the endocervix and exocervix to ensure adequate collection of cervical and vaginal secretions.

From all eligible students tested for HPV, we collected two endocervical specimens from each participant: one was used for HPV DNA detection and genotyping in the present study, while the second was stored for potential future analyses. The samples were immediately placed in a cooler containing ice packs and transported to the "Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA)", where they were stored at 4˚C pending molecular analysis.

2.4. HPV Detection and Genotyping

The detection and genotypic characterization of HPV were performed without prior DNA extraction using the HPV Direct Flow Chip kit (Vitro Master Diagnóstica, Spain). This assay enables the simultaneous detection of 36 HPV genotypes, including 18 high-risk (HR-HPV) types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and 18 low-risk (LR-HPV) types (6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81, 84, and 89). Undetermined genotype refers to samples testing positive for HPV DNA in which the specific genotype could not be identified because it is not included in the detection panel of the assay used.

This kit allows direct amplification from clinical samples. Following sample pretreatment with bidistilled water, 30 µL of each specimen was added to the lyophilized PCR Master Mix. Amplification was carried out using the GeneAmp PCR System 9700 (Applied Biosystems) under the following cycling conditions: initial incubation at 25˚C for 10 minutes, followed by denaturation at 94˚C for 3 minutes; 15 cycles of 94˚C for 30 seconds, 47˚C for 30 seconds, and 72˚C for 30 seconds; then 35 cycles of 94˚C for 30 seconds, 65˚C for 30 seconds, and 72˚C for 30 seconds; with a final extension at 72˚C for 5 minutes. The resulting amplicons were heated at 95˚C for 10 minutes to obtain single-stranded DNA. Immediately after heating, thermal shock was induced by transferring the PCR products onto ice for 5 minutes to prevent reannealing of denatured DNA strands. The denatured PCR products were subsequently subjected to a hybridization step using the HybriSpot12 system (Vitro Master Diagnóstica). Signal detection, result interpretation, and report generation were performed using HybriSoft version 2.2.0.

2.5. Statistical Data Processing

The socio-demographic data, HPV test results, and genotypes were described providing frequencies and percentages. All analyses were performed using Excel 2016 and R version 4.4.2 software.

2.6. Ethical Considerations

This research was conducted following the ethical regulations in effect in Burkina Faso. The study protocol received approval from the Health Research Ethics Committee (CERS) under registration number 2023-12-288. Additionally, an infor-

mation notice was sent to the management of CENOU in Ouagadougou and to the regional health departments of Bobo-Dioulasso and Koudougou.

3. Results

3.1. Characteristics of the Students

The study sample consisted of 469 female students: 224 from the Joseph Ki-Zerbo University and the Thomas Sankara University in Ouagadougou; 118 from the Nazi Boni University in Bobo-Dioulasso, and 127 from the Norbert Zongo University in Koudougou. The overall mean age of participants was 23 ± 2.93 years and the 18 - 24-year age group was the most represented in the overall study population, accounting for 63.97% (300/469) of participants.

3.2. Prevalence of HPV Infection

All samples tested were positive for the β-globin gene, confirming specimen adequacy and the absence of PCR inhibition. Of the 36 HPV genotypes targeted by the assay, 31 were detected, including 18 high-risk (HR-HPV) and 13 low-risk (LR-HPV) types. The overall prevalence of HPV infection in the study population n = 469 was 35.82% (168/469). Prevalence varied across cities as presented in Figure 1.

Figure 2 presents an age-stratified analysis showing a predominance of infection in the 18 - 24 years age group, which accounted for 67.26% (113/168) of HPV-positive cases.

Among the 168 infected students, 31 distinct genotypes were identified. Out of the 36 HPV genotypes targeted by the assay, 31 were detected, including 18 high-risk (HR-HPV) and 13 low-risk (LR-HPV) types, yielding a total of 332 detected genotypes. Of the positive cases, 124 (73.8%) harbored at least one HR-HPV

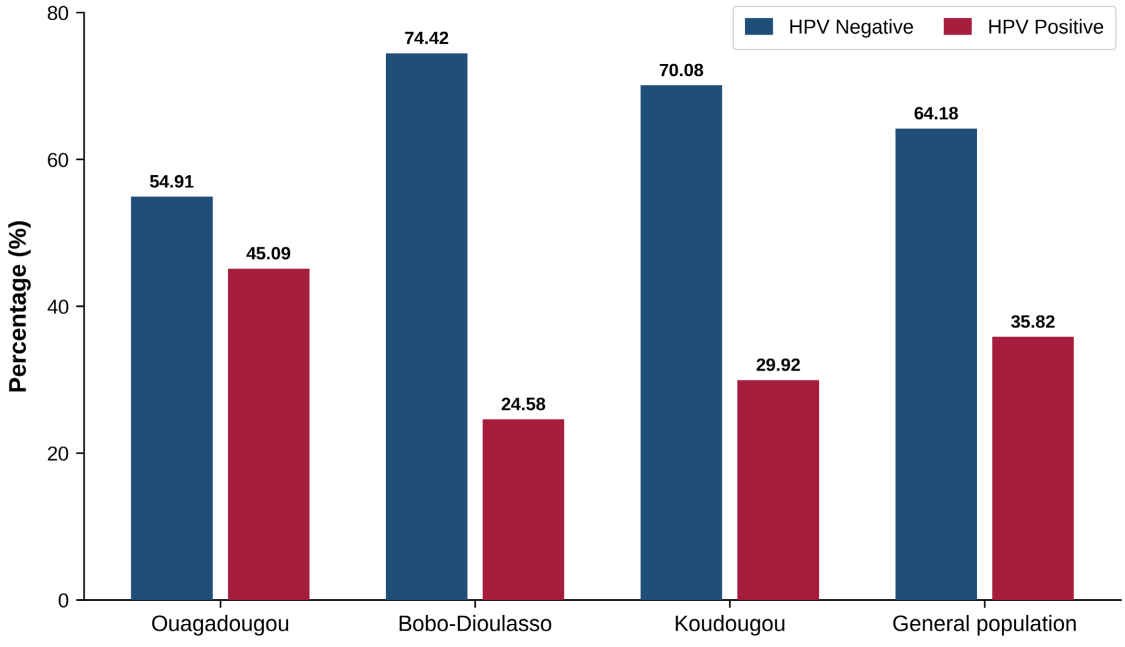

Figure 1. Prevalence of HPV infections among Burkinabe female students in our study.

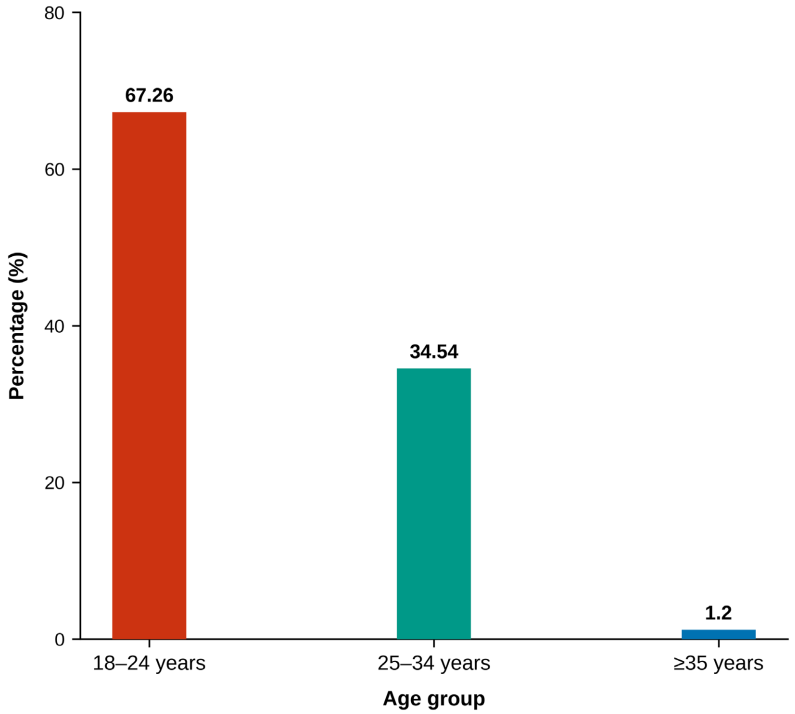

Figure 2. Age group distribution among HPV-positive.

genotype, while 96 (57.1%) carried at least one LR-HPV genotype. The most frequent oncogenic genotypes were HPV16 (25/178), followed by HPV56 (23/178) and HPV52 (22/178). Among LR-HPV types, HPV62 (36/124), HPV81 (34/124), and HPV42 (19/124) were the most commonly detected. No HPV11 genotype was identified in this study. The complete distribution of HR-HPV and LR-HPV genotypes is presented in Table 1.

Table 1. Distribution of all detected HPV genotypes (n = 332).

HPV Genotypes		Number (n)	Percentage (%)
High-risk HPV		**178**	**53.61**
	HPV 16	25	7.53
	HPV 56	23	6.93
	HPV 52	22	6.63
	HPV 66	15	4.52
	HPV 51	12	3.61
	HPV 58	12	3.61
	HPV 18	11	3.31
	HPV 31	9	2.71
	HPV 45	9	2.71
	HPV 68	8	2.41
	HPV 39	7	2.11

Continued

	HPV 53	5	1.51
	HPV 59	5	1.51
	HPV 82	5	1.51
	HPV 33	4	1.20
	HPV 35	4	1.20
	HPV 26	1	0.30
	HPV 73	1	0.30
Low-risk HPV		**154**	**46.39**
	HPV 62	36	10.84
	HPV 81	34	10.24
	HPV 42	19	5.72
	HPV 6	15	4.52
	HPV 67	11	3.31
	HPV 43	10	3.01
	HPV 40	8	2.41
	HPV 44	7	2.11
	HPV 54	4	1.20
	HPV 55	4	1.20
	HPV 61	3	0.90
	HPV 69	2	0.60
	HPV 84	1	0.30

The total number of genotypes detected per infected woman ranged from 1 to 10. Table 2 summarizes the distribution of single- and multiple-genotype HPV infections. Among the 168 HPV-positive cases, 67 (39.88%) had a single HPV genotype infection, while 93 (55.35%) had multiple HPV genotype infections. Multiple infections included 44 cases involving two genotypes, 27 involving three genotypes, and 22 involving more than four genotypes. In 8 cases (4.76%), the genotype could not be determined.

Table 2. Distribution of isolated and multiple HPV infections among positive female students.

Type of infection	Number (n)	Percentage (%)
Single infection	67	39.88%
Multiple infection with 2 genotypes	44	26.19%
Multiple infection with 3 genotypes	27	16.07%
Multiple infection with ≥ 4 genotypes	22	13.10%
Indeterminate genotypes	8	4.76%

4. Discussion

The overall prevalence of HPV infection among female students from public universities tested in this study was 35.82%. Our results align with previous studies in Burkina Faso, where similar prevalence rates of 35.42% and 34.4% were found among sexually active women [12] [13] from higher age groups. Other studies conducted in similar settings showed mixed results. In fact, much higher rates have been reported in a study at the University of Gaborone in Botswana, where the prevalence reached 63.0% among 493 male and 500 female students [14], and in a study conducted in Mexico, which reported a prevalence of 74.4% among 129 female students [15]. However, the prevalence found in our study remains higher than the 15.2% found among 171 female students in Busan, South Korea [16], 4.2% in a cohort of 1491 female students in Vietnam [17], and 11.5% among 435 adolescent girls and female university students in northern Portugal [18].

The age-specific distribution of HPV infection in our study showed a considerable prevalence among young women aged 18 - 24 years, corresponding to the period shortly after sexual debut. Similar findings have been reported in Maputo (Mozambique) among young adults aged 18 - 24 years [19]; likewise, an American survey indicated that the prevalence of HPV infections in women aged 15 to 24 was significantly higher than in older age groups [20]. In contrast, our study found that the 25 - 34-years age group had a lower infection rate. However, this period aligns with a stage where HPV persistence becomes more prominent. Indeed, after the age of 25 [21], viral persistence rather than new acquisition tends to increase, likely due to less effective viral clearance or possible reinfection with high-risk types. This persistence is a key factor in the progression to precancerous and invasive cervical lesions, making this age group an important target for systematic screening of precancerous lesions and the implementation of catch-up vaccination strategies.

Although HPV-16 and HPV-18 are the main vaccine targets, several African studies show that they are not always the most common circulating genotypes. A multicenter study [22] reported relatively low prevalences of HPV-16 and HPV-18, similar to those observed in our study. HPV-56 has already been reported in Burkina Faso as one of the predominant types. A high prevalence of this genotype has already been reported in sexually active women in the Middle East region of Burkina Faso, significantly higher than that observed in our cohort [23]. Our HPV-56 prevalence is lower than that reported in Kara, Togo [24] and in Parakou, Benin [25]. However, our results remain significantly higher than the 3.9% reported in Jos, Nigeria [26]. Our results are consistent with and extend the observations previously reported in Burkina Faso. Indeed, a systematic review conducted in 2024 [27], which analyzed 24 studies covering three regions of the country, highlights that although HPV-16 and HPV-18 genotypes are major targets for vaccines, their prevalence remains relatively low compared to that of other high-risk genotypes such as HPV-52 and HPV-56. Another systematic review conducted for West Africa [28] also highlighted a high frequency of HPV-52 and

HPV-56 in the general population and is consistent with the results of our study.

Regarding low-risk genotypes, the most common in our study were HPV-62, HPV-81, and HPV-42. No cases of HPV-11 were observed. HPV-62 and HPV-81, although less extensively researched, have previously been reported in Burkina Faso among pregnant women [29] and in patients with chronic hepatitis [30]. The high frequency of low-risk genotypes in our cohort highlights the importance of not limiting surveillance solely to oncogenic types. Although classified as low-risk oncogenic, these genotypes can cause benign lesions, including genital warts and condylomas, which may significantly impact patients' quality of life and place a notable burden on healthcare systems [31] [32].

The high prevalence of multiple infections observed in our study is about finding that warrants particular attention. Infection with multiple HPV types may be implicated in persistence and a higher likelihood of progression to precancerous lesions and cancer [33] [34]. In the present study, more than half of the participants infected with HPV had multiple genotype infections, and two in five female students had a single genotype infection. These co-infections involved both high-risk and low-risk genotypes. The prevalence of multiple infections in our study population was higher than that found in Morocco [35] but close to that reported in Mexico [36]. The coexistence of multiple HPV genotypes with variable oncogenic potentials may promote competitive or synergistic viral interactions and could increase, compared to single-genotype infections, the susceptibility to the development of cervical lesions [37]. The presence of such undetermined genotypes in our study underscores the technical limitations of current diagnostic assays. This proportion may mask the circulation of rare, uncommon, or emerging HPV variants, whose potential role in cervical carcinogenesis remains to be fully elucidated.

The quadrivalent Gardasil®4 vaccine (covering HPV types 6, 11, 16, 18), currently available free of charge in Burkina Faso, corresponds only partially to the HPV types observed in our study population. Notably, HPV-56, which was frequently detected in our cohort, is not included in any currently available vaccine. These findings highlight the differences between circulating HPV genotypes and those targeted by existing vaccines, underscoring the relevance of local genotype surveillance for guiding vaccination strategies. While our results cannot be used to infer the real-world effectiveness of HPV vaccination in preventing cervical cancer, they provide a descriptive overview of potential vaccine coverage in this population. They also support consideration of the nonavalent Gardasil®9 vaccine, which targets a broader spectrum of HPV types, for inclusion in future prevention programs in Burkina Faso, in line with previous recommendations from West African studies [12] [38] [39].

Some limitations of our study relate to its descriptive nature and the fact that it included only female students who voluntarily participated in sexually transmitted disease screening. In addition, only age and university affiliation were collected; information on vaccination status, sexual behavior, and other clinical fac-

tors was not available. Nevertheless, our large sample size and the inclusion of students from diverse regions of the country constitute a strength of the study and provide fundamental epidemiological data on HPV infection and the distribution of specific genotypes among female university students in Burkina Faso.

5. Conclusion

Our study revealed a high prevalence and diverse distribution of both high-risk and low-risk HPV genotypes among young female students in Burkina Faso, with frequent multiple infections and notable geographic variation. These findings highlight the need for continuous monitoring of circulating HPV genotypes, and further research on molecular and genotypic changes in precancerous and cancerous cervical lesions to guide evidence-based cervical cancer prevention strategies.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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