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Genotyping of Human Papillomaviruses (HPV) by Real-Time PCR in Patients at the Thies Regional Hospital Centre in Senegal

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Abstract

Background: Cervical cancer remains a major global public health concern, with an estimated 662,301 new cases and 348,874 deaths in 2022 (GLOBOCAN). Persistent infection with high-risk human papillomaviruses (HPV), particularly genotypes 16 and 18, accounts for approximately 70% of precancerous lesions. The genetic diversity of HPV influences disease progression, making genotyping essential for effective screening and prevention strategies. This study aimed to determine the prevalence and distribution of HPV genotypes among women attending the gynecology department of the Thies Regional Hospital Centre (CHRT), Senegal. **Methods:** A prospective, descriptive, and analytical study was conducted with 27 women consulting for cervical cancer screening or follow-up of precancerous lesions. Cervico-vaginal samples were analyzed using real-time PCR with the Allplex™ HPV28 Detection Kit (Seegene) on CFX96™ Bio-Rad. Sociodemographic and clinical data were collected via Google Forms, processed with Excel, and analyzed using RStudio 2025. **Results:** Among the 27 participants, only one tested positive for the HPV 53/82 complex, corresponding to a prevalence of 3.7%. The mean age was 44.8 years (range 30 - 64). Most participants were married, had never undergone prior HPV testing, and none had received HPV vaccination. **Conclusion:** The

low levels of prior screening and the absence of HPV vaccination observed in this cohort reflect limited awareness and uptake of cervical cancer prevention measures. Although based on a small sample size, these preliminary findings suggest the need for further investigation into local prevention strategies. Further well-powered studies are needed to accurately characterize circulating HPV genotypes in THIES and to inform context-specific screening and vaccination programs.

Keywords

Human Papillomavirus, Cervical Cancer, Genotyping, Screening, Prevention, Senegal

1. Introduction

Cervical cancer represents a major public health problem, ranking as the fourth most common cancer among women worldwide [1]. In 2022, GLOBOCAN reported 662,301 new cases and 348,874 deaths, highlighting the persistent global burden of this disease [2]. Persistent infection with high-risk human papillomavirus (HPV-HR) genotypes, including types 16 and 18, accounts for approximately 70% of cervical cancers [3] [4]. HPV is a double-stranded DNA virus belonging to the Papillomaviridae family, with more than 200 identified genotypes.

In sub-Saharan Africa, and particularly in Senegal, cervical cancer is the leading female cancer in terms of both incidence and mortality (2064 new cases and 1324 deaths in 2022) [2]. High mortality is driven by persistent structural constraints, including the high cost of molecular tests, limited availability of specialized equipment, insufficient qualified personnel, and geographic disparities hindering access to screening and specialized care.

Primary prevention relies on HPV vaccination, integrated into the Senegalese Expanded Program on Immunization (EPI) since 2018 following a pilot phase. However, vaccination coverage remains insufficient, reaching 47% for the first dose and 25% for the complete schedule in 2023 [5]. The World Health Organization (WHO) set a target of 90% coverage among girls under 15 years old by 2030 [6].

Secondary prevention through screening is a cornerstone of the global cervical cancer elimination strategy [7]. Initially based on cytology (Papanicolaou smear), screening has progressively shifted towards molecular methods, including the detection of high-risk HPV DNA and mRNA as well as HPV oncoproteins [8] [9]. High-risk HPV DNA tests demonstrate high sensitivity and excellent negative predictive value for precancerous lesions and cervical cancer [10] [11], making them the recommended method for primary screening.

Molecular genotyping techniques using real-time PCR, such as the Allplex™ HPV28 kit, allow simultaneous detection of 19 high-risk HPV genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82) and 9 low-risk genotypes (6, 11, 40, 42, 43, 44, 54, 61, 70), with integrated quality controls ensuring

optimal performance. These tools are essential for monitoring the prevalence and distribution of circulating genotypes.

In Senegal, particularly in the THIES region, data on HPV genotype distribution remain limited. A better understanding of local genotypes is crucial to adapt screening strategies, optimize vaccination, and strengthen the management of at-risk women.

This study aimed to determine the prevalence and distribution of HPV genotypes among women attending the gynecology department of the Regional Hospital Center of THIES for cervical cancer screening or follow-up of precancerous lesions.

2. Methodology

2.1. Study Design and Participants

This prospective, descriptive, analytical study included 27 women attending the gynecology department of the Thies Regional Hospital Centre (CHRT), Senegal, for cervical cancer screening or follow-up of precancerous lesions. Written informed consent was obtained from all participants.

2.2. Cervical-Vaginal Sample Collection

Cervical-vaginal samples were collected using sterile cytobrushes to recover both endocervical and exocervical cells. Prior to sampling, participants were checked for the absence of interfering substances (creams, gels, vaginal douches). Excess mucus was removed, and the cytobrush was inserted 1 - 1.5 cm into the cervix and rotated three times. Samples were placed in PreservCyt® tubes, transported at 2˚C - 30˚C, and stored at −20˚C until DNA extraction.

2.3. DNA Extraction and HPV Genotyping

DNA was extracted using the Quick-DNA™ Miniprep Plus kit (Zymo Research) according to the manufacturer's instructions, with sterile RNase-free water as extraction control. Briefly, 200 µL of sample were lysed with 200 µL BioFluid & Cell buffer and 20 µL Proteinase K, vortexed, and incubated at 55˚C for 10 min. Lysates were processed through Zymo-Spin™ IIC-XLR columns, washed sequentially with DNA Pre-Wash and g-DNA Wash buffers, and DNA was eluted with ≥50 µL elution buffer. Extracted DNA was stored at ≤−20˚C or used immediately for PCR.

HPV genotyping was performed using the Allplex™ HPV28 Detection kit on a CFX96™ Bio-Rad thermocycler, enabling simultaneous detection of 19 high-risk and 9 low-risk HPV genotypes with an integrated internal control. PCR reactions (20 µL) contained 15 µL Master Mix (5 µL HPV28 A or B MOM, 5 µL EM4, and 5 µL EM4 buffer) and 5 µL DNA. Negative, positive, and extraction controls were included. Tubes were sealed and centrifuged to ensure complete contact of the mixture with the tube bottom. Thermal cycling consisted of initial denaturation at 95˚C for 15 min, followed by 45 cycles of 95˚C (3 s), 60˚C (10 s), and 72˚C (10 s), with fluorescence readings at 60˚C, 72˚C, and 83˚C. Samples were considered

positive if HPV targets were detected (Ct ≤ 43), and results were validated using internal and external controls.

2.4. Data Collection and Analysis

Sociodemographic and clinical data were collected using structured questionnaires administered by a trained interviewer. A translator (midwife) assisted in cases of language barriers (French or Wolof). Data were recorded in Google Forms, exported to Excel, and analyzed using RStudio (version 2025).

3. Results

Among the 27 participants, the mean age was 44.8 ± 9.6 years. Most were married (74%) and had varying levels of education, with a substantial proportion either engaged in diverse occupations or being homemakers. The majority had experienced multiple childbirths (81%) and had never undergone cervical cancer screening (70%). None of the participants had received HPV vaccination.

The most frequently reported symptoms were pelvic or abdominal pain (56%) and intermenstrual or postcoital bleeding (41%). Cervical examination showed a predominantly normal appearance (37%) or signs of bleeding and/or inflammation (41%).

HPV PCR testing identified a single positive case (3.7%), with genotypes belonging to the HPV 53/82 complex; the remaining 26 samples were negative (Table 1).

Table 1. Sociodemographic, behavioral, clinical, and biological characteristics of participants (N = 27).

Variable	Category	N	n (%) or Mean ± SD
Age (years)	Mean ± SD	27	44.8 ± 9.6
Educational level		27	
	Other/None		7 (25.9)
	Primary		8 (29.6)
	Secondary		5 (18.5)
	University		7 (25.9)
Occupation		27	
	Other		12 (44.4)
	Trader		6 (22.2)
	Teacher		2 (7.4)
	Housewife		7 (25.9)
Marital status		27	
	Married		20 (74.1)
	Divorced		4 (14.8)
	Widowed		2 (7.4)
	Married/Widowed		1 (3.7)

Continued

Multiparity (≥2 deliveries)	27	
	Yes	22 (81.5)
	No	5 (18.5)
Previous screening	27	
	Yes	7 (25.9)
	No	19 (70.4)
	Do not know	1 (3.7)
History of STIs	27	
	Yes	3 (11.1)
	No	15 (55.6)
	Do not know	9 (33.3)
Use of oral contraceptives	27	
	Yes	6 (22.2)
	No	21 (77.8)
HPV vaccination	27	
	Yes	0 (0.0)
	No	27 (100.0)
Regular physical activity	27	
	Yes	7 (25.9)
	No	20 (74.1)
Pelvic pain	27	
	Yes	15 (55.6)
	No	12 (44.4)
Abnormal genital bleeding	27	
	Yes	11 (40.7)
	No	16 (59.3)
Vaginal discharge	27	
	Yes	5 (18.5)
	No	22 (81.5)
Reason for consultation	27	
	Screening	22 (81.5)
	HSIL follow-up	2 (7.4)
	LSIL follow-up	3 (11.1)
HPV PCR result	27	
	Negative	26 (96.3)
	Positive	1 (3.7)

4. Discussion

This study aimed to analyze the frequency and distribution of HPV genotypes among women attending the Thies Regional Hospital Centre (CHRT) for cervical cancer screening or follow-up of precancerous lesions, in order to characterize circulating genotypes and provide insights for cervical cancer prevention strategies. Viral DNA detection was performed at UMRED using the Allplex™ HPV 28 Detection kit (Seegene), which identifies 28 HPV genotypes, including high-risk oncogenic types on CFX96™ Bio-Rad. HPV testing, used as a first-line screening method, has been shown to outperform cytology in detecting high-grade lesions and provides a negative predictive value close to 100%, justifying extended screening intervals in accordance with international and national recommendations

4.1. HPV Prevalence and Genotype Distribution

The HPV prevalence observed in this study was low (3.7%), with only one positive case detected, belonging to the HPV 53/82 complex, classified as high-risk but more commonly associated with low-grade cervical lesions. This prevalence is substantially lower than reported in previous African and international studies: 85.9% in a Senegalese cohort of women specifically diagnosed with cervical intraepithelial neoplasia (CIN), a population inherently at higher risk, in which HPV genotypes 16, 18, 45, and 58 predominated [12]; and 37.2% in Kumasi, Ghana, with HPV 52, 56, 35, 18, and 58 being most frequently detected [13].

The low prevalence observed in the present study may be explained by several factors:

- **Small sample size (n = 27)**, limiting statistical power.
- **Sociodemographic profile and sexual behavior**: The majority of participants were married and monogamous, reflecting low exposure to high-risk sexual behaviors. Previous studies have reported an association between the number of lifetime sexual partners and the risk of high-risk HPV infection (HPV-HR) [14] [15]. Moreover, Shi et al. showed that, according to marital status, HPV prevalence was lowest among married women (29.4%) and highest among single, divorced, or never-married women (>47%) [16]. These findings suggest that sociodemographic characteristics and sexual behavior patterns may contribute to the low prevalence observed in this cohort.
- **Hospital-based recruitment**, introducing potential selection bias, as most participants were recruited for routine screening (22/27; 81.5%), with fewer women undergoing follow-up for high-grade squamous intraepithelial lesions (HSIL: 2/27; 7.4%) or low-grade squamous intraepithelial lesions (LSIL: 3/27; 11.1%).
- It is also important to note that cross-sectional studies reflect a single time point and do not capture viral clearance dynamics. Consequently, a low point prevalence does not necessarily indicate a low lifetime risk or cumulative incidence of HPV infection.

HPV-53 and HPV-82, both possibly carcinogenic to humans (Group 2B), are

frequent causes of precancerous lesions but rarely lead to cervical cancer [17]-[19]. Previous studies, including those by *Zappacosta et al.*, suggest that HPV-53 may sustain the malignant phenotype induced by HPV-16, potentially promoting the progression of high-grade intraepithelial lesions to invasive cervical cancer [20]. HPV-82 can lead to cancer in cases of high viral load or co-infection with a high-risk HPV type, with a reported prevalence of 7 % among 198 women with normal endocervical cytology, according to *Faye B. et al.,* 2020 [21].

4.2. Sociodemographic Characteristics

The mean age of participants was 44.8 ± 9.6 years (range: 30 - 64 years), consistent with World Health Organization (WHO), Haute Autorité de Santé (HAS), and national guidelines for cervical cancer screening [22] [23]. A large proportion of women were married (74.1%), reflecting prevailing local sociocultural norms. Low educational attainment was identified as a major barrier, influencing:

- Awareness and understanding of cervical cancer and the importance of screening;
- Adherence to screening recommendations;
- Access to healthcare services.

This observation is consistent with previous studies identifying educational level as a key determinant of cervical cancer screening uptake and adherence to preventive programs.

4.3. Screening Practices

A concerning finding was that 70% of participants had never undergone an HPV test, indicating:

- Persistent opportunistic screening;
- Absence of a structured national screening programme;
- Insufficient awareness and health education.

The lack of knowledge regarding individual screening status also reflects gaps in medical record traceability, poor communication between healthcare providers and patients, and limited retention of health information. These findings underscore the need for digitalization of medical records to improve follow-up, longitudinal monitoring, and continuity of care.

4.4. Risk Factors

Traditional risk factors were infrequently reported:

- Multiple sexual partners: 33.3% (likely underreported);
- Unknown HIV status: 40.7%;
- History of sexually transmitted infections (STIs): 11.1%.

No participant reported HPV vaccination, despite its introduction in the national Expanded Programme on Immunization (EPI) in 2018. The lack of coverage may be explained by the initial target population of girls born between 2004 and 2009, highlighting a gap in vaccination among adult women.

4.5. Study Limitations

The main limitations of this study include:

- Small sample size, limiting generalizability;
- Cross-sectional design, preventing assessment of viral persistence or temporal dynamics;
- Enrolment challenges related to participants' low educational levels;
- Limited knowledge of medical history among participants, potentially affecting data reliability.

Despite these limitations, the study provides preliminary insights into HPV genotype distribution in a Senegalese hospital-based population and highlights critical areas for improving cervical cancer prevention, including structured screening, health education, and expanded vaccination coverage.

5. Conclusions

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HPV infections are common, usually asymptomatic, and often transient; however, persistent infection with high-risk genotypes can lead to precancerous lesions and cervical cancer in the absence of adequate screening and follow-up. In this preliminary study, a low HPV prevalence (3.7%) was observed, with detection limited to the HPV 53/82 complex, indicating the circulation of high-risk HPV genotypes within the studied population.

Given the small sample size, these findings should be interpreted with caution and do not allow for definitive conclusions regarding population-level HPV prevalence. Nevertheless, they highlight potential gaps in cervical cancer prevention strategies, particularly regarding screening uptake. The study also underscores the need to improve access to HPV testing and to implement digital traceability of diagnostic and clinical procedures, especially in low-resource settings.

Larger, adequately powered studies are needed to accurately characterize circulating HPV genotypes, assess the role of molecular biomarkers in the screening and monitoring of high-risk cervical lesions, and guide evidence-based public health interventions tailored to the epidemiological context of THIES, Senegal.

Conflicts of Interest

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

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