

Original Research Article

DOI: <https://dx.doi.org/10.18203/2320-6012.ijrms20252012>

Epidemiology, genotypic diversity and age-specific risk pattern of circulating human papillomavirus in Dhaka City, Bangladesh: a five-year study (2020-2024)

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Received: 15 May 2025

Revised: 05 June 2025

Accepted: 21 June 2025

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ABSTRACT

Background: Human papillomavirus (HPV) is among the most prevalent sexually transmitted illnesses globally, particularly with certain high-risk genotypes known as the predominant etiological agents of cervical carcinoma, which remains a considerable public health threat, especially in low- and middle-income countries (LMICs), like Bangladesh.

Methods: This study investigated the frequency, characteristics, and distribution of HPV genotypes among women in Dhaka City, Bangladesh, from 2020 to 2024. The HPV genotypes were detected by real-time polymerase chain reaction (PCR) from extracted DNA of cervical swab samples.

Results: Among 2049 tested participants, the overall HPV prevalence was 4.01%, with significant temporal variations observed throughout the study period. The highest number of positive cases, 29 (4.71%) out of 587, was recorded in 2024, while 2020 showed the lowest incidence, 1 (2.5%) positive case out of 39 cases. Seasonal analysis revealed higher case numbers during Winter (29%) and Autumn (30%), followed by Summer (26%), with Spring showing the lowest incidence (15%). Age distribution analysis indicated that middle-aged adults (35-50 years) represented the largest group with 37 cases, followed by adults aged 25-34 years (25 cases). The most prevalent genotype combination was HPV 16/31 (55 cases), followed by HPV 18/45 (16 cases).

Conclusions: This unique genotype distribution pattern differs from global trends and has important implications for vaccination strategies in Bangladesh. The findings suggest the need for broader-spectrum vaccines and age-specific screening programs, while highlighting the importance of considering seasonal variations in public health interventions.

Keywords: HPV, Real-time PCR, Genotype distribution, Prevalence, Urban health, Temporal distribution, Seasonal variation, Women's health, Cervical swab, Epidemiology

INTRODUCTION

Human papillomaviruses (HPV) are highly prevalent DNA viruses that only affect humans. Papilloma refers to a tiny wart-like development on the skin or mucous membrane. HPV is regarded as the most prevalent sexually transmitted infection (STI) in the world. Nearly everyone will have HPV at least once in their lives.^{1,2} The 16 most cancer-causing HPV forms are: 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. Types 16 and 18 are most typically linked to cancer development, accounting for approximately 70% of all invasive cervical malignancies.^{3,4} Moreover, HPV 16 was the most prevalent kind in every nation except Indonesia, where HPV 18 was more prevalent.⁵ Over 200 HPV genotypes have been discovered, approximately 40 of which infect the vaginal tract.^{6,7} However, other oncogenic types such as HPV 31, 33, 45, and 58 are emerging as key contributors to disease, especially in parts of Asia.⁸ The worldwide burden of cervical cancer remains concentrated in low- and middle-income countries (LMICs), where over 85% of new cases occur, and access to prevention strategies such as HPV vaccination, screening, and healthcare resources remains uneven.^{7,9} Despite this, countries like Bangladesh face a lack of comprehensive data. In Dhaka, the capital city with over 21 million people, rapid urbanization, dense populations, and socioeconomic disparities present unique public health challenges. Yet, the distribution and prevalence of HPV genotypes in this context remain understudied.^{10,11} In this context, genotyping of HPV plays a critical role in understanding epidemiological trends and designing effective public health interventions, including tailored vaccination and screening programs. Furthermore, HPV genotyping makes it possible to accurately identify women who have had an infection for an extended period, which might be a significant risk factor for the development of higher-grade lesions.¹²⁻¹⁴

In light of these concerns, cervical cancer, primarily caused by chronic exposure to high-risk HPV subtypes, has emerged as the fourth most frequent malignancy in women worldwide, with an estimated 660,000 new cases and 350,000 deaths recorded in 2022.¹⁵⁻¹⁷ In the same year, about 94% of the 350,000 cervical cancer-related fatalities occurred in low and middle-income countries.^{18,19} The disparity in mortality is largely driven by limited access to early screening, timely diagnosis, and treatment services.^{20,21} The disease disproportionately affects women in less developed countries, where it accounts for about 90% of new cases and deaths globally.²² In regions like South Asia, cervical cancer contributes significantly to the disease burden among women, exacerbated by low rates of screening and HPV vaccination.^{22,23}

The situation in Bangladesh is particularly concerning since HPV-related infections remain a serious public health issue. Despite worldwide breakthroughs in cervical cancer prevention and treatment, including the introduction of national HPV vaccine, screening, and treatment programs, it remains the country's second most

frequent malignancy among women. This is mostly due to deficiencies in healthcare infrastructure, limited access to medical services, and inadequate coverage of preventative measures.¹¹ Although a nationwide program began in 2016, it primarily targets adolescent girls, leaving many women of reproductive age unvaccinated and exposed to risk. The lack of region-specific data on circulating genotypes limits the development of effective, localized prevention strategies.^{24,25} Previous studies in South Asia have consistently shown that HPV 16 and 18 dominate the genotype landscape but regional variations are increasingly evident.¹⁰ These variations could influence the efficacy of vaccines such as Gardasil and Cervix, which mainly target HPV 16 and 18.^{23,26} Understanding the local genotype distribution is thus vital for refining vaccine strategies, especially in densely populated urban hubs like Dhaka.

Despite its size and significance, epidemiological data from Dhaka City remain scarce. Existing studies have not adequately addressed age-specific risk factors, demographic influences, or the impact of urbanization on HPV transmission dynamics.⁸ The World Health Organization (WHO) has delineated a worldwide approach to eradicate cervical cancer through vaccination, screening, and treatment.^{24,27} However, implementing these measures effectively in resource-limited settings remains a formidable challenge. Consequently, comprehending the regional distribution of HPV genotypes is critical to optimizing vaccination strategies and ensuring comprehensive protection.²⁸

To address this gap, this study investigates the prevalence and distribution of HPV genotypes in women in Dhaka city from 2020 to 2024. By analyzing local trends and identifying the most significant high-risk genotypes across age groups and risk profiles, we aim to generate evidence that supports enhanced public health strategies, including expanded vaccination coverage and targeted screening programs. These efforts are essential to alleviate the load of HPV-related diseases and better serve the health needs of women in Bangladesh.

METHODS

Study design and subjects for the study

The retrospective research was carried out in a laboratory setting in Dhaka. The study was conducted with all 2049 patients with their HPV genotypes analyzed at the Ibn Sina Diagnostic and Imaging Centre, Dhanmondi, Dhaka, based on a doctor's advice of testing between January 2020 and December 2024. This study only included female participants, regardless of their age. Data on age and HPV genotypes were extracted from the diagnostic center's laboratory database, following explicit written authorization. Ensuring an adequate level of privacy during or after data collection that prevents the researcher from identifying particular patients. The data was accessed at the end of every month during the study period.

Exclusion criteria included: prior cervical precancer or cancer treatment, history of hysterectomy, cervical lesion treatment, cervical amputation, and pregnancy.

The research was conducted in accordance with the principles described in the Declaration of Helsinki.²⁹ Jagannath University's Institutional Ethical Review Board (IERB) approved the study ethically.

Sample collection and transport

Cervical swab was collected in a viral transport medium by expert female phlebotomists. During sample collection, the following conditions were considered: approximately two weeks (10-18 days) following the initial day of her previous menstrual cycle, refrain from douching for 48 hours before the exam, avoid using tampons, contraceptive foams, jellies, or any vaginal creams or drugs for 48 hours before to the test, and avoid sexual intercourse for 48 hours before the test.

Specimens were transported from different branches of Ibn Sina Trust at 2-30°C comply with the country and local regulations for the transport of etiologic agents.

Sample processing and DNA extraction

Human papillomavirus is a deoxyribonucleic acid (DNA) virus. First, we extracted viral DNA from the cell preservation solution. The DNA purification kit was the QIAamp DSP virus kit. The kit instructions were followed wherever the sample volume was 200 µl, and the extracted DNA was eluted in 100 µl.

PCR amplification

Amplification and detection of HPV DNA were performed using the AmpliSens HPV HCR genotype-titre-FRT PCR kit. The kit detects 14 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) using real-time PCR with fluorescent detection. Four PCR reactions were set up for each sample using different primer/probe mixes to detect different HPV genotype groups. The total reaction volume was 25 µl, containing 10 µl of the extracted DNA sample. Amplification was performed on a Bio-Rad CFX96™ real-time PCR system using the following cycling conditions: 95°C for 15 min, followed by five cycles of 95°C for 5 s, 60°C for 20 s, 72°C for 15 s, then 40 cycles of 95°C for 5 s, 60°C for 20 s (with fluorescence detection), 72°C for 15 s. Fluorescence was detected in FAM, JOE, ROX, and Cy5 channels. Results were analyzed using the kit instructions to determine the presence/absence of each HPV genotype. Appropriate positive and negative controls were included in each run.

Statistical analysis

The statistical package for social sciences (SPSS) was used for the data analysis. The frequency of genotypes, age

distribution, years, and seasonal impact were used to display the category.

RESULTS

Overall HPV prevalence

Among the study participants, 95.99% (1967 women) tested negative for HPV while 4.01% (82 women) were positive, indicating a prevalence rate of 4.01% in the study population from Dhaka city during 2020-2024 (Figure 1).

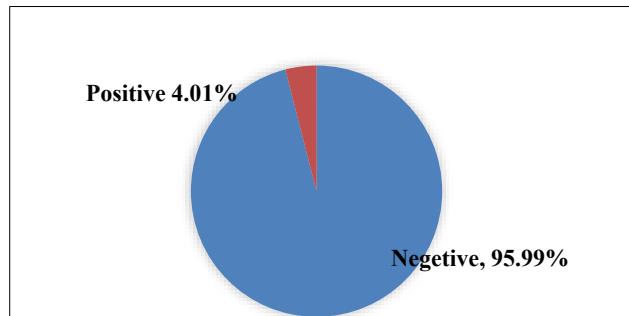


Figure 1: Overall prevalence of the percentage of HPV infection from 2020 to 2024.

Temporal distribution

Annual trends

The distribution of HPV infections showed notable variation across the study period (2020-2024). The highest number of positive cases 29 (4.71%) was recorded in 2024, followed by 27 (4.94%) cases in 2023. The years 2022 and 2021 reported 19 (3.7%) and 6 (2.15%) cases, respectively, while 2020 had the lowest incidence with only 1 (2.5%) case. However, the affected percentage was highest in 2023 (Figure 2).

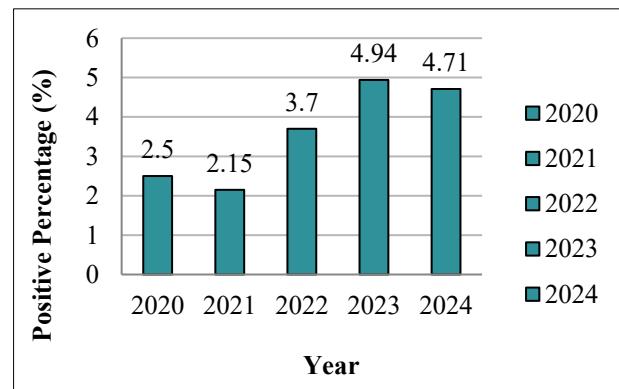


Figure 2: Annual positive trend of HPV for five consecutive years in the study population.

Seasonal pattern

The analysis of the seasonal distribution of cases revealed notable trends across the four seasons. Autumn had the

highest number of cases, with a total of 25 (30%). Winter and Autumn showed almost similar numbers, each reporting 24 (29%) and 25 (30%) cases, suggesting that the cooler seasons may correlate with higher case counts. On the other hand, Spring recorded the lowest number of cases at 12 (15%), indicating a possible increase (Table 1).

Table 1: Seasonal pattern of HPV infection for the consecutive 5 years.

Season	HPV positive cases	%
Autumn	25	30
Spring	12	15
Summer	21	26
Winter	24	29
Total	82	100

There was a decline in incidence during this season. This distribution suggests that there may be a slight seasonal pattern, with Winter and Autumn seeing the highest numbers, followed closely by Summer, and Spring showing the lowest incidence.

Monthly distribution

The monthly distribution of cases displayed considerable variation. November recorded the highest incidence with 17 cases, indicating a peak in this month. February, December and March followed with moderate numbers, reporting 11, 9 and 8 cases respectively, showing a consistent presence of cases at the beginning of the year. April marked the lowest incidence with only 1 case, suggesting a significant drop. The remaining months showed fluctuating case numbers, ranging from 2 to 7 instances, reflecting an uneven distribution throughout the year with occasional peaks and troughs. This variability suggests potential seasonal influences or other factors affecting monthly incidence rates (Figure 3).

Demographic characteristics

Age distribution

The age-specific distribution of HPV infections highlights those middle-aged adults (35-50 years) represented the largest group, with a total of 37 cases, suggesting that HPV infections may be more prevalent in this age range. Adults aged 25-34 followed, accounting for 25 cases, indicating a moderately high incidence among individuals in this age group. Young adults (15-24 years) represented a smaller number, with 12 cases, which may reflect early-stage exposure. Older adults (50+ years) had the lowest incidence, with only 8 cases (Figure 4).

This age-specific distribution points to a peak in HPV infections in middle-aged adults, with declining rates in both younger and older populations.

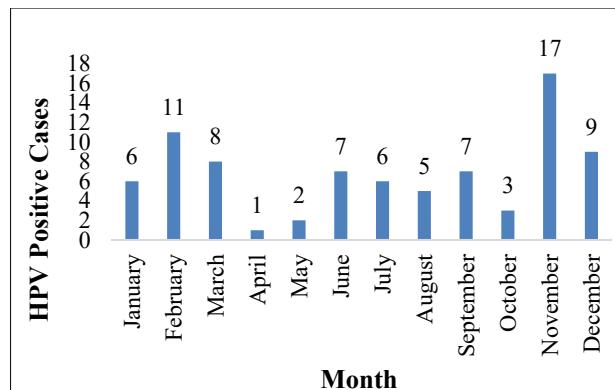


Figure 3: Month and year-wise distribution of HPV positive infections from 2020 to 2024.

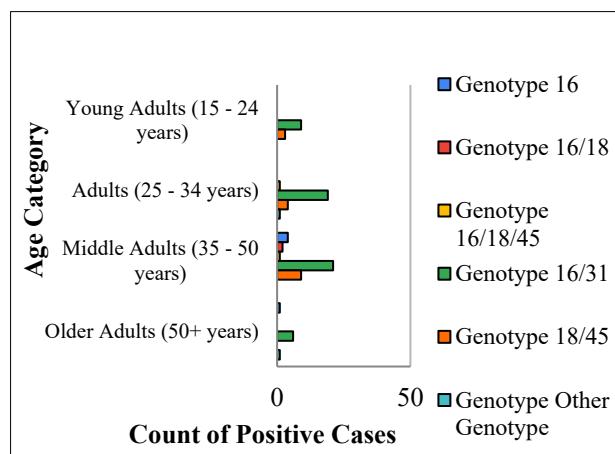


Figure 4: Age group distribution of HPV infection from 2020 to 2024.

HPV genotype distribution

The analysis of HPV genotypes revealed a diverse pattern of infections, with certain types showing higher prevalence. The predominant genotype combination was HPV 16/31, detected in 55 cases, suggesting a strong presence of these high-risk types. HPV 18/45 was the next most frequent in 16 cases, indicating another significant combination. A single HPV 16 infection was identified in 5 cases, underscoring the high prevalence of HPV 16 even in isolated infections. The HPV 16/18 combination appeared in 2 cases, while HPV 16/18/45 was detected in 2 cases, showing less common multi-genotype infections. Other genotypes contributed to 2 cases, reflecting the diversity of HPV types, but with a clear predominance of high-risk strains like HPV 16 and 18 in various combinations. This pattern underscores the importance of targeting these prevalent genotypes in HPV prevention and vaccination strategies. This detailed distribution of HPV genotypes suggests a predominance of HPV 16/31 co-infection in the study population, followed by HPV 18/45 as the second most common variant (Figure 5).

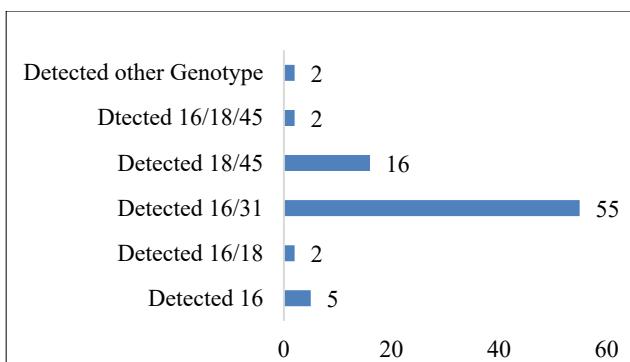


Figure 5: Genotype distribution of HPV infection from 2020 to 2024.

DISCUSSION

Our study provides critical insights into the epidemiology of HPV infections among women in Dhaka City, Bangladesh, from 2020 to 2024. The overall HPV prevalence of 4.01% is lower than estimates from other South Asian regions, which have reported prevalence rates ranging from 6% to 12%. This relatively low prevalence rate may reflect regional variations in exposure, cultural norms, healthcare-seeking behavior, or limitations in diagnostic outreach and screening coverage.

The temporal analysis revealed a significant increase in HPV cases from 2021 through 2024, with a notable peak in 2024 with the highest number of cases 29 (4.71%), suggesting improved testing infrastructure and greater awareness in recent years. The exceptionally low case number in 2020 (only 1 (2.5%) case) may be attributed to pandemic-related disruptions in healthcare access and elective screenings, a phenomenon observed globally during COVID-19.⁹

Seasonal variation in HPV incidence was a notable finding. Higher case counts in Winter and Autumn 24 (29%) and 25 (30%) cases and Summer 21 (26%) instances suggest potential immunological or behavioral dynamics, such as reduced mucosal immunity during colder months or increased clinic attendance during non-harvest/agricultural seasons. Although global literature offers limited insight into HPV seasonality, these findings align with localized observations suggesting seasonal immune modulation may influence infection susceptibility.³⁰

The age distribution pattern observed in our study reveals several significant findings. This analysis showed that women aged 35–50 had the highest number of infections (37 cases). This diverges from global patterns, where the highest incidence often appears in women under 25.^{23,31} Possible explanations include delayed screening uptake, persistent infections in older women, and cultural reluctance to seek gynecological care at younger ages. The lower infection rate among women aged +50 (8 cases) could also suggest under-screening in that demographic or

underreporting due to stigma and limited access to adolescent health services.

The most striking observation was the predominance of HPV 16/31 co-infection (55 cases), which comprised nearly 67% of all positive cases. This finding diverges from global trends, where HPV 16 and 18 are typically most prevalent.³² The high prevalence of HPV 16/31 raises concerns about vaccine coverage, as the bivalent and quadrivalent HPV vaccines may not provide sufficient protection in this population. Screening strategies should account for the high prevalence of type 31, and additional inquiry is required to comprehend the elements leading to this unique genotype distribution. HPV 18/45, as the second most common variant (16 cases), aligns more closely with global patterns and supports the continued inclusion of these types in vaccine formulations.

Our findings have important implications for public health strategies in Bangladesh. The high prevalence of HPV genotypes 16 and 31 suggests a need for broader-spectrum vaccines to protect against multiple high-risk genotypes (HPV 31, 33, 45, 52, and 58 in addition to HPV 16 and 18). Such formulations may offer enhanced protection in populations like Dhaka's, where non-16/18 high-risk genotypes appear to play a more prominent role.²⁴ Additionally, Public health planning should consider not only genotype prevalence but also seasonal and demographic factors. Screening programs should be strengthened for middle-aged women, who appear to be at elevated risk, while improving access for younger age groups to enable early detection and intervention. Furthermore, the seasonal patterns observed in HPV prevalence could be used to optimize the timing of screening campaigns and other public health interventions, ensuring resources are allocated efficiently and vulnerable populations are reached at critical times. Together, these strategies support a targeted approach to mitigating the impact of HPV-associated illnesses in Bangladesh.

In terms of future directions, studies with larger and more representative sample sizes across diverse regions would enhance the robustness of these findings. Examining the impacts of socioeconomic status and access to healthcare in more detail could also provide insights into targeted intervention strategies, helping to address disparities more effectively. Finally, post-pandemic studies could offer a clearer view of healthcare without the influence of COVID-19, providing more accurate baseline data for public health planning.

In summary, these findings clearly provide a foundation for more targeted prevention and control strategies that address the specific dynamics of HPV in Bangladesh, contributing valuable insights to the field of HPV epidemiology in this unique urban setting.

While the current findings are robust and offer actionable insights, certain limitations must be acknowledged. The study was confined to a single diagnostic center in Dhaka

city, which may not capture the full epidemiological variability across urban and peri-urban areas. Additionally, socioeconomic condition, behavioral status, life style patterns, smoking, hormonal contraceptive use and immunological factors of the participants were not examined, which could better explain variations in age and seasonal prevalence. Broader population-based studies must authenticate these results across Bangladesh's diverse regions.

CONCLUSION

This study provides novel and context-specific insights into the prevalence, seasonal distribution, and genotype characteristics of HPV infections among women in Dhaka, Bangladesh, from 2020 to 2024. The overall prevalence rate of 4.01 % underscores a relatively lower burden than regional estimates.

Yet, the dominance of high-risk genotypes such as HPV 16/31 and 18/45 suggests substantial potential for oncogenic progression if left unaddressed. A steady rise in HPV cases over the study period, peaking in 2024, suggests increased detection and awareness, while the COVID-19 pandemic may have affected the minimal occurrence in 2020.

The significant prevalence among women aged 35–50 highlights the need to extend screening and vaccination efforts beyond adolescent girls, as current national strategies may miss a high-risk population segment. Similarly, the seasonal concentration of cases in Autumn and Winter provides a potential opportunity for targeted public health campaigns, including mobile screening units or vaccination drives during these peak periods.

Most importantly, the predominance of HPV 31 alongside HPV 16 supports the urgent consideration of broader-spectrum vaccines like the nonvalent formulation, which can offer improved protection in this population. These findings directly affect tailoring Bangladesh's HPV prevention strategy based on local epidemiological evidence.

Future research should incorporate diverse urban and rural cohorts, explore behavioral and socioeconomic determinants, and assess post-vaccine genotype shifts. Longitudinal studies are also necessary to evaluate infection persistence and outcomes over time. Strengthening health education, reducing stigma, and improving vaccine accessibility, especially in middle-aged women, will be critical steps toward reducing HPV-related disease burden in Bangladesh.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the PCR lab, Ibn Sina Diagnostic and Imaging Center, Dhanmondi, Dhaka, Bangladesh for allowing to access their laboratory database as a part of research work.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Rahman MA, Islam MR, Hasan MN, Ullah MS, Jahan I, Morshed RA, et al. Epidemiology, genotypic diversity and age-specific risk pattern of circulating human papillomavirus in Dhaka City, Bangladesh: a five-year study (2020–2024). *Int J Res Med Sci* 2025;13:2781-7.