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Detection of High-Risk Human Papillomavirus Using the cobas® 4800 System among Women Screened for Cervical Cancer in Western Province, Zambia: A Retrospective Laboratory-Based Study

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Abstract

Background: High-risk human papillomavirus (HR-HPV) infection is the cause of cervical cancer, but its distribution varies across populations and geographical settings. Evidence on HR-HPV infection across districts of Western Province, Zambia, remains limited. This study determined the prevalence and distribution of HR-HPV infection and examined variation by age and district among women screened for cervical cancer.

Methods: We conducted a retrospective laboratory-based cross-sectional study using routinely collected cervical cancer screening records from women tested for HR-HPV between 9 May 2025 and 29 June 2026. Data were extracted from the laboratory information system of the Kaoma PCR Laboratory, which serves seven districts in Western Province. HR-HPV testing was performed using the cobas® 4800 HPV Test. test. The primary outcome was detection of at least one HR-HPV target. HR-HPV prevalence and target distribution were summarized using proportions with 95% confidence intervals (CIs). Differences across age groups and districts were assessed using Pearson's chi-square test. Logistic regression was used to estimate crude and adjusted odds ratios (aORs) with 95% CIs for factors associated with HR-HPV positivity.

Results: Among 1,623 women, the median age was 40 years (IQR 31–49; range 15–89). HR-HPV was detected in 529 women, giving an overall prevalence of 32.59% (95% CI 30.35–34.92). Prevalence was highest among women aged <25 years (46.58%) and decreased to 26.67% among those aged 45–54 years before increasing to 34.32% among those aged 55–64 years (χ^2=26.96, p<0.001). HR-HPV prevalence ranged from 21.84% in Kalabo to 37.04% in Nkeyema (χ^2=12.70, p=0.048). Among HR-HPV-positive women, 456 (86.20%) had other HR-HPV targets, 88 (16.64%) had HPV16, and 48 (9.07%) had HPV18/45. Compared with women aged <25 years, adjusted odds of HR-HPV positivity were lower among those aged 25–34 years (aOR 0.66, 95% CI 0.45–0.97), 35–44 years (0.43, 0.29–0.64), 45–54 years (0.38, 0.25–0.57), 55–64 years (0.55, 0.35–0.88), and ≥65 years (0.50, 0.26–0.97). Women from Kalabo had lower odds of HR-HPV positivity than those from Kaoma (aOR 0.55, 95% CI 0.37–0.83).

Conclusions: HR-HPV infection affected approximately one-third of women screened across seven districts of Western Province, with substantial variation by age and district. The predominance of other HR-HPV targets highlights the importance of broad HR-HPV detection in cervical cancer screening. District-level surveillance using routinely generated laboratory data can provide useful evidence for monitoring and strengthening cervical cancer prevention services in Western Province.

1. Introduction

Cervical cancer remains a major public health problem, particularly in the low- and middle-income countries, where most cases and deaths occur [1]. Globally, approximately 604,000 new cases and 280,000 deaths from cervical cancer were estimated in 2024, with the highest burden occurring in countries with limited access to human papillomavirus (HPV) vaccination, screening, and treatment

services [2]. Persistent infection with high-risk HPV (HR-HPV) is the cause of virtually all cervical cancers, although most HPV infections are transient and clear spontaneously [3].

HPV-based screening has become central to cervical cancer prevention because HPV deoxyribonucleic acid (DNA) testing provides an objective method for identifying women at risk of cervical precancer before invasive disease develops [4, 5]. The World Health Organization (WHO) recommends HPV DNA testing as the preferred primary screening approach, with strategies incorporating triage and treatment according to HPV status and population risk [6]. Partial genotyping is increasingly relevant to screening programmes because HPV16 and HPV18 carry particularly high oncogenic risk, while infections with other carcinogenic HPV types also contribute substantially to cervical cancer burden [7].

In sub-Saharan Africa, HR-HPV prevalence and genotype distribution vary considerably between populations and geographical settings [7–9]. Evidence from Zambia has demonstrated substantial HR-HPV burden among women undergoing cervical screening, including differences in HPV distribution according to age, HIV status, and clinical characteristics. A recent large programme-based study in Southern Province reported HPV infection in 35.56% of 4,612 women screened between 2021 and 2022, highlighting the importance of geographically specific surveillance of HPV infection in Zambia [10]. Earlier studies from Lusaka have also demonstrated substantial HR-HPV prevalence and considerable genotype diversity among women living with HIV [11].

Despite increasing availability of HPV-based screening in Zambia, evidence from Western Province remains limited, particularly from routinely generated laboratory data covering multiple districts [12]. Characterising the burden and distribution of HR-HPV infection in this setting can provide locally relevant evidence for cervical cancer prevention and screening programme planning. We therefore, determined the prevalence and distribution of HR-HPV infection and examined variation by age and district among women screened for cervical cancer in Western Province, Zambia, using routinely collected laboratory data.

2 Methods

2.1 Study design and setting

We conducted a retrospective laboratory-based cross-sectional study using routinely collected cervical cancer screening records from women tested for HR-HPV between 9 May 2025 and 29 June 2026. The study was conducted in Western Province, Zambia, and included women from seven districts: Kalabo, Kaoma, Luampa, Lukulu, Mitete, Nkeyema, and Sikongo. Western Province is predominantly rural, with geographic differences in population distribution and access to health services [13].

The Kaoma PCR Laboratory at Kaoma District Hospital serves as the molecular HPV testing site for these districts. Cervical specimens collected through the cervical cancer screening programme were referred to the laboratory for HR-HPV testing. The Kaoma District Hospital laboratory is registered with the Health Professions Council of Zambia (HPCZ; Registration No. HPCZ/101/3/301), and the Kaoma

PCR Laboratory is accredited to ISO 15189:2022 by the Southern African Development Community Accreditation Service (SADCAS; Facility Accreditation Number: MED 208).

2.2 Data source, study population and unit of analysis

De-identified HPV testing records were extracted from the laboratory information system (LIS; DISA Version 04.18.02.977). The study population comprised women who underwent cervical cancer screening at participating health facilities and whose cervical specimens were processed for HR-HPV testing at the Kaoma PCR Laboratory during the study period.

Eligible records were from women residing in the study districts and contained interpretable information on age, district, HPV testing result, and HPV target results. The unit of analysis was the individual woman. All eligible records generated during the study period were included, resulting in 1,623 women in the final analysis.

2.3 Specimen referral, processing and quality assurance

Cervical swab specimens were collected at participating health facilities and placed in PreservCyt® transport medium (Hologic, Inc., Marlborough, MA, USA) before referral to the Kaoma PCR Laboratory. Specimens were transported under temperature-monitored conditions within the validated range of 2–30°C for cervical specimens collected in PreservCyt® for cobas® HPV testing [14].

Upon receipt, specimens were checked for identification, integrity, and transport conditions before processing. HPV testing was performed using the cobas® 4800 HPV Test on the cobas® 4800 System (Roche Molecular Systems, Pleasanton, CA, USA), according to the manufacturer's instructions and the laboratory's validated procedures [15]. Internal quality controls were included in each testing run, and results were accepted only when the required assay controls met the manufacturer's validity criteria [16].

2.4 Study variables and outcome definitions

The primary outcome was HR-HPV infection, defined as detection of at least one high-risk HPV target by the cobas® HPV assay. HR-HPV status was classified as positive or negative for the primary analysis. HPV-positive results were further classified according to detection of HPV16, HPV18/45, and other HR-HPV targets.

The explanatory variables were age group and district of residence. Age was categorized as < 25, 25–34, 35–44, 45–54, 55–64, and ≥ 65 years. District was categorized as Kalabo, Kaoma, Luampa, Lukulu, Mitete, Nkeyema, and Sikongo.

For analysis of infection patterns, individual HPV target results were not mutually exclusive because women could have more than one target detected. Mutually exclusive patterns were classified as HPV16 only, HPV18/45 only, other HR-HPV only, HPV16 plus HPV18/45 only, HPV16 plus other HR-HPV, HPV18/45 plus other HR-HPV, and all three target groups.

2.5 Data management and cleaning

Data were exported from the LIS and cleaned using Stata version 18 (StataCorp LLC, College Station, TX, USA). Records were assessed for completeness, consistency, duplicate entries, and valid coding of key study variables. Records with missing or uninterpretable information required for the primary analysis were excluded.

2.6 Statistical analysis

Analyses were performed using Stata version 18 (StataCorp LLC, College Station, TX, USA). Continuous variables were summarized using the median and interquartile range (IQR), with the range reported where appropriate. Categorical variables were summarized using frequencies and percentages.

HR-HPV prevalence and the prevalence of individual HPV targets were calculated as proportions of women tested, with corresponding 95% confidence intervals (CIs). Pearson's chi-square test was used to assess differences in HR-HPV positivity and individual HPV target detection across age groups and districts.

Logistic regression was used to assess factors associated with HR-HPV positivity. Crude odds ratios (cORs) and adjusted odds ratios (aORs) with 95% CIs were estimated. Age group and district were included in the multivariable model, with women aged < 25 years and those residing in Kaoma District as the respective reference categories. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

3 Results

3.1 Study characteristics

A total of 1,623 women were included in the analysis. The median age was 40 years (IQR 31–49), with a range of 15–89 years. By age group, 146 (9.00%) women were aged < 25 years, 402 (24.77%) were aged 25–34 years, 462 (28.47%) were aged 35–44 years, 390 (24.03%) were aged 45–54 years, 169 (10.41%) were aged 55–64 years, and 54 (3.33%) were aged ≥ 65 years. By district, 174 (10.72%) women were from Kalabo, 520 (32.04%) from Kaoma, 121 (7.46%) from Luampa, 425 (26.19%) from Lukulu, 16 (0.99%) from Mitete, 243 (14.97%) from Nkeyema, and 124 (7.64%) from Sikongo (Table 1).

Table 1
Characteristics of women screened for high-risk human papillomavirus

Characteristic	Number (N = 1,623)	Percentage (%)
Age, years		
Median (IQR)	40	31–49
Range	15–89	
Age group, years		
< 25	146	9.00
25–34	402	24.77
35–44	462	28.47
45–54	390	24.03
55–64	169	10.41
≥ 65	54	3.33
District		
Kalabo	174	10.72
Kaoma	520	32.04
Luampa	121	7.46
Lukulu	425	26.19
Mitete	16	0.99
Nkeyema	243	14.97
Sikongo	124	7.64

3.2 Prevalence of high-risk HPV infection

Among the 1,623 women screened, 529 (32.59%; 95% CI: 30.35–34.92%) tested positive for high-risk HPV, while 1,094 (67.41%; 95% CI: 65.08–69.65%) tested negative (Table 2).

Table 2
Prevalence of high-risk HPV infection among women screened for cervical cancer

High-risk HPV status	Number, n	Prevalence, % (95% CI)
Negative	1,094	67.41 (65.08–69.65)
Positive	529	32.59 (30.35–34.92)
Total	**1,623**	**100.00**

3.3 Distribution of high-risk HPV targets and infection patterns

Among the 529 women who tested positive for high-risk HPV, 88 (16.64%; 95% CI: 13.69–20.07%) were positive for HPV16, 48 (9.07%; 95% CI: 6.90–11.85%) for HPV18/45, and 456 (86.20%; 95% CI: 82.98–88.89%) for other high-risk HPV. Because individual HPV targets were not mutually exclusive, infection patterns were further classified into mutually exclusive combinations. Other HR-HPV only was the most common pattern, occurring in 395 (74.67%; 95% CI: 70.77–78.20%) women, followed by HPV16 only in 47 (8.88%; 95% CI: 6.73–11.64%), HPV16 plus other HR-HPV in 39 (7.37%; 95% CI: 5.43–9.94%), HPV18/45 only in 26 (4.91%; 95% CI: 3.36–7.13%), HPV18/45 plus other HR-HPV in 20 (3.78%; 95% CI: 2.45–5.80%), and all three targets in 2 (0.38%; 95% CI: 0.09–1.51%) women. No woman had HPV16 plus HPV18/45 without other HR-HPV **(**Table 3**).**

Table 3
Distribution of high-risk HPV targets and infection patterns among HR-HPV-positive women (n = 529)

HPV result	n	% (95% CI)
Individual HPV targets		
HPV16 positive	88	16.64 (13.69–20.07)
HPV18/45 positive	48	9.07 (6.90–11.85)
Other HR-HPV positive	456	86.20 (82.98–88.89)
Mutually exclusive HPV infection patterns		
HPV16 only	47	8.88 (6.73–11.64)
HPV18/45 only	26	4.91 (3.36–7.13)
Other HR-HPV only	395	74.67 (70.77–78.20)
HPV16 + HPV18/45 only	0	0.00
HPV16 + other HR-HPV	39	7.37 (5.43–9.94)
HPV18/45 + other HR-HPV	20	3.78 (2.45–5.80)
HPV16 + HPV18/45 + other HR-HPV	2	0.38 (0.09–1.51)
Total	**529**	**100.00**

3.4 High-risk HPV positivity by age group and district

High-risk HPV positivity differed significantly across age groups (Pearson χ^2 (5) = 26.96, $p < 0.001$). Positivity was 46.58% (68/146; 95% CI: 38.61–54.72%) among women aged < 25 years, 37.31% (150/402; 95% CI: 32.71–42.16%) among those aged 25–34 years, 28.57% (132/462; 95% CI: 24.63–32.87%) among those aged 35–44 years, and 26.67% (104/390; 95% CI: 22.50–31.29%) among those aged 45–54 years. Positivity was 34.32% (58/169; 95% CI: 27.53–41.82%) among women aged 55–64 years and 31.48% (17/54; 95% CI: 20.46–45.08%) among those aged ≥ 65 years.

High-risk HPV positivity also differed significantly across districts (Pearson $\chi^2(6) = 12.70$, $p = 0.048$). Prevalence was 21.84% (38/174; 95% CI: 16.30–28.62%) in Kalabo, 31.73% (165/520; 95% CI: 27.87–35.87%) in Kaoma, 34.71% (42/121; 95% CI: 26.74–43.64%) in Luampa, 34.12% (145/425; 95% CI: 29.76–38.77%) in Lukulu, 31.25% (5/16; 95% CI: 13.23–57.55%) in Mitete, 37.04% (90/243; 95% CI: 31.18–43.30%) in Nkeyema, and 35.48% (44/124; 95% CI: 27.54–44.32%) in Sikongo (Table 4, Fig. 1).

Table 4
Association between demographic characteristics and high-risk HPV positivity

Characteristic	Total, n	HR-HPV positive, n (%)	HR-HPV negative, n (%)	P value
Age group, years				**< 0.001**
< 25	146	68 (46.58)	78 (53.42)	
25–34	402	150 (37.31)	252 (62.69)	
35–44	462	132 (28.57)	330 (71.43)	
45–54	390	104 (26.67)	286 (73.33)	
55–64	169	58 (34.32)	111 (65.68)	
≥ 65	54	17 (31.48)	37 (68.52)	
District				**0.048**
Kalabo	174	38 (21.84)	136 (78.16)	
Kaoma	520	165 (31.73)	355 (68.27)	
Luampa	121	42 (34.71)	79 (65.29)	
Lukulu	425	145 (34.12)	280 (65.88)	
Mitete	16	5 (31.25)	11 (68.75)	
Nkeyema	243	90 (37.04)	153 (62.96)	
Sikongo	124	44 (35.48)	80 (64.52)	
Total	**1,623**	**529 (32.59)**	**1,094 (67.41)**	

3.5 Distribution of HPV targets by district and age group

The district-specific prevalence of HPV16 ranged from 1.65% (2/121; 95% CI: 0.41–6.41%) in Luampa to 6.58% (16/243; 95% CI: 4.07–10.49%) in Nkeyema. HPV16 prevalence did not differ significantly across districts (Pearson $\chi^2(6) = 5.64$, $p = 0.465$). HPV18/45 prevalence ranged from 0% in Luampa and Mitete to 4.71% (20/425; 95% CI: 3.05–7.19%) in Lukulu, with no significant difference across districts (Pearson $\chi^2(6) = 9.98$, $p = 0.126$). Other high-risk HPV was more frequently detected and ranged from 17.24% (30/174; 95% CI: 12.31–23.62%) in Kalabo to 33.88% (41/121; 95% CI: 25.98–42.80%) in Luampa. The distribution of other high-risk HPV differed significantly across districts (Pearson $\chi^2(6) = 14.34$, $p = 0.026$). Overall high-risk HPV prevalence ranged from 21.84% in Kalabo to 37.04% in Nkeyema (Table 5, Fig. 2).

Table 5
District-specific prevalence of HPV16, HPV18/45, other high-risk HPV and any high-risk HPV

District	n	HPV16, n (%)	HPV18/45, n (%)	Other HR-HPV, n (%)	Any HR-HPV, n (%)
Kalabo	174	10 (5.75; 3.11–10.37)	3 (1.72; 0.55–5.23)	30 (17.24; 12.31–23.62)	38 (21.84; 16.30–28.62)
Kaoma	520	29 (5.58; 3.90–7.91)	16 (3.08; 1.89–4.97)	144 (27.69; 24.01–31.70)	165 (31.73; 27.87–35.87)
Luampa	121	2 (1.65; 0.41–6.41)	0 (0.00)	41 (33.88; 25.98–42.80)	42 (34.71; 26.74–43.64)
Lukulu	425	26 (6.12; 4.20–8.84)	20 (4.71; 3.05–7.19)	122 (28.71; 24.60–33.20)	145 (34.12; 29.76–38.77)
Mitete	16	1 (6.25; 0.82–35.07)	0 (0.00)	4 (25.00; 9.38–51.77)	5 (31.25; 13.23–57.55)
Nkeyema	243	16 (6.58; 4.07–10.49)	6 (2.47; 1.11–5.40)	75 (30.86; 25.36–36.97)	90 (37.04; 31.18–43.30)
Sikongo	124	4 (3.23; 1.21–8.32)	3 (2.42; 0.78–7.27)	40 (32.26; 24.60–41.01)	44 (35.48; 27.54–44.32)
Total	**1,623**	**88 (5.42; 4.39–6.71)**	**48 (2.96; 2.24–3.91)**	**456 (28.10; 25.99–30.32)**	**529 (32.59; 30.35–34.92)**

High-risk HPV positivity varied significantly across age groups (Pearson χ^2 (5) = 26.96, $p < 0.001$). Positivity was 46.58% (68/146; 95% CI: 38.61–54.72%) among women aged < 25 years, 37.31% (150/402; 95% CI: 32.71–42.16%) among those aged 25–34 years, 28.57% (132/462; 95% CI: 24.63–32.87%) among those aged 35–44 years, and 26.67% (104/390; 95% CI: 22.50–31.29%) among those aged 45–54 years. Positivity increased to 34.32% (58/169; 95% CI: 27.53–41.82%) among women aged 55–64 years and was 31.48% (17/54; 95% CI: 20.46–45.08%) among those aged ≥ 65 years. Other HR-HPV also differed significantly across age groups ($p < 0.001$), whereas HPV16 ($p = 0.225$) and HPV18/45 ($p = 0.181$) did not show significant differences by age group **(**Table 6, Fig. 3**).**

Table 6
Prevalence of high-risk HPV infection and HPV targets by age group

Age group	N	HPV16, n (%)	HPV18/45, n (%)	Other HR-HPV, n (%)	Any HR-HPV, n (%)
< 25	146	5 (3.42; 1.43–7.99)	4 (2.74; 1.03–7.10)	65 (44.52; 36.64–52.69)	68 (46.58; 38.61–54.72)
25–34	402	30 (7.46; 5.26–10.48)	18 (4.48; 2.84–7.00)	129 (32.09; 27.70–36.82)	150 (37.31; 32.71–42.16)
35–44	462	20 (4.33; 2.81–6.62)	9 (1.95; 1.02–3.71)	114 (24.68; 20.95–28.82)	132 (28.57; 24.63–32.87)
45–54	390	22 (5.64; 3.74–8.42)	10 (2.56; 1.38–4.70)	84 (21.54; 17.73–25.91)	104 (26.67; 22.50–31.29)
55–64	169	10 (5.92; 3.21–10.67)	7 (4.14; 1.98–8.45)	47 (27.81; 21.56–35.07)	58 (34.32; 27.53–41.82)
≥ 65	54	1 (1.85; 0.26–12.22)	0 (0.00)	17 (31.48; 20.46–45.08)	17 (31.48; 20.46–45.08)
Total	**1,623**	**88 (5.42)**	**48 (2.96)**	**456 (28.10)**	**529 (32.59)**
P value		**0.225**	**0.181**	**< 0.001**	**< 0.001**

3.6 Factors associated with high-risk HPV positivity

In multivariable logistic regression, age group and district were associated with high-risk HPV positivity. Compared with women aged < 25 years, the odds of high-risk HPV positivity were lower among women aged 25–34 years (aOR 0.66, 95% CI: 0.45–0.97; p = 0.037), 35–44 years (aOR 0.43, 95% CI: 0.29–0.64; p < 0.001), 45–54 years (aOR 0.38, 95% CI: 0.25–0.57; p < 0.001), 55–64 years (aOR 0.55, 95% CI: 0.35–0.88; p = 0.012), and ≥ 65 years (aOR 0.50, 95% CI: 0.26–0.97; p = 0.042). Compared with women from Kaoma District, those from Kalabo had lower odds of high-risk HPV positivity (aOR 0.55, 95% CI: 0.37–0.83; p = 0.005). No significant associations were observed for Luampa, Lukulu, Mitete, Nkeyema, or Sikongo compared with Kaoma District **(**Table 7**).**

Table 7
Factors associated with high-risk HPV positivity among women screened for cervical cancer

Characteristic	cOR (95% CI)	P value	aOR (95% CI)	P value
Age group				
< 25 years	Reference	—	Reference	—
25–34 years	0.68 (0.47–1.00)	0.051	0.66 (0.45–0.97)	0.037
35–44 years	0.46 (0.31–0.67)	< 0.001	0.43 (0.29–0.64)	< 0.001
45–54 years	0.42 (0.28–0.62)	< 0.001	0.38 (0.25–0.57)	< 0.001
55–64 years	0.60 (0.38–0.94)	0.027	0.55 (0.35–0.88)	0.012
≥ 65 years	0.53 (0.27–1.02)	0.057	0.50 (0.26–0.97)	0.042
District				
Kaoma	Reference	—	Reference	—
Kalabo	0.60 (0.40–0.90)	0.014	0.55 (0.37–0.83)	0.005
Luampa	1.14 (0.75–1.74)	0.528	1.18 (0.77–1.81)	0.437
Lukulu	1.11 (0.85–1.46)	0.437	1.13 (0.86–1.49)	0.385
Mitete	0.98 (0.33–2.86)	0.968	0.87 (0.29–2.57)	0.800
Nkeyema	1.27 (0.92–1.74)	0.148	1.30 (0.94–1.80)	0.109
Sikongo	1.18 (0.78–1.79)	0.423	0.99 (0.65–1.52)	0.969

4 Discussion

This study provides contemporary laboratory-based evidence of HR-HPV infection among women screened for cervical cancer across seven districts of Western Province, Zambia. Among 1,623 women tested between May 2025 and June 2026, 529 (32.59%; 95% CI: 30.35–34.92%) had detectable HR-HPV. Infection was most frequent among women aged < 25 years (46.58%) and declined through middle age, reaching 26.67% among women aged 45–54 years, before increasing to 34.32% among those aged 55–64 years. HR-HPV prevalence also varied geographically, from 21.84% in Kalabo to 37.04% in Nkeyema. In adjusted analysis, all age groups had lower odds of HR-HPV positivity than women aged < 25 years, while women from Kalabo had lower odds than those from Kaoma. The predominance of pooled non-16/18/45 HR-HPV targets further demonstrates the breadth of oncogenic HPV infection detected through routine screening in this population.

4.1 Prevalence of high-risk HPV infection

The overall HR-HPV prevalence of 32.59% falls within the range reported across sub-Saharan Africa. A systematic review of 27 studies estimated a pooled HR-HPV prevalence of 34% (95% CI: 29–39%), although heterogeneity between studies was very high (I^2=98.3%), reflecting differences in populations, geographical settings and laboratory methods [9]. The estimate observed in Western Province therefore fits within the broader epidemiological pattern reported for the region.

The finding is also close to recent programme-based evidence from Zambia. In Southern Province, 4,612 women screened between 2021 and 2022 had an HPV prevalence of 35.56%, compared with 32.59% in the present study; the median ages were 39 and 40 years, respectively [10]. The difference of 2.97 percentage points is relatively small despite the studies being conducted in different provinces and periods. However, the Southern Province population included 2,734 women living with HIV (59.28%), whereas HIV status was not available in the present dataset, limiting direct adjustment for this potentially important determinant [10].

Earlier Zambian studies reported substantially higher HPV prevalence, but these studies involved more selected populations. HPV infection was detected in 65% of 70 women in one early Zambian study, while a study restricted to 145 HIV-infected women attending a tertiary centre in Lusaka reported HR-HPV in 90.3% [11, 17]. These estimates should not be interpreted as evidence of a temporal increase in HPV prevalence because the populations differed markedly from the predominantly programme-screened population in the present study. Differences in HIV status, clinical selection, sample size, assay coverage and study setting are likely to account for much of the observed variation. The present estimate is therefore more appropriately interpreted alongside contemporary programme-based studies than with highly selected clinical populations.

The broader African literature also demonstrates substantial variation in HPV prevalence. Among women with normal cervical cytology, pooled HPV prevalence has been estimated at 29.3% overall, although substantially higher estimates have been reported in Southern Africa [18]. Such variation reinforces the importance of generating geographically specific surveillance data rather than assuming that national or regional estimates apply uniformly across populations.

4.2 Factors associated with high-risk HPV positivity

Age was strongly associated with HR-HPV positivity. Prevalence was 46.58% among women aged < 25 years, decreased to 37.31% at 25–34 years, 28.57% at 35–44 years and 26.67% at 45–54 years, then increased to 34.32% at 55–64 years. The overall association was statistically significant (χ^2=26.96, $p < 0.001$), and the adjusted odds of infection were lower in every age category compared with women aged < 25 years.

This pattern is consistent with the established epidemiology of HPV infection, in which prevalence is generally highest among younger women and declines with increasing age. A global analysis of 346,160 women demonstrated a marked peak in HPV prevalence at younger ages, particularly among women aged ≤ 25 years, followed by a decline across subsequent age groups [19]. African pooled estimates

similarly showed HPV prevalence of 48.2% among women aged 15–24 years, 50.5% among those aged 25–34 years, 36.1% among those aged 35–44 years and 31.6% among those aged 45–54 years [18]. The corresponding estimates in Western Province were 46.58%, 37.31%, 28.57% and 26.67%, respectively, indicating a particularly pronounced decline after the youngest age group.

The adjusted analysis quantified this gradient. Relative to women aged < 25 years, the odds of HR-HPV positivity were 34% lower among women aged 25–34 years (aOR 0.66), 57% lower among those aged 35–44 years (aOR 0.43), 62% lower among those aged 45–54 years (aOR 0.38), 45% lower among those aged 55–64 years (aOR 0.55), and 50% lower among those aged ≥ 65 years (aOR 0.50). This pattern is compatible with the predominance of newly acquired, transient HPV infection among younger women, followed by increasing clearance or reduced detection with age. However, the cross-sectional design cannot distinguish newly acquired infection from persistent infection or reactivation.

The increase from 26.67% at 45–54 years to 34.32% at 55–64 years is noteworthy. Secondary increases in HPV prevalence at older ages have been described in several populations, particularly in Africa and the Americas [20–22]. Such patterns may reflect differences in sexual behaviour, persistence or reactivation of previously acquired infection, immune changes, or population-specific screening characteristics, but these mechanisms cannot be established from the present data. The finding nonetheless indicates that HR-HPV infection was not confined to younger women and supports continued screening among women in older age groups.

4.3 Distribution of HPV targets

The distribution of HPV targets was dominated by the pooled other HR-HPV category. Among the 529 HR-HPV-positive women, 456 (86.20%) had other HR-HPV detected, compared with 88 (16.64%) with HPV16 and 48 (9.07%) with HPV18/45. Because these target categories were not mutually exclusive, the proportions cannot be summed and the 86.20% estimate should not be interpreted as the prevalence of a single genotype or a specific genotype group.

The predominance of non-16/18/45 targets is consistent with evidence that carcinogenic HPV types beyond HPV16 and HPV18 contribute substantially to HPV infection in African populations. In a Zambian study of HIV-infected women, HPV52 was detected in 37.2%, HPV58 in 24.1% and HPV53 in 20.7%, while HPV16 and HPV18 were detected in 17.2% and 13.1%, respectively [11]. Another Zambian study detected HPV16 and HPV18 in 21.6% each among 70 women tested [17]. Because those studies used assays reporting individual genotypes whereas the present analysis grouped several targets as other HR-HPV, direct genotype-specific comparisons are not appropriate.

The broader African evidence similarly indicates considerable genotype diversity. Among African women with normal cervical cytology, HPV16, HPV52, HPV35, HPV18 and HPV58 have been identified among the most frequently detected genotypes [18]. In invasive cervical cancer, HPV16 accounted for 61.7% of cases globally, followed by HPV18 at 15.3% and HPV45 at 4.8%, while additional carcinogenic types including HPV31, HPV33, HPV52 and HPV58 accounted for an important proportion of the remaining

cases [23]. The contribution of HPV16 and HPV18 to invasive cervical cancer was estimated at 71.9% in Africa, indicating that a substantial proportion of African cervical cancers is attributable to types other than these two major genotypes [23].

The infection-pattern analysis in Western Province further showed that 395 (74.67%) HR-HPV-positive women had other HR-HPV only, compared with 47 (8.88%) with HPV16 only and 26 (4.91%) with HPV18/45 only. Coinfection involving HPV16 and other HR-HPV occurred in 39 (7.37%) women, while HPV18/45 plus other HR-HPV occurred in 20 (3.78%). Only two women (0.38%) had all three target groups. These findings indicate that most HPV-positive women in this screening population would not be characterised solely by the presence of HPV16 or HPV18/45.

This distinction is relevant to HPV-based screening because detection of HR-HPV establishes infection but does not confer the same immediate risk across all genotypes. WHO screening strategies therefore incorporate HPV testing with risk-based triage and, where available, partial genotyping to distinguish women at different levels of risk for cervical precancer [24]. The high frequency of pooled other HR-HPV in this study supports maintaining broad HR-HPV detection rather than restricting screening to HPV16 and HPV18.

4.4 Geographic variation of high-risk HPV

HR-HPV prevalence varied significantly across the seven districts, ranging from 21.84% in Kalabo to 37.04% in Nkeyema (χ^2=12.70, p = 0.048). Prevalence was 31.73% in Kaoma, 34.71% in Luampa, 34.12% in Lukulu, 31.25% in Mitete and 35.48% in Sikongo. After adjustment for age, women from Kalabo had 45% lower odds of HR-HPV positivity than women from Kaoma (aOR 0.55, 95% CI: 0.37–0.83; p = 0.005), while differences involving the other districts were not statistically significant.

Geographical heterogeneity in HPV prevalence has been documented across Africa, where differences in population structure, sexual behaviour, HIV prevalence, screening access and laboratory methods can contribute to variation between settings [12, 25–27]. The present findings demonstrate that such heterogeneity can also occur within a single provincial screening network. The approximately 15-percentage-point difference between Kalabo and Nkeyema indicates that aggregating all districts into a single provincial estimate may obscure meaningful local variation.

The geographical association should nevertheless be interpreted cautiously. District of residence is a marker of place rather than a direct biological determinant of HPV infection. The present dataset did not contain information on sexual behaviour, parity, contraceptive use, HIV status, HPV vaccination, socioeconomic characteristics, previous screening or other factors that could explain the observed differences. Differences in the characteristics of women accessing screening and, in the intensity, or reach of screening services may also contribute. The particularly small Mitete sample of 16 women resulted in wide confidence intervals and limits precision for that district.

The district-specific target distribution provides additional context. HPV16 prevalence did not differ significantly across districts (χ^2=5.64, p = 0.465), nor did HPV18/45 (χ^2=9.98, p = 0.126), whereas other

HR-HPV differed significantly (χ^2=14.34, p = 0.026). This suggests that the geographical variation in overall HR-HPV prevalence was driven predominantly by differences in the pooled other HR-HPV category rather than by HPV16 or HPV18/45. This observation should be interpreted as a programme-level epidemiological signal rather than evidence of district-specific genotype ecology because individual genotypes within the pooled category were not resolved.

4.5 Implications for cervical cancer prevention

The findings have direct relevance to cervical cancer prevention in Western Province. Nearly one in three women screened had HR-HPV detected, and the burden was particularly high among women younger than 25 years. At the same time, substantial positivity persisted among women in older age groups, including 34.32% among those aged 55–64 years. The distribution across districts indicates that screening programmes operating at provincial level may benefit from monitoring local uptake and positivity rather than relying exclusively on aggregated estimates.

The predominance of other HR-HPV targets also has implications for screening strategy. Although HPV16 and HPV18/45 warrant particular attention because of their association with cervical cancer risk, 86.20% of HR-HPV-positive women in this study had at least one other HR-HPV target detected. Broad molecular detection therefore remains important for identifying women who may otherwise be missed by approaches focused only on HPV16 and HPV18. However, because the assay grouped several genotypes together, the present data cannot determine which individual non-16/18/45 genotypes accounted for the largest proportion of infections or which had the greatest contribution to precancer risk.

The use of routinely generated laboratory data also demonstrates the value of molecular surveillance within cervical cancer screening programmes. Laboratory information systems can provide geographically disaggregated estimates of HR-HPV positivity and target distribution without requiring a separate population-based survey. Such surveillance could support monitoring of screening coverage, identification of areas with lower screening uptake and evaluation of changes in HPV positivity over time.

4.6 Strengths and limitations

This study has several strengths. It included 1,623 women from seven districts and provides contemporary evidence from a provincial HPV screening programme rather than a highly selected tertiary clinical population. The use of a common molecular testing platform and individual-level analysis enabled assessment of overall HR-HPV prevalence, target distribution, age-specific patterns and district-level variation within the same laboratory network. The study also provides evidence from Western Province, where geographically disaggregated HPV data remain limited.

Several limitations should be considered. The retrospective cross-sectional design prevents assessment of HPV acquisition, persistence or clearance and does not establish temporal or causal relationships. The study population consisted of women who accessed cervical cancer screening and therefore may

not represent women who had not been screened. Important potential determinants of HPV infection, including HIV status, HPV vaccination, sexual behaviour, reproductive history and socioeconomic characteristics, were unavailable. The assay also reports several high-risk HPV types as a pooled category, limiting detailed genotype-specific interpretation. Finally, the small number of women from Mitete limits the precision of its prevalence estimate.

5 Conclusion

Among women screened for cervical cancer in seven districts of Western Province, Zambia, 32.59% had detectable HR-HPV. Infection was most frequent among women younger than 25 years, declined through middle age, and increased again among women aged 55–64 years. HR-HPV prevalence also varied geographically, with Kalabo having lower adjusted odds of infection than Kaoma. Most HR-HPV-positive women had pooled non-16/18/45 targets, highlighting the contribution of a broader range of carcinogenic HPV types to the screening burden. These findings provide contemporary district-level evidence to support monitoring of HPV screening services and inform cervical cancer prevention planning in Western Province.

Abbreviations

HR-HPV: High-Risk Human Papillomavirus

PCR: Polymerase Chain Reaction

HPV: Human Papillomavirus

CI: Confidence Interval

aOR: adjusted Odds Ratio

IQR: Interquartile Range

WHO: World Health Organization

DNA: Deoxyribonucleic Acid

SADCAS: Southern African Development Community Accreditation Service

HPCZ: Health Professions Council of Zambia

LIS: Laboratory Information System

cOR: crude Odds Ratio

Declarations

Ethics approval

Ethical approval for this study was obtained from the Levy Mwanawasa Medical University Research Ethics Committee, Zambia (approval number: LMMU-REC 0000718-25). The study was subsequently submitted to the National Health Research Authority, Zambia, and registered under reference number NHRA-2591/16/08/2025. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Consent to participate

The requirement for informed consent was waived by the Levy Mwanawasa Medical University Research Ethics Committee because the study involved retrospective analysis of routinely collected, de-identified programme data and posed no risk to participants. The waiver also applied to participants younger than 16 years of age; therefore, parental or legal guardian consent was not required.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

FP conceived the study, developed the study design, conducted data analysis, and drafted the manuscript. MM (Michael Mokha) and MM (Mukubesa Matakala) contributed to the study design, data interpretation, and critical revision of the manuscript. PS contributed to data interpretation and critical revision of the manuscript. DKM contributed to study supervision, data interpretation, and critical revision of the manuscript. All authors read and approved the final manuscript.

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Not applicable.

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Figures

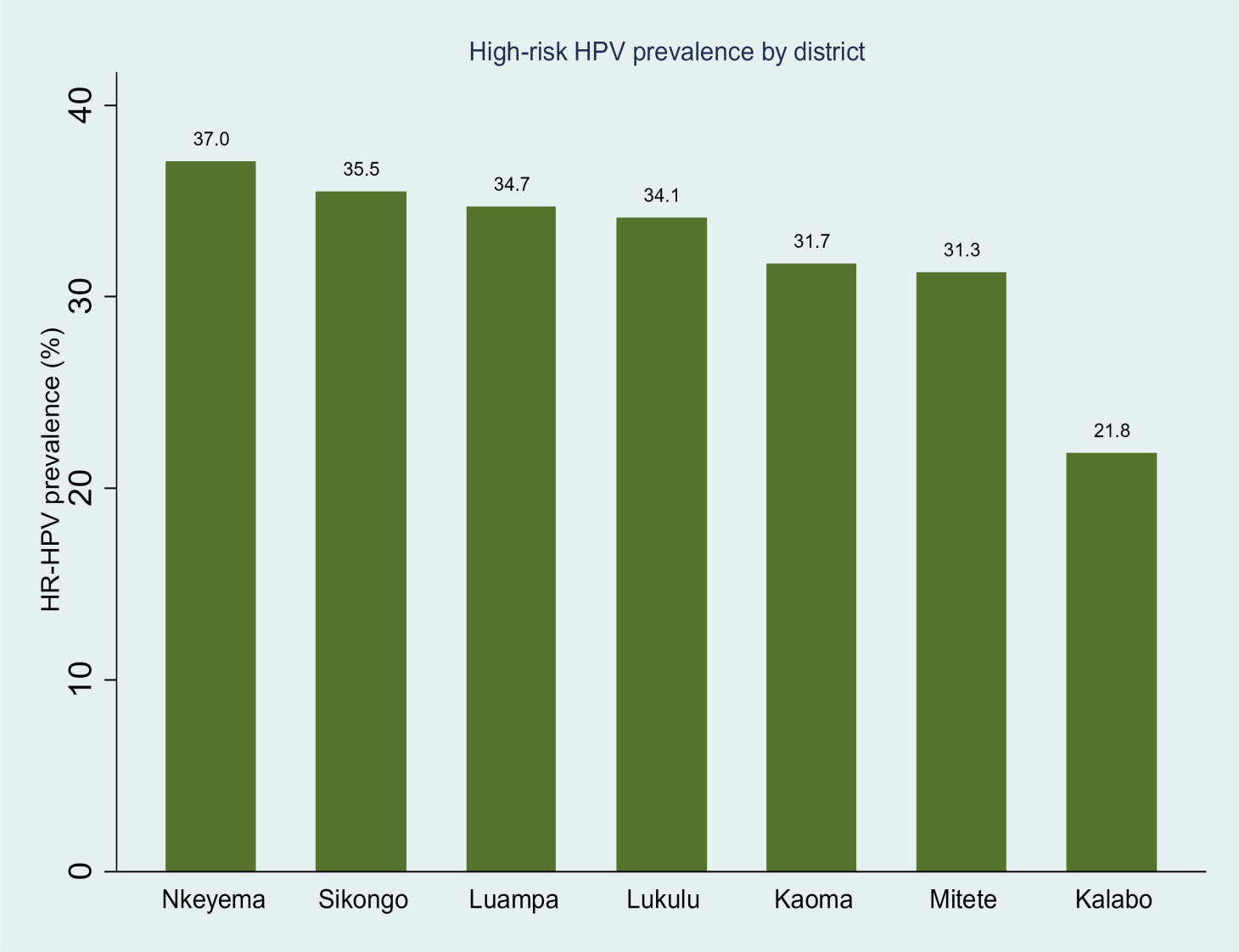


Figure 1

High-risk HPV prevalence by district

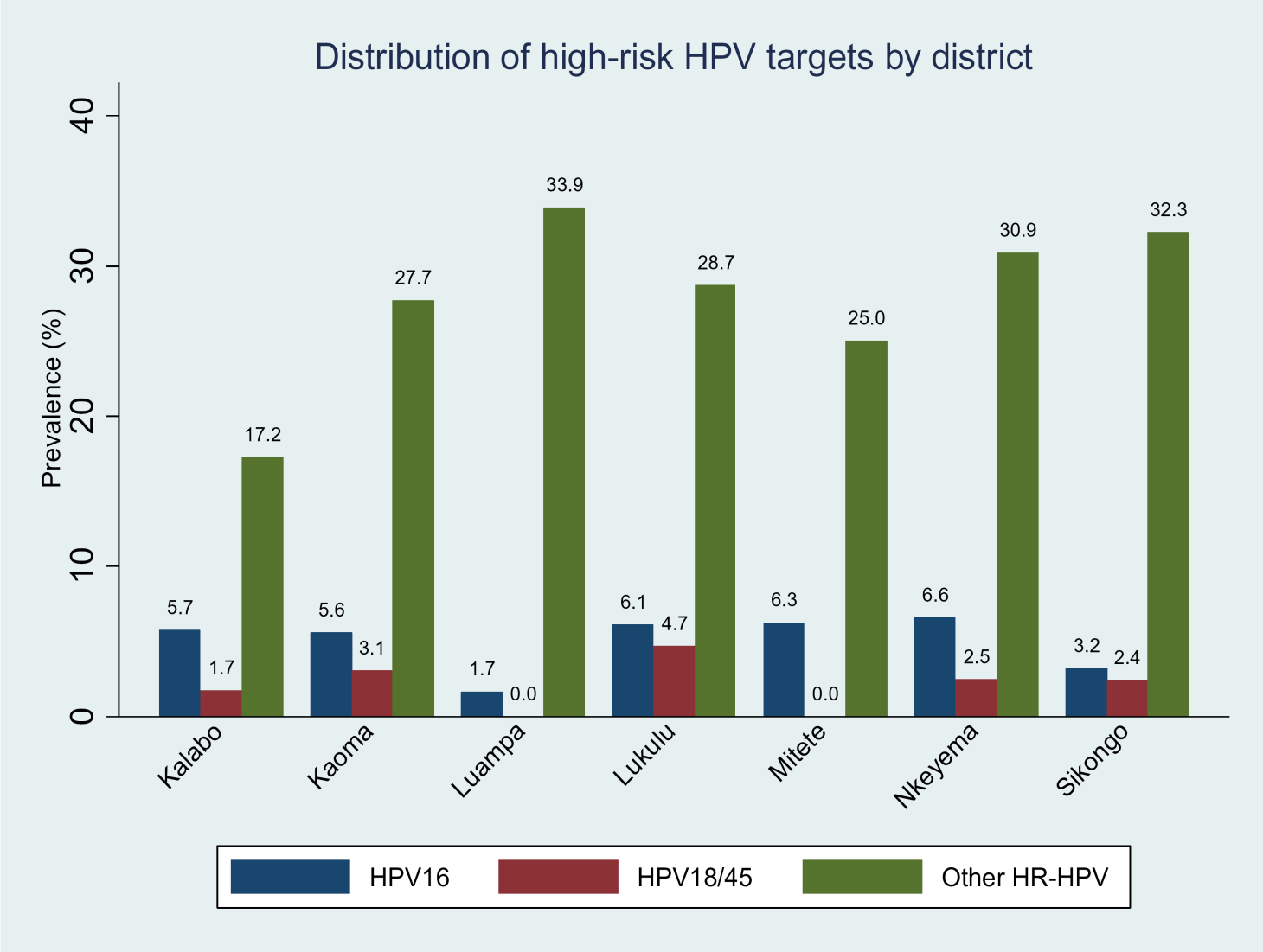


Figure 2

Distribution of HPV16, HPV18/45, and other high-risk HPV by district

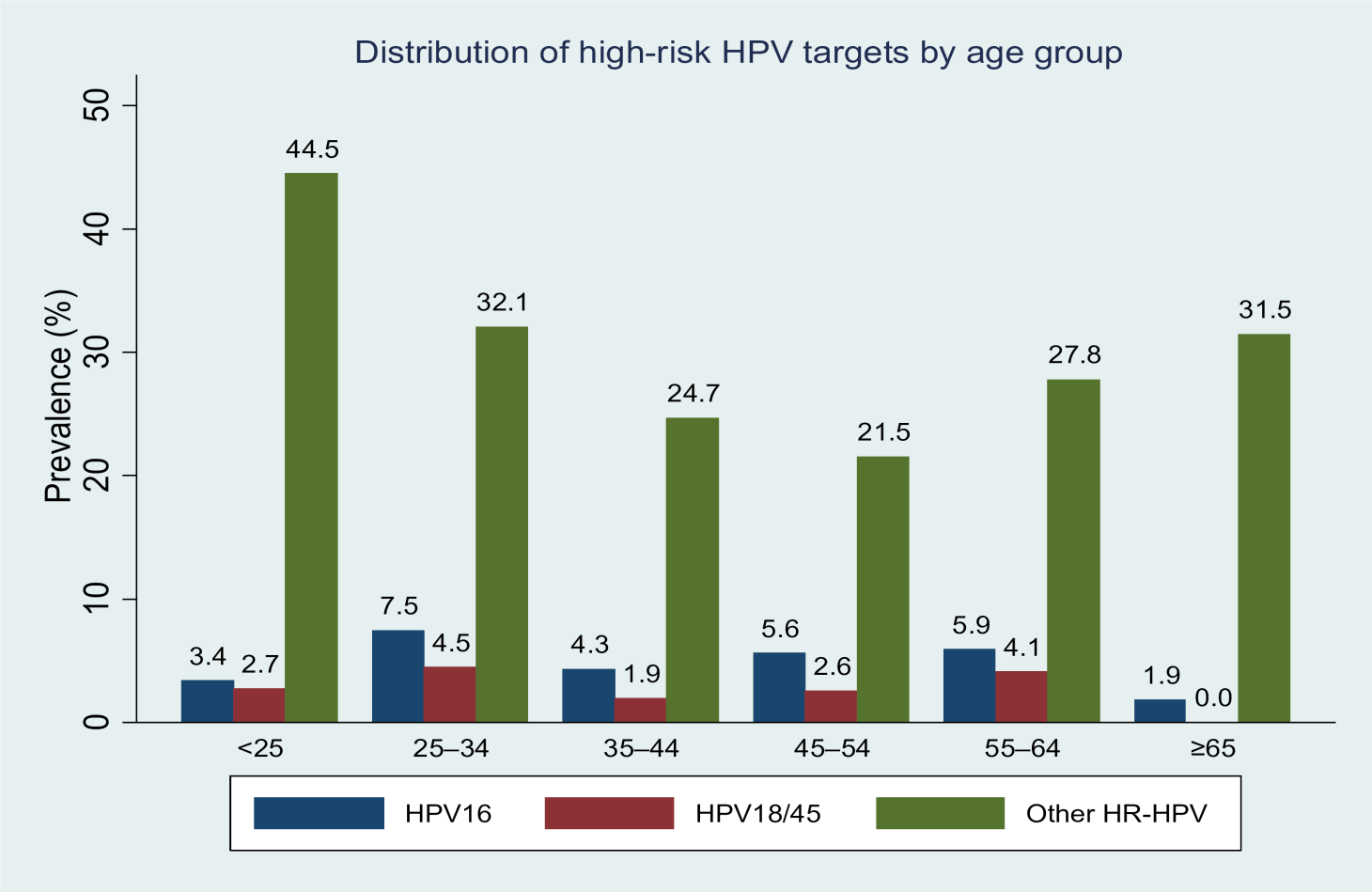


Figure 3

Distribution of high-risk HPV targets by age group