SENTINEL SURVEILLANCE OF HUMAN PAPILLOMAVIRUS GENOTYPES AMONG YOUNG WOMEN ATTENDING PUBLIC HEALTHCARE FACILITIES IN SOUTH AFRICA, 2017

Zizipho Mbulawa[1-4], Tendesayi Kufa-Chakezha [2,5], Anna-Lise Williamson[1, 3, 4], Ranmini Kularatne[2,6]

[1]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa
[2]Center for HIV and STIs, NICD
[3]Division of Medical Virology, Department of Pathology, University of Cape Town, South Africa
[4]UCT-MRC Clinical Gynaecological Cancer Research Centre, University of Cape Town, South Africa
[5]School of Public Health, University of the Witwatersrand, Johannesburg, South Africa
[6]Deparment of Clinical Microbiology & Infectious Diseases, University of the Witwatersrand, Johannesburg, South Africa

Executive summary

Human papillomavirus (HPV), a sexually transmitted virus, is associated with a number of cancers, with cervical cancer being the most important. HPV vaccination should occur prior to exposure to HPV infection as the current HPV vaccines are prophylactic. As part of the HPV vaccination strategy in South Africa it is important to have baseline data on HPV in adolescent girls and young women so that the impact of vaccination can be assessed. Therefore, women between the ages of 18 and 20 years attending public healthcare facilities in South Africa during 2017 were recruited to participate in HPV surveillance. Cervical swabs were taken for HPV genotyping. The overall HPV prevalence was 64.4% (181/281). HPV-16 (11.7%, 33/281) was the most commonly detected HPV type. When compared with HIV-negative women, HIV-positive women not on antiretroviral treatment were found to have a higher risk of HPV infection (RR: 1.32, 95% CI: 1.10-1.58, p=0.003), but this was not observed among those on antiretroviral treatment (RR: 1.10, 95% CI: 0.78-1.57, p=0.585). HPV types targeted by the Cervarix® HPV vaccine, (HPV-16/18, currently used in the South African school-based HPV vaccination program), were detected in 16.7% (47/281) of women, while those found in the Gardasil (HPV-6/11/16/18) were detected in 23.8% (67/281) of women; and those in the Gardasil® 9 (HPV-6/11/16/18/31/33/45/52/58) were detected in 35.9% (101/281) of women. The high prevalence of HPV types targeted by Gardasil® 4 and Gardasil® 9 HPV encourages the introduction of vaccines targeting a higher number of HPV types in South Africa.

Introduction

In 2014, South Africa introduced a school-based vaccination with Cervarix® against human papillomavirus (HPV)16/18 in public schools. Cervarix® vaccination is given in two doses (six-months apart) within the academic calendar. The school-based HPV vaccination program currently targets girls aged 9 or older in grade 4. HPV is sexually transmitted and HPV vaccination should therefore be given prior to sexual debut. The median age of sexual debut among South African women ranges between 16 and 19 years.[1-4] HPV vaccination coverage of up to 93% has been observed within the HPV school-based vaccination program.[5]

The aim of HPV vaccination is to reduce the incidence of infection that will result in a reduction of HPV-associated cancers, including those of the cervix, anus, vulva, vagina, penis and oropharynx. Cervical cancer is the most common cancer in South African women between the ages of 15 and 44 years with an age standardized rate (ASR) of 30.2 per 100 000 population. HPV16 (50.7%), HPV18 (13.5%), HPV33 (7.3%), HPV35 (6.0%) and HPV45 (5.6%) are the most common high-risk (HR) HPV types observed in cervical cancer cases in South Africa.[5]

HPV typing data in an unvaccinated population is important to inform vaccination campaigns as well as to establish a baseline to monitor the impact on prevalence of HR-HPV types after vaccination. This report is an update of previous study by Mbulawa et al.[6], and reports on overall HPV prevalence, prevalence of HPV genotypes and prevalence of HPV types targeted by current HPV vaccines, namely, Cervarix®, Gardasil® and Gardasil® 9, among young women attending public healthcare facilities in South Africa during 2017. Factors associated with HPV infection, particularly HIV infection and associated ART use, were also investigated.

Methods

Participant recruitment, data and specimen collection. A total of 286 sexually active young women between the ages of 18 -20 years was recruited from the family planning units of primary healthcare clinics (PHCs) in four provinces namely, Gauteng (Alexandra community health centre), Free State (Heidedal clinic), Western Cape (Site C Youth Clinic, Khayelitsha) and Eastern Cape (Zwide clinic) during 2017. During the informed consent process, a detailed description of study procedures was discussed with each of the participants. A questionnaire designed to record demographic, behavioural and clinical data was administered by surveillance nurses. An endocervical swab for HPV testing was collected by a surveillance nurse during speculum examination. Endocervical swabs were transported at 2-8°C to the Centre for HIV and STIs of the National Institute for Communicable Diseases (NICD) laboratories in Johannesburg and Cape Town where HPV testing was performed.

HPV testing and genotyping. DNA from endocervical swabs was extracted by a MagNA Pure Compact (Roche) using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche). DNA was stored at -80°C until processing. HPV genotyping was performed on extracted DNA using the Roche Linear Array HPV genotyping test which identifies 37 different HPV genotypes; HR-HPV types included HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58 and -59; probable or possible HR-HPV types included HPV-26, -53, -66, -67, -68, -70, -73 and -82; and low-risk (LR) HPV types included HPV-6, -11, -40, 42, -54, -55, -61, -62, -64, -69, -71, -72, -81, -83, -84, -89 (HPV-CP6108) and HPV-82. HPV-52 was recorded positive only in the absence of HPV-33, -35 and -58 due to the combined probe used for these four types. The Roche Linear Array HPV genotyping test also amplifies the β-globin gene as an internal control for cell adequacy, extraction and amplification in each specimen. Samples with negative β-globin results were considered invalid and excluded from the analysis.

Data management and statistical analysis. Completed questionnaires were couriered to the data centre at the NICD, entered into an Access database and exported into Stata® 14.2 [Stata Corporation, College Station, Texas, United States] for analysis. Descriptive statistics were used to describe demographic, behavioural and clinical characteristics of enrolled participants as well as HPV prevalence. Binomial univariable and multivariable regression was used to determine factors associated with the detection of any HPV genotypes. Variables with p-values < 0.2 in univariable

analyses were included in the multivariable analysis. The target sample size per site was 100 participants, estimated using the Wald test, assuming a prevalence of any HPV infection between 58% and 70% power of 80% and α-level of 0.05. Assuming a power of 80% and α-level of 0.05, a sample size of 286 was required to measure a prevalence of any HPV genotype of 64% +/- 4% across all the sites.

Results

Description of surveillance population. A total of 286 women between the ages of 18 and 20 years (median 19 years) participated in HPV surveillance. The majority were enrolled at the Western Cape site – 80(28%), followed by the Gauteng Province site - 77 (26.9%), the Eastern Cape site – 69 (24.1%) and the Free State site - 60 (21%). The median age at first sex was 17 years (IQR 16-17 years). The majority of women self-reported heterosexual activity (99.3%, 280/282). Only 32.2% (92/194) reported the use of a condom at last sexual encounter. Vaginal sex was the most common sex act reported by participants (97.9%, 278/284), followed by oral sex (11.3%, 32/284) and receptive anal sex (2.5%, 7/284). At least one in five participants - 21.2% (50/236) - was HIV-positive; with 16 of the 50 (32%) reporting that they were taking antiretroviral treatment (ART).

HPV prevalence and impact of HIV status. Of the 286 women enrolled, 281 (98.3%) had a valid HPV genotype result. The overall HPV prevalence was 64.4% (181/281). Infection with multiple (2-10) HPV types was more common ((42.0% (118/281)) than single HPV infections (22.4%, 63/281, p<0.001, Figure 1). HPV-16 (11.7%, 33/281) was the most commonly detected HPV type (Figure 2). HIV-positive women not on ART had a higher risk of HPV infection compared to HIV-negative women (RR: 1.32, 95% CI: 1.10-1.58, p=0.003). A slightly increased but statistically insignificant risk of HPV infection was also observed among HIV-positive women who reported taking ART compared to HIV negative women (RR: 1.10, 95% CI: 0.78-1.57, p=0.585, Table 1).

Prevalence of HPV types targeted by bivalent, quadrivalent and nonavalent HPV vaccines. HPV types targeted by the bivalent HPV vaccine (HPV-16/18) were detected in 16.7% (47/281) of women, while those found in the quadrivalent vaccine (HPV-6/11/16/18) were detected in 23.8% (67/281) of women, and those in the nonavalent vaccine (HPV-6/11/16/18/31/33/45/52/58) were detected in 35.9% (101/281) of women (Figure 3). The bivalent HPV vaccine (Cervarix®) is currently being used in school-based vaccination in South Africa. HR-HPV types that are not targeted by bivalent, quadrivalent or nonavalent HPV vaccines, and not cross-protective, were observed in 18.9% (53/281) of the women (HPV-35/39/56/59, Figure 3).

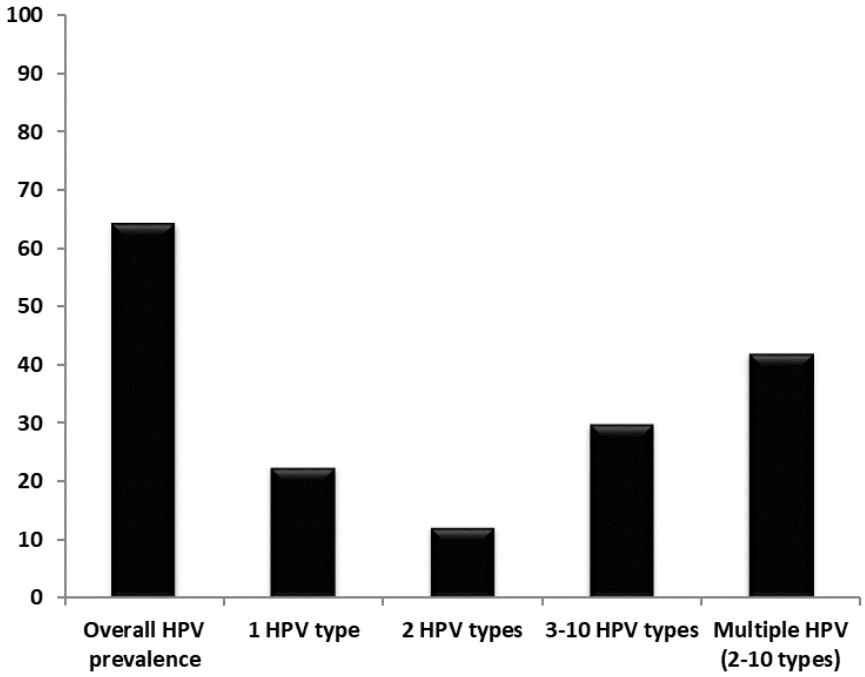

Figure 1. Prevalence of overall human papillomavirus (HPV), single infection and multiple infections among young women attending GERMS sentinel sites in South Africa, 2017.

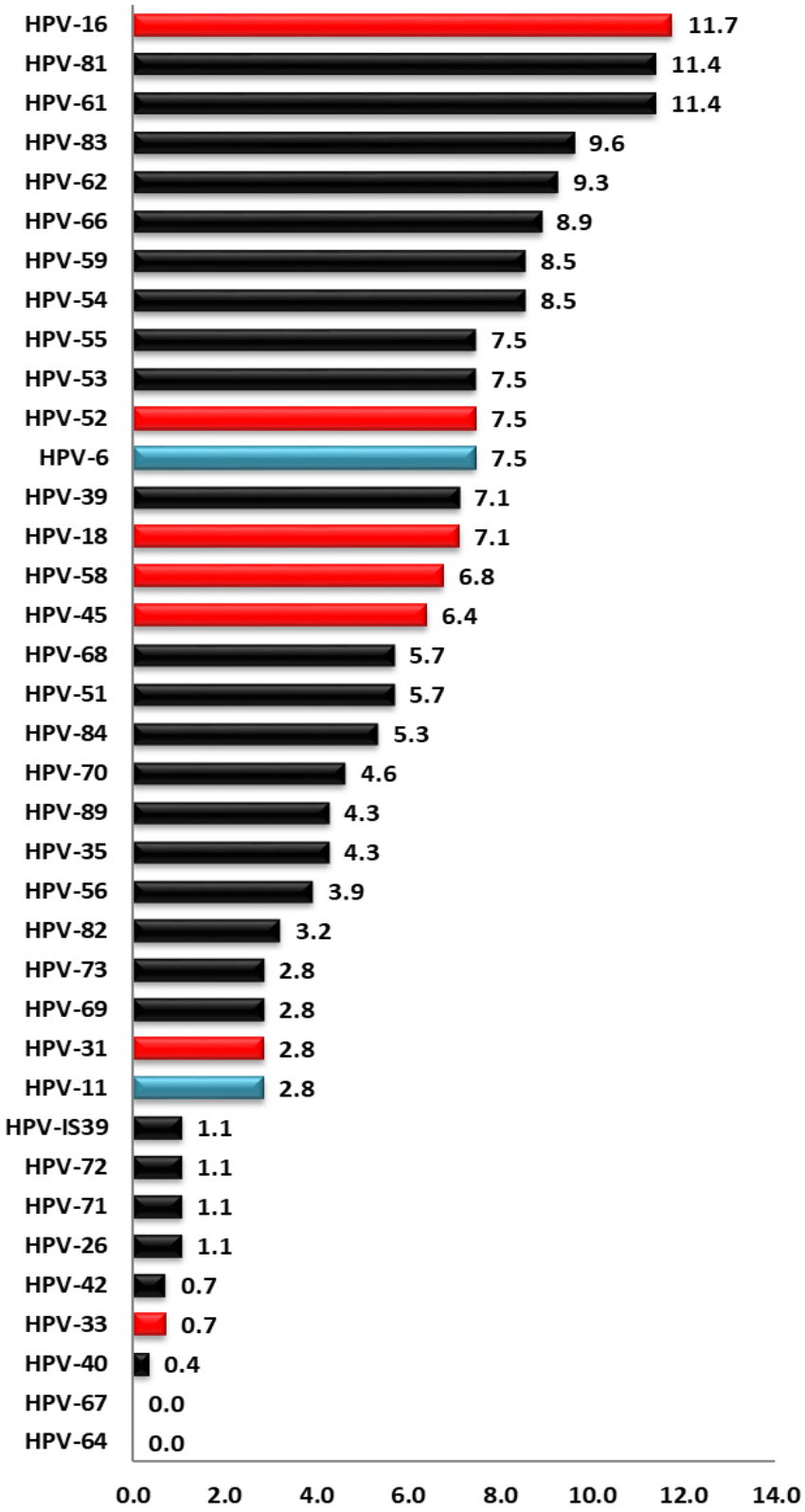

Figure 2. Prevalence of different human papillomavirus (HPV) genotypes detected among young women attending GERMS sentinel sites in South Africa, 2017. HPV types targeted by three current HPV vaccines are indicated by red bars (HR-HPV-16, -18, -31, -33, -45, -52 and 58) and blue bars (LR: HPV-6 and -11).

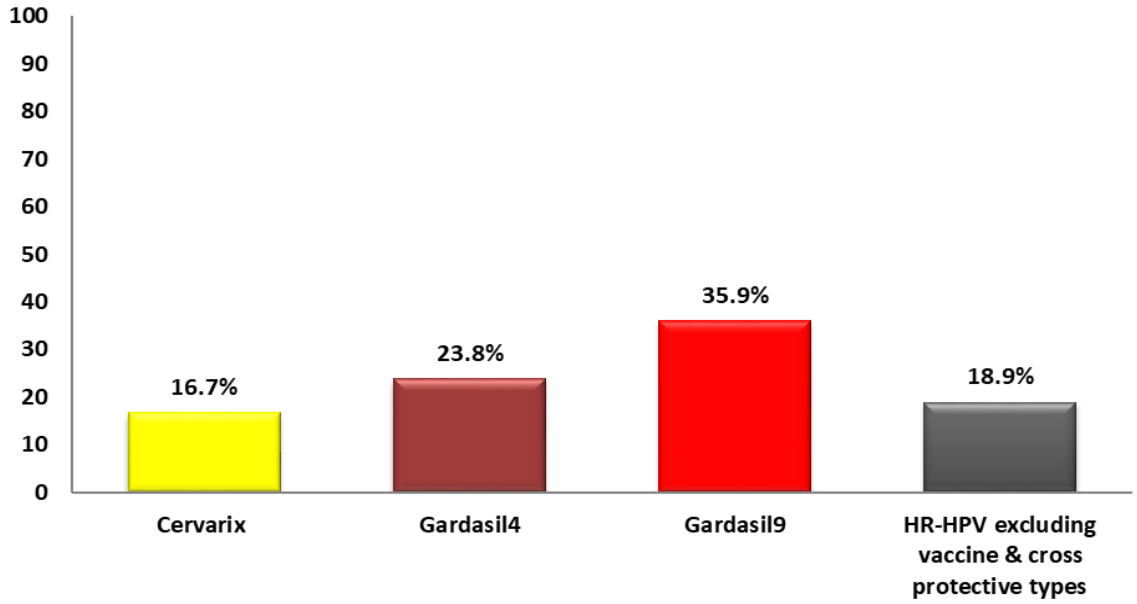

Figure 3. Prevalence of human papillomavirus (HPV) types targeted by current HPV vaccines and HR-HPV non-vaccine types detected among young women attending GERMS sentinel sites in South Africa, 2017.

Table 1. Factors associated with the prevalence of any human papillomavirus (HPV) genotype among enrolled women (N= 281), South Africa, 2017.

Factor	HPV n/N	%HPV infection	Univariable RR (95% CI)	p-value	Multivariable RR (95% CI)	p-value
Age						
18 years	46/74	58.2	0.96 (0.77- 1.19)	0.692		
19 years	57/87	65.5	1.01 (0.82- 1.23)	0.938		
20 years	78/120	65.0	reference			
HIV status						
Negative	141/231	61.0	reference		reference	
Positive, on ARVs	11/16	68.8	1.13 (0.80- 1.59)	0.500	1.10 (0.78 - 1.57)	0.585
Positive, not on ARVs	29/34	85.3	1.38 (1.17- 1.66)	**<0.001**	1.32 (1.10- 1.58)	**0.003**
Condom use						
Yes	129/190	67.9	reference		reference	
No	52/91	57.1	0.84 (0.69- 1.03)	0.096	0.90 (0.73- 1.10)	0.321
Age at first sex						
17-20 years	91/139	65.5	reference			
≤16 years	87/134	64.9	0.99 (0.83- 1.18)	0.925		
missing	3/8	37.5	0.57 (0.23- 1.51)	0.226		
Sex Act						
Vaginal only	158/240	65.8	reference			
Vaginal &/receptive anal & /oral	20/34	58.8	0.89 (0.66- 1.20)	0.455		
missing/none	3/7	42.9	0.65 (0.28- 1.54)	0.328		
Study site						
Inland Provinces (GP/FS)	80/135	59.3	reference		reference	
Cape Provinces (EC/WC)	101/146	69.2	1.17 (0.98- 1.39)	0.086	1.11 (0.93- 1.32)	0.25

ARVs=Antiretrovirals. GP=Gauteng Province. FS=Free State Province. EC=Eastern Cape Province. WC=Western Cape Province

Discussion and conclusions

High overall HPV prevalence (64.4%) was observed among young women attending family planning services at primary healthcare clinics. The prevalence of the most common HPV type in HPV-associated cancers, HPV-16, was 11.7%. It is well documented that HIV infection increases the risk of HPV acquisition and persistence. In this study, HIV-infected women not on ART were found to have higher risk of HPV infection compared to HIV-negative women, but this was not observed among those women on ART. A systematic review by Kelly et al.[7] reported that HIV infected women on ART had lower prevalence of HR-HPV than those not on ART. This may be as a consequence of early ART initiation and sustained adherence which results in functional mucosal immune reconstitution and HPV clearance. All HIV positive young women should be initiated on ART as early as possible to reduce direct morbidity and mortality from HIV infection and also to promote HPV clearance and prevent progression of HPV infection to cervical intraepithelial neoplasia and cancer of the cervix.

The study was conducted at a few surveillance sites in four provinces, limiting the generalisability of the current study findings to other settings and provinces. Even though none of the facilities met their target sample size of 100 participants per site, a reasonable sample size was achieved overall. Strengths of this study include the fact that participants were recruited from geographically diverse locations, and the availability of risk factor data. HPV-16/18 targeted by Cervarix® were detected in 16.7% women, while HPV-6/11/16/18/31/33/45/52/58 targeted by Gardasil® 9 were detected in 35.9% of women. The high prevalence of HPV types targeted by Gardasil® 9 was previously observed in NICD GERMS-SA HPV surveillance that was conducted in young among women accessing family planning services in Gauteng, Mpumalanga, KwaZulu-Natal and North West provinces between 2015 and 2016.[6] These observations encourage the introduction of vaccine targeting high numbers of HPV types such as Gardasil® 9 in South Africa.

In conclusion, this surveillance programme provides useful HPV baseline prevalence data for assessing the effectiveness of HPV vaccination, i.e. by enabling comparisons over time to detect significant changes in the prevalence of HR-HPV types (both vaccine and phenotypically related non-vaccine HPV genotypes). It also enables monitoring for genotype replacement and guides the formulation of enhanced HPV vaccination strategies, including catch-up HPV vaccination programmes.

Acknowledgements

We thank the all the young women who kindly participated in the surveillance, the clinical staff at the sentinel sites and the NICD GERMS-SA surveillance team who recruited participants and collected specimens. This surveillance was supported by grants from South African Research Chairs Initiative of the Department of Science and Technology and funding from the National Department of Health.

References

1. Pettifor AE, Rees HV, Kleinschmidt I, Steffenson AE, MacPhail C, Hlongwa-Madikizela L, et al. Young people's sexual health in South Africa: HIV prevalence and sexual behaviors from a nationally representative household survey. *Aids*. 2005;19(14):1525-34.
2. Jaspan HB, Berwick JR, Myer L, Mathews C, Flisher AJ, Wood R, et al. Adolescent HIV prevalence, sexual risk, and willingness to participate in HIV vaccine trials. *J Adolesc Health.* 2006;39(5):642-8.

3. McGrath N, Nyirenda M, Hosegood V, Newell ML. Age at first sex in rural South Africa. *Sexually transmitted infections*. 2009;85 Suppl 1:i49-55.
4. Bruni L, Barrionuevo-Rosas L, Albero G, Serrano B, Mena M,Gómez D, Muñoz J, Bosch FX, deSanjosé S. ICO/ IARC Information Centre on HPV and Cancer (HPV Information Centre). *Human Papillomavirus and Related Diseases in South Africa*. Summary Report 27 July 2017.
5. Jordaan S, Michelow P, Richter K, Simoens C, Bogers J. (2016). **A** review of cervical cancer in South Africa: previous, current and future. *Health Care Curr Rev*, 4, p. 180.
6. Mbulawa, Z.Z.A, Kularatne, R., Kufa-Chakezha, T., & Williamson, A.-L. (2017). Sentinel surveillance of human papillomavirus genotypes among patients attending public healthcare facilities in South Africa, 2015-2016. *Communicable Disease Surveillance Bulletin*, Vol 15, Issue 2, page 72-77
7. Kelly, H., Weiss, H. A., Benavente, Y., de Sanjose, S. & Mayaud, P. (2018). Association of antiretroviral therapy with high-risk human papillomavirus, cervical intraepithelial neoplasia, and invasive cervical cancer in women living with HIV: a systematic review and meta-analysis. *The lancet HIV* 5, e45-e58.