Unusual and unique distribution of anal high-risk human papillomavirus (HR-HPV) among men who have sex with men living in the Central African Republic

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Abstract

Background
High-risk (HR) human papillomavirus (HPV) infection remains a great concern in relation to African men who have sex with men (MSM), especially those infected with HIV. The prevalence of HR-HPV and associated risk factors was estimated in a cross-sectional observational study covering MSM living in Bangui, Central African Republic.

Methods
MSM receiving care at the Centre National de Référence des Infections Sexuellement Transmissibles et de la Thérapie Antirétrovirale, Bangui, were included. HIV serostatus and socio-demographic and behavioral characteristics were collected. HPV DNA was detected and genotyped on anal swabs using Anyplex™ II HPV28 test (Seegene, South Korea), and HSV DNA by in-house real-time PCR. Logistic regression analyses were used to determine risk factors associated with HPV outcomes.

Results
42 MSM (mean age, 23.2 years; range, 14–39) including 69.1% HIV-1-positive and 30.9% HIV-negative were prospectively enrolled. The prevalence of anal HPV was 69.1%,
including 82.7% of HR-HPV which were multiple in 52.0%. The most prevalent genotypes were HPV-35, HPV-58, HPV-59 and HPV-31. While, HPV-16 and HPV-18 were present in a minority of samples. Multiple HR-HPV infection was more frequent in HIV-positive MSM (41.4%) with 2.7 genotypes per anal samples than in HIV-negative (7.7%) with 1.5 genotypes per anal samples. HPV types included in the prophylactic Gardasil-9® vaccine were detected in 68.9% of specimens and HPV-58 was the most frequently detected. MSM infected by HPV-16 and HPV-18 were all infected by HIV-1. Few anal swabs (11.9%) contained HSV-2 DNA without relationship with HPV detection. Condomless receptive anal intercourse was the main risk factor to being infected with any type of HPV and condomless insertive anal intercourse was significantly less associated with HPV contamination than receptive anal intercourse (Odd ratio = 0.02).

Conclusion
MSM in Bangui are at-risk of HIV and HR-HPV anal infections. The unusual distribution of HPV-35 as predominant HPV suggests possible geographic specificities in the molecular epidemiology of HR-HPV in sub-Saharan Africa. Scaling up prevention strategies against HPV infection and related cancers adapted for MSM in Africa should be prioritized. Innovative interventions should be conceived for the MSM population living in Bangui.

Introduction
Men who have sex with men (MSM) in sub-Saharan Africa constitute a core group for several sexual transmitted infections (STI), including human immunodeficiency virus (HIV) [1–4], human papillomavirus (HPV) and herpes simplex virus type 2 (HSV-2) infections [5–8]. HPV infection is the most common viral STI in the world and high-risk oncogenic (HR)-HPV genotypes are responsible for 7.7% of all cancers in developing countries [9,10]. Therefore, anal HR-HPV infection is a steady increasing health problem for MSM because it causes anal cancer. Indeed, MSM are about 20 times more likely susceptible than heterosexual men to develop HPV-related anal cancer and HIV-infected MSM are at an even greater risk [11,12]. Indeed, HIV infection is considered as an independent risk factor strongly associated with increased risk for acquiring HR-HPV anal infection [13–15]. In vitro interactions between HSV-2 and HPV suggest that HSV-2 infection could constitute a cofactor of anal carriage for HPV infection [16], especially in the epidemiological context of sub-Saharan Africa, where HSV-2 infection is highly prevalent [17,18], and constitutes the first cause of genital ulcer [17–19].

The burden of HPV infection in MSM living in sub-Saharan Africa has nonetheless been poorly documented [8]. However, recent reports from South Africa [6] and Nigeria [7] emphasize very high prevalences of anal HR-HPV infection, ranging from 57.6 to 70.1%, in MSM living in sub-Saharan Africa, especially in those co-infected with HIV [6,7]. The reported prevalences of anal HR-HPV DNA among MSM living in sub-Saharan Africa appear higher than those usually recorded in studies conducted in developed countries, which range from 20.9% to 65% [20–23]. Anal HR-HPV in African MSM was strongly associated with high-risk sexual behaviors such as having sex with men only, engaging in group sex and practicing condomless receptive anal intercourse [6,7]. Interestingly, a wide diversity of predominant HPV genotypes was observed in South African and Nigerian studies, suggesting the possibility of unique spatial distributions of HPV diversity by regions within sub-Saharan Africa [6,7]. Thus, Müller
et al. described in South Africa a distribution quite similar to that commonly observed throughout the world with HPV-16 as predominant genotype [6]. In contrast, Nowak et al. depicted in Nigeria an atypical distribution profile with the non-vaccine HR-HPV-35 as the predominant genotype circulating in MSM [7]. Although limited, these observations highlight that MSM in sub-Saharan Africa constitute a high-risk core group for HR-HPV infection and that the distribution of the main HPV genotypes involved in anal cancers in African MSM could be relatively different from that generally observed. Finally, in order to implement effective HPV vaccine-based prevention adapted to every sub-Saharan African region, it is important to establish the molecular distribution of predominant HR-HPV genotypes circulating in African MSM.

Little data is available on MSM living in the Central African Republic (CAR). One recent preliminary serosurvey conducted on MSM in Bangui highlighted that MSM are an identifiable core group accumulating high-risk sexual behaviors for STI, and high prevalence of HIV (25%), hepatitis B (17%) and syphilis (4%) [24]. Herein, we designed a cross-sectional study to assess the prevalence and type distribution of anal HPV infection and associated risk factors, including sexual behavior and HSV-2 infection, in a population of HIV-infected and HIV-uninfected MSM living in Bangui, the capital city of the CAR.

**Material and methods**

**Study population, medical interventions and data collection**

The Centre National de Référence des Infections Sexuellement Transmissibles et de la Thérapie Antirétrovirale (CNRIST/TAR) of Bangui includes both care for general population and specific care towards the MSM population of Bangui. MSM regularly attend the STI clinic for HIV and STI screening and care, to receive specific treatment, HIV counseling and for those positive, for HIV global support. For purposes of the study, a specific strategy involving peer educators was adopted in order to confirm the accuracy of homosexuality of the included MSM attending the CNRIST/TAR. Thus, inclusion criteria were to be in majority age (age ≥18 years), to be approved as having sex with men by his peers, to get possible follow up for at least 3 months, and to have a fully informed medical and socio-demographic record. At inclusion, a standardized interview was conducted to collect socio-demographic characteristics and behavioral data, including age, ethnic group, number of sexual male partners in the last 6 months, sexual orientation, frequency of condom use, sexual practices, HIV status and antiretroviral treatment (ART) for those already aware of their positive HIV status and finally to advise participants about HIV and associated STIs.

After the interviews, MSM undertook medical appointments including clinical examinations and biological investigations for the diagnosis of the most common STIs including HIV (for those who did not know their HIV-serological status), syphilis and hepatitis B. Biological results were returned 72 hours after and those positive for STIs received adapted treatment. HIV-positive MSM were enrolled in the HIV cohort followed in the CNRIST/TAR. The medical intervention package consisted of HIV/STI counseling, condom distribution, clinical examination, biological monitoring, and medical care for patients infected with STIs and HIV. A Medical professional carried out physical and clinical examinations to check patients for symptoms of potential diseases. For HIV/STI counseling and condom distribution, a 7 to 10-minute interactive conversation on HIV, STIs, their modes of transmission and effective prevention mean with a special emphasis on condom use as an easy and effective prevention tool, was carried out by health care assistants. At the end, the health care assistants distributed as many condoms as required to the participants.
Samples and processing

Plasma or serum samples from blood collected by venipuncture in each MSM were used for serological testing of HIV infection, as recommended by national guidelines [25]. HIV-positive status was determined by the national serological testing algorithm using Genscreen ULTRA Combo HIV Ag/Ab test (Bio-Rad Laboratories, Washington, USA), an enzyme immunoassay kit for the simultaneous detection of HIV p24 antigen and antibodies to HIV-1 (group M and O) and HIV-2 in serum or plasma. The positive tests were confirmed by an enzyme-linked immunosorbent assay [ELISA] (Vironostika® HIV Uni-Form II Ag/Ab, bio-Mérieux Marcy l’Etoile, France). Specimens for molecular testing were obtained by inserting a moistened polyester swab into the anal canal, rotating 5 times and then removing. The swab was immediately placed into a sampler tube and frozen at -80°C before the DNA extraction procedure.

HPV detection and genotyping

DNA was extracted from the anal swab specimen using the DNeasyBlood and Tissue kit, as recommended by the manufacturer (Qiagen, Hilden, Germany). The detection of HPV DNA and the distribution of genotypes were done using Anyplex™ II HPV28 detection test (Seegene, Seoul, South Korea). Anyplex™ II HPV28 detection test was performed as recommended by the manufacturer with 5μl of DNA in each of the two reaction mixtures (20μl) with the primers A and B [26]. According to the International Agency for Research on Cancer (IARC) nomenclature [27], Anyplex™ II HPV28 detection test distinguishes 28 HPV genotypes, including 13 high-risk types (HR-HPV -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68), 9 low-risk (LR) types (LR-HPV -6, -11, -40, -42, -43, -53, -54 and -70) and then, 6 genotypes reported as possibly carcinogenic (HPV-26, -61, -66, -69, -73 and -82). The process is carried out in 2 reactions by taking advantage of the 5 dyes that can be resolved on the CFX96™ real-time PCR instrument (Bio-Rad, Marnes-la-Coquette, France) [26]. Anyplex™ II HPV28 has been evaluated for several years and is as suitable for HPV genotyping as other molecular assays commonly used for genotyping [26, 28–32]. Data recording and interpretation were automated with Seegene viewer software (Seegene, Seoul, South Korea), according to the manufacturer’s instructions. A swab sample was considered positive for any HPV if containing any of the 28 types included in the Anyplex™ II HPV28 detection test; positive for multiple HPV when containing at least 2 types of the 28 HPV types included in genotypic test; HR-HPV positive and multiple HR-HPV positive when containing respectively at least 1 HR-HPV type and at least 2 HR types of the 19 HR types of the Anyplex™ II HPV28 detection test, irrespective of the presence of LR-HPV.

HSV detection in the anal tract

HSV DNA was detected from 1μg of DNA extracted from the anal swab specimen by in-house real-time PCR using LightCycler® 480 Real-Time PCR (Roche molecular diagnostics, California, USA), as previously described [33,34]. HSV genotypes (HSV-2 versus HSV type 1) were assessed by the final melting curve, [33,34].

Statistical analyses

Statistical analyses were conducted using IBM® SPSS® Statistics 20 software (IBM, SPSS Inc, Armonk, New York, USA). P-values were calculated using Pearson’s χ² test or Fisher’s exact test for categorical variables and the non-parametric Mann-Whitney U-test for quantitative variables. Logistic regression models were assessed to evaluate the association of each
independent variable [i.e., age at enrollment, HIV-1 infection, sex of sexual partner (MSM-exclusively or MSMW), the number of sexual male partners in the last 6 months, sexual practices in the last 6 months (condomless receptive anal sex; condomless insertive anal sex; regular receptive oral sex) and the HSV-2 DNA detection in anal swab] with the HPV type-specific anal infections (i.e., anal infection by any type of HPV, multiple types of HPV, HR-HPV and multiple HR-HPV). All variables statistically significant (P < 0.05) in univariate analyses were entered into multivariate logistic regression models. Crude Odds ratio (cOR) and adjusted Odds ratio (aOR) were calculated, as appropriate along with 95% confidence intervals (CI). For variable giving infinite OR, the Odds ratios and their confidence intervals were recalculated, using the statistical software package R (available at https://www.r-project.org/) and the hypothesis test inversion method, as previously described [35]. The final multivariate model for any HPV outcome included condomless receptive and insertive anal sex and regular receptive oral sex. For multiple HPV outcome, the final multivariate model included condomless receptive and insertive anal sex and regular receptive oral sex. For HR-HPV outcomes, the final multivariate model included condomless insertive anal sex and regular receptive oral sex. Finally, for the multiple HR-HPV outcomes variable, the final multivariate model included HIV infection and condomless insertive anal sex.

Ethics statement

The study was formally approved by the Scientific Committee Faculty of Health Sciences of Bangui (“Comité Scientifique Chargé de la Validation des Protocoles d’Etudes et des Résultats”/ ”CSCVPER”) (agreement UB/FACSS/CSCVPER), which constitutes the National Ethical Committee. All MSM participants were of majority age and gave their informed oral consent to participate in the study. For each MSM, the record of the consent was documented in each questionnaire. This consent procedure was formally approved by the National Ethical Committee.

Results

Characteristics of study population

Forty-two participants were included and their socio-demographic, sexual behavior, clinic-biological characteristics are shown in the Table 1. Among them, 29 (69.1%) were infected by HIV-1 whereas 13 (30.9%) were HIV-negative.

Overall, the study population was exclusively constituted by black native people and comprised mainly young men (mean age: 23.2 years; range, 18–39). All were living in 4 (out of 10) districts of the capital city Bangui which were neighboring. The majority of participants (34/42; 80.9%) reported having sex in the last 6 months with both men and women (MSMW), whereas a minority (8/42; 19.1%) reported having sex in the last 6 months exclusively with men (MSM-exclusively). The prevalence of HIV-1 infection was higher in MSM-exclusively (8/8; 100%; 95%CI: 100–100%) than in MSMW (21/34; 61.7%; 95%CI: 45.4–78.1%) (P = 0.04). At inclusion, only 10 of 29 (34.5%) HIV-infected MSM, including 4 MSM-exclusively and 6 MSMW, were tacking antiretroviral treatment (ART) according to the 2015-World Health Organization (WHO) consolidated guidelines [36]. Among them, only 6, including 4 MSM-exclusively and 2 MSMW, showed a baseline CD4 cell count above 500 cells/μl. Most (40/42; 95.3%) of the MSM reported having sexual intercourse with at least 1 to 5 partners (median: 4; range, 1–5) in the last 6 months and most of them (30/42; 71.4%) had condomless receptive anal sex during the past 6 months. The group of MSM-exclusively reported having 7-time more receptive anal sex than insertive anal sex [receptive anal sex: 7/8 (87.5%), 95%CI: 64.6–100.0%; insertive anal sex: 1/8 (12.5%), 95%CI: 0.0–35.4%]. Furthermore, MSM-exclusively reported more likely to have receptive oral sex than MSMW [MSM-exclusively: 8/8 (100.0%),
95%CI: 100.0–100.0%; MSMW: 20/34 (58.8%), 95%CI: 42.28–75.37%; P < 0.03]. Lastly, HIV-infected MSM reported practicing more regularly receptive oral sex than HIV-negative MSM [25/29 (86.2%); 95%CI: 73.6–98.7% versus 3/13 (23.1%); 95%CI: 0.2–45.9%; P < 0.001].
Finally, the study MSM were generally free of clinical STI symptoms at admission. Thus, only 5 MSM showed herpetic genital recurrences (n = 2), syphilis genital ulcer (n = 1), anal warts (n = 1) and anal infection due to Neisseria gonorrhoea (n = 1). The seroprevalences of syphilis and hepatitis B (HBs antigen) at inclusion were 12% and 14%, respectively.

### HSV DNA detection

Only 5 (11.9%) of the study MSM were positive for HSV-2 in anal samples (1 MSM-exclusively and 4 MSMW); all of them were coinfected with HIV-1 and 4 of them showed multiple anal HR-HPV infections.

### HPV prevalence and genotype distribution

As shown in Table 1, the overall HPV prevalence in study population was 69.1% (29/42) with 82.7% (24/29) of HR-HPV DNA-positive samples. Most (25/42; 59.5%) anal swabs contained multiple HPV genotypes and 52.0% (13/25) of them contained an average of 2.7 HR-HPV (range, 1 to 4) per anal swab. About 24 out of 29 (82.7%; 95%CI: 69.01–96.51%) HPV-positive anal specimens showed at least 1 HR-HPV and only 37.9% (11/29) showed single HR-HPV infections. The distribution of HPV genotypes in HPV DNA-positive anal samples is depicted in Fig 1.

HR-HPV-35 was the predominant genotype (8/29; 27.6%), followed by LR-HPV types 42 and 53 with a prevalence of 24.1% (7/29), HR-HPV types 58 and 59 with 20.7% (6/29) and HR-HPV types 31 and 61 with 17.2% (5/29). HPV-16 and HPV-18 were found in a minority of HPV-positive swabs [4/29 (13.8%) and 3/29 (10.3%), respectively] (Fig 1).

Among the 21 HIV-1-infected MSM with anal HPV infection, 19 (90.5%) had multiple HPV and 20 (95.3%) were co-infected with HR-HPV (Fig 2 and Table 1). Multiple anal HR-HPV types were detected more frequently in HIV-1-infected than HIV-negative MSM (41.4% versus 7.7%; P<0.01). The mean number of HR-HPV genotypes more frequently detected in HR-HPV-positive anal swabs was 2.7 (range 1–4) in HIV-positive MSM and 1.5 (range 1–4) in HIV-negative MSM (P = 0.07). MSM positive for HPV-16, HPV-18 and HPV-
35 were all HIV-1-infected (Fig 2) and only one sample was simultaneously infected with both HPV-16 and HPV-18.

Possible efficiencies of anal HPV prevention by 4- and 9-valent Gardasil® vaccines were further assessed. 37.9% (11/29) of the HPV-positive anal swabs were infected with at least 1 of the 4 genotypes covered by the Gardasil-4 vaccine. Regarding the Gardasil-9 vaccine, 68.9% (20/29) of HPV-positive anal samples contained at least 1 HPV type included in the 9-valent HPV vaccine and 45% (9/20) of them contained multiple HPV genotypes (Fig 2 and Table 1). The predominant HR-HPV genotype included in the 9-valent HPV vaccine detected in study anal swabs was the type 58 with a prevalence of 20.7% (20/29) (Fig 2).

Risk factors associated with HPV infection. The associations between anal HPV infection, including anal infection by any type of HPV, multiple types of HPV, and HR-HPV type and multiple types HR-HPV, with their potential risk factors were assessed by logistic regression analysis, as shown in Table 2.

No significant differences could be observed between MSM-exclusively and MSMW regarding to the prevalence of HPV DNA detection in the anal samples [7/8 (87.5%) versus 22/34 (64.7%), respectively; P＞0.05]. However, the MSM-exclusively group had the highest rates of anal detection of HPV infections, including LR-HPV and HR-HPV with high prevalence of HPV-16 and HPV-18, multiple HPV as well as HPV types included in the 9-valent HPV prophylactic vaccine.

In the univariate analysis, anal infections by any type of HPV and multiple type of HPV were significantly associated with the practice of condomless receptive anal sex (cOR: 14.1, 95%C.I: 2.9–68.4%; P = 0.001; cOR: 5.9, 95%C.I: 1.4–24.7%; P = 0.014, respectively). Likewise, anal infections by any type of HPV, multiple type of HPV and anal infections with HR-HPV were significantly associated with the practice of receptive oral sex (cOR: 11.0, 95%C.I: 2.3–49.5%; P = 0.002; cOR: 7.5, 95%C.I: 1.8–31.7%; P＜0.05 and cOR: 16.9, 95%C.I: 3.4–83.7%; P＜0.001, respectively). The anal carriage of multiple anal HR-HPV infection was significantly associated with being infected with HIV (cOR: 15.0, 95%C.I: 1.4–161.1%; P = 0.003). Insertive anal sex was significantly associated with a slightly decreased risk of being infected with any HPV type, HR-HPV type and multiple HR-HPV type in the anal canal (cOR: 0.01, 95%C.I: 0.002–0.130%; P＜0.01; cOR: 0.03, 95%C.I: 0.0–0.2%; P＜0.001 and cOR: 0.01, 95%C.I: 0.02–0.65%; P = 0.008, respectively).
Table 2. Univariate and multivariate logistic regression analyses for HPV-associated risk factors in study men who have sex with men (MSM) living in Bangui, Central African Republic.

| Risk factor                      | Any HPV (%) | Multiple HPV (%) | HR-HPV (95% CI) | Any HPV (%) | Multiple HPV (%) | HR-HPV (95% CI) | Any HPV (%) | Multiple HPV (%) | HR-HPV (95% CI) | Any HPV (%) | Multiple HPV (%) | HR-HPV (95% CI) |
|----------------------------------|-------------|------------------|-----------------|-------------|------------------|-----------------|-------------|------------------|-----------------|-------------|------------------|----------------|
| Age                              |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| 18–19                            | 8 (0.7%)    |                  |                 | 0.7          |                  |                 | 0.4          |                  |                 | 0.9         |                  |                 |
| 20–29                            | 14 (0.9%)   |                  |                 | 0.9          |                  |                 | 0.2          |                  |                 | 0.6         |                  |                 |
| 30–39                            | 21 (1.4%)   |                  |                 | 1.4          |                  |                 | 0.3          |                  |                 | 0.7         |                  |                 |
| HIV-1 infection                  |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| Yes                              | 21 (2.25)   |                  |                 | 2.25         |                  |                 | 0.2         |                  |                 | 0.6         |                  |                 |
| No                               |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| MSM-exclusively                  |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| Yes                              | 7 (3.8%)    |                  |                 | 3.8          |                  |                 | 0.2         |                  |                 | 1.7         |                  |                 |
| No                               |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| MSMW                             |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| Yes                              | 29 (1.4%)   |                  |                 | 1.4          |                  |                 | 0.3          |                  |                 | 0.7         |                  |                 |
| No                               |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| Anal sexual partners             |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| MSM                             | 22 (0.9%)   |                  |                 | 0.9          |                  |                 | 0.1          |                  |                 | 0.5         |                  |                 |
| No                               |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| Condomless insertive anal sex    |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| MSM                             | 7 (3.8%)    |                  |                 | 3.8          |                  |                 | 0.2         |                  |                 | 0.9         |                  |                 |
| No                               |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| Condomless receptive anal sex    |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| MSM                             | 0.3         |                  |                 | 0.2          |                  |                 | 0.0          |                  |                 | 0.9         |                  |                 |
| No                               |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| HSV-2 DNA in swab                |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |
| MSM                             | 0.3         |                  |                 | 0.2          |                  |                 | 0.0          |                  |                 | 0.9         |                  |                 |
| No                               |             |                  |                 |             |                  |                 |             |                  |                 |             |                  |                 |

**P-value calculated using Pearson’s X²-test or Fisher’s exact test for categorical variables and the non-parametric Mann-Whitney U-test for non-categorical variables.**

**NA:** Not attributable for variables giving crude Odds ratio not significant in univariate analysis (P > 0.05).

**ART:** Antiretroviral treatment; aOR: adjusted Odds ratio; cOR: crude Odds ratio; HIV-1: Human immunodeficiency virus-1; HSV-2: Herpes simplex virus-2; HR-HPV: high-risk human papillomavirus; LR-HPV: low-risk human papillomavirus; MSM: men who have sex with men; MSM-exclusively: men who have sex only with men; MSMW: men who have sex with both men and women; n: Number (size of study group); NA: Not attributable; CI: Confidence Interval.
In the multivariate analysis, condomless insertive anal sex and having regular receptive oral sex were the only factors associated with an HPV outcome after adjusting other significant variables in the univariate analysis. Thus, having regular receptive oral sex was a substantial risk factor for being infected with the HR-HPV in the anal canal (aOR: 22.3, 95%CI: 2.0–244.1%; P = 0.01). Likewise, the weak protective effect of practicing insertive anal sex against the acquisition of any HPV type, HR-HPV type and multiple HR-HPV type in the anal canal observed in univariate analysis (cOR: 0.01, 95%CI: 0.002–0.13%; P<0.01; cOR: 0.03, 95%CI: 0.0–0.2%; P<0.001 and cOR: 0.1, 95%CI: 0.02–0.65%; P = 0.008, respectively) was maintained in multivariate analysis (aOR: 0.02, 95%CI: 0.001–0.18%; P<0.01; aOR: 0.02, 95%CI: 0.0–0.24%; P = 0.002 and aOR: 0.20, 95%CI: 0.02–1.1%; P = 0.05, respectively). In addition, the effect of insertive anal sex against the acquisition of multiple type of HPV in the anal canal, which was not significant in univariate analysis (cOR: 0.4, 95%CI: 0.03–5.3%; P<0.05) was upgraded as a weak protective effect in the multivariate analysis (aOR: 0.05, 95%CI: 0.01–0.3%; P = 0.01). In the other hand the strong risk effect of being infected with any HPV and multiple HPV observed in univariate analysis for MSM practicing condomless receptive anal intercourse was downgraded in multivariate analysis until being not significant anymore (aOR: 4.9, 95%CI: 0.5–47.1%; P = 0.16 and aOR: 1.4, 95%CI: 0.2–11.3%; P = 0.669; respectively). Finally, the other explicative variables such as age group, sexual orientation (MSM-exclusively or MSMW), number of sexual male partners and anal HSV-2 infection were not significantly associated with each of the four variables characterizing anal HPV infections that were taken into account in the analysis.

**Discussion**

In the present series, the study of MSM living in the Central African Republic and overseen at the Centre National de Référence des Infections Sexuellement Transmissibles et de la Thérapie Antirétrovirale of Bangui showed remarkable findings. Firstly, the prevalence of HIV-1 infection in the MSM was notably high (#70%). Secondly, the prevalence of anal HPV was also particularly high (#70%) and unique due to the high prevalence of HR-HPV (82.7%), its high genotypes diversity and the frequent (52%) multiple HR-HPV infections. Thirdly, the distribution of anal HPV in anal samples appeared unusual, the most prevalent genotypes being HPV-35, HPV-58, HPV-59 and HPV-31, while the more classical HPV-16 and HPV-18 were present only in a minority of samples (#25%), likely indicating possible regional clusterization in the diffusion of HR-HPV within the MSM community living in Bangui. Fourthly, HPV types included in the prophylactic Gardasil-9® vaccine were detected in the majority of HPV-positive anal samples (#70%) suggesting that the current 9-valent vaccine could be beneficial for the prevention of HPV-associated disease in this MSM community, although one-third of HPV anal infection would not be prevented. Finally, anal HSV-2 shedding was not associated with anal HPV shedding in our study population, in which the only negative modulatory cofactor for HPV anal carriage was behavioral, the insertive anal intercourse being relatively protective in comparison with receptive anal intercourse. Taken together, our observations indicate for the first time that the MSM community living in Bangui should be at very high-risk for HIV infection as well as HR-HPV anal infections, and strongly suggest that scaling up prevention strategies against HPV infection and related cancers adapted to this highly vulnerable MSM community should be urgently prioritized with innovative interventions.

Very high prevalence of HIV-1 was observed in the study MSM, suggesting that the HIV prevalence in the MSM community of Bangui could be high. This observation is reminiscent to similar reports on MSM living in sub-Saharan Africa. Studies conducted in sub-Saharan African countries in the last 10 years show that HIV prevalence among MSM is more than...
5–18 times higher as compared to general population [37, 38]. In Tanzania, the prevalence of HIV in MSM was 17.4% and 3.7% among the general population [3]. Similarly, in Malawi, the prevalence of HIV was 21.4% in MSM and 6.1% in the general population [39]. In Kenya, the HIV prevalences in MSM ranged from 12.3% to 43.0% as compared to 6.1% in the general population [40]. In Ivory Coast, the prevalence of HIV in MSM was as high as 50% as compared to 3.2% in the general population [41].

The prevalence of anal HPV was particularly high (≈70%) in this sample of MSM from the Bangui’s community. The high prevalence of anal HR-HPV in our study MSM appeared quite similar to the prevalence reported in MSM living in South Africa and Nigeria, ranging from 58% to 72% [6, 7], but lower than the prevalence reported in young Black American MSM (87%) [42]. Other reports conducted outside Africa showed lower anal HR-HPV prevalence rates, ranging from 29% to 56% [43–46]. Previous reports have clearly demonstrated that the elevated risk for anal HPV in MSM is increased by HIV infection. Thus, anal HR-HPV infection was up to 4–10 times more frequent in MSM living in many countries outside Africa than in heterosexual men [47, 48]. Finally, our observations confirm that anal HPV constitutes a major infectious health concern in the MSM living in Bangui, highly escalated by HIV infections, and that each MSM community is characterized by local epidemiological specificities rendering necessary their research before intervention.

In our series, the distribution of anal HPV in anal samples appeared quite atypical, with HPV-35 being the predominant genotype. Furthermore, HPV-16 and HPV-18 were very poorly represented in the study population with the most prevalent 9-valent vaccine HR-HPV being HPV-58. This unusual distribution mirrors the previous observations by Nowak and colleagues, reporting that anal samples from MSM living in Nigeria harbored HPV-35 as the predominant genotype, HPV-16 and HPV-18 as minor genotypes, and HPV-58 as the most prevalent 9-valent vaccine genotype [7]. Interestingly, MSM infected by HPV-35 in our series and in MSM living in Nigeria [7], were all co-infected with HIV likely suggesting that HIV infection may play a role in the persistence of this unusual HPV type as a predominant genotype. Likewise, another recent study conducted in the United States of American highlighted the low proportion of HPV-16 and HPV-18 in anal samples from young black American MSM [42]. In contrast, anal HPV-16 and HPV-18 were the most prevalent in MSM living in South Africa, including a majority (67%) of mixed race/colored and white people and only (31%) one-third of black individuals [6]. Taken together, these observations suggest the possibility of a regional distribution in molecular epidemiology of HR-HPV within the diverse MSM communities inside the sub-Saharan African continent [49]. Thus, it is possible to hypothesize that anal cancers in certain black African MSM populations may be due to other HR-HPV rather than HPV-16 and HPV-18, which constitute the HR-HPV types involved in more than 89% of all anal cancers in MSM living in Western countries [50, 51]. Interestingly, others studies on HPV infection in women living in Nigeria have also reported HPV-35 as the predominantly isolated genotype [52,53]. In the Central African Republic, there is no data on the HPV type specific prevalence in the general population. It would be interesting to check whether the unusual HPV genotype distribution found in our Central African MSM series is similar to that in the female or general population. Further studies are nevertheless needed to determine the natural history and the burden of HPV-associated diseases in black African MSM in order to confirm our observations and to formulate effective and adapted HPV vaccine strategies towards young African MSM.

HPV types included in the prophylactic Gardasil-9® vaccine were detected in the majority of HPV-positive anal samples. Around 70% of all anal HPV-positive individuals harbored at least 1 of the 9-valent HPV vaccine genotypes and 45% of HPV-positive anal specimens contained multiple HPV vaccine types. High rates of 9-valent HPV vaccine types in anal canal of
MSM were previously reported in South Africa (57%) [6]. These observations indicate that MSM living in Bangui, as other MSM populations, constitute a key target population for HPV vaccination with the current prophylactic Gardasil-9® vaccine, which would potentially prevent most of HPV infections and associated anal diseases. However, anal HR-HPV not included in the prophylactic nonavalent vaccine, including the unusual HPV-35, were found in one-third of the study’s MSM, indicating that the current HPV vaccine may be insufficient to prevent HPV-related diseases in a significant proportion of the MSM community living in Bangui. Thus, the guidelines on HPV immunization recommended in 2015 by the American Cancer Society (ACS), which integrate HPV vaccination up to 26 years for young MSM with the current two large spectrum HPV vaccines [54, 55], because HPV-16 and HPV-18 are mostly involved in HPV-associated anal cancer in Western countries [50, 51], may be poorly adapted to the MSM community living in the Central African Republic and other sub-Saharan African settings.

In vitro interactions between HPV and HSV-2 [16], in the context of high HSV-2 prevalence in sub-Saharan Africa [18], prompted us to evaluate the possible association between anal HSV-2 DNA shedding and anal HPV detection. The rate of anal HSV-2 DNA detection in our series (11.9%) was similar to that (10.6%) reported in a recent meta-analysis on MSM living in China [56]. However, no association between anal shedding of HSV-2 and HPV detection could be found.

Multiple HR-HPV was frequently detected in anal swabs from Bangui’s MSM, mainly in HIV-infected individuals. Multiple HR-HPV in MSM is yet to be well documented [6, 21, 42, 51, 57]. High rates (91–94%) of multiple anal HPV infections with numerous different HPV genotypes ranging from 0 to 18 (mean, 4.8–5.0) were reported in HIV-positive MSM living outside Africa, such as North Canada and Thailand [58, 59]. In the present series, multiple HR-HPV infections were more frequently detected in HIV-infected than HIV-negative MSM and multiple anal HR-HPV infection was 15-times more frequent in HIV-positive than in HIV-negative MSM (univariate analysis). Indeed, high-risk sexual behavior, including exclusive sex with other men while being HIV-infected, constitutes a significant cofactor strongly associated with increased risk of multiple anal infections with HR-HPV genotypes [13–15]. In our study MSM population, MSM-exclusively showed 7-times more frequently receptive than insertive anal intercourse (88% versus 13%). Furthermore, MSM-exclusively were all HIV-positive (100%), thus more frequently infected by HIV than those having sex with both men and women (62%).

The principal negative modulatory cofactor for HPV anal carriage was behavioral, the MSM practicing condomless receptive anal intercourse being at more risk to be infected with HPV than those practicing insertive anal intercourse. Condomless receptive anal intercourse in MSM is well known to constitute the main sexual risk factor associated with HPV infection [6, 14, 15].

These findings are reminiscent to previous reports on HIV transmission demonstrating that MSM practicing receptive anal intercourse are at higher risk for HIV acquisition than their insertive partner [60, 61]. More generally, a receptive partner is more vulnerable to HPV infection than insertive partner in anal intercourse, as previously demonstrated in a recent study on 733 HIV-infected individuals (538 MSM, 195 heterosexual) that showed the prevalence, clearance and incidence of HPV infection was higher in the anal mucosa (73%, 30% and 36%, respectively) than in penile mucosa (26%, 56% and 17%, respectively) [62]. Indeed, the rectal mucosa, which consists of a single layer of epithelial cells, may break easily, exposing the epidermoid cells of the basal lamina, thus facilitating their infection by HPV [63]. Furthermore, the anal mucosa is relatively large and the rectal receptacle may retain pathogens [63, 64]. Circumcised men, as MSM living in Bangui, have keratinized external mucosa of the penis that provides strong epithelial barrier hampering HPV infection [63, 64].
Finally, HIV-positive MSM reported practicing condomless receptive oral sex were more frequent than HIV-negative MSM, and this sexual practice constituted a risk factor was strongly associated with having anal infection with any type of HPV and particularly HR-HPV. Hu and colleagues have previously reported in HIV-infected Chinese MSM that oral sex, even protected, is closely linked with anal HPV infection, suggesting that other high-risk practices of the sexual repertoire, yet unvalued, may be associated [44]. Taken together, these observations are similar to a previous report in South Africa where the population of HIV-positive MSM-exclusively constituted a high-risk group accumulating several risky sexual behaviors and multiple anal HR-HPV infections [6]. In short, these observations emphasize the urgent need to implement adapted intervention strategies towards African MSM to reduce sexual risk behaviors. Intervention strategies such as counseling for HIV and sexual risk behaviors have been shown to reduce the incidence of HIV and STI among MSM [65–68]. Furthermore, scaling up prevention strategies against HR-HPV infections and associated cancers adapted for this at-risk vulnerable population should be prioritized. Targeted behavioral interventions may include promotion of consistent condom use [65–68], clinical examination with digital ano-rectal touching (“DARE”) which is an important tool for the early detection of precancerous lesions and anal cancer [69], medical male circumcision to reduce HIV incidence, as well as prevalence and persistence of anal HR-HPV infections [70], and finally HPV prophylactic vaccination of young MSM [55].

In conclusion, MSM community living in Bangui constitutes a very high risk population for both HIV infection and HR-HPV anal infections, and should urgently receive adapted STI and anal cancer screening and care.

Our study had some limitations. Indeed, the recruitment of participants from only the CNRIST/TAR of Bangui as well as the small sample size of our study population, may have introduced selection and information bias. Thus, the study participants may be not completely representative of the MSM community of the Central African Republic, especially regarding prevalences of HIV and anal HPV, and the genotypes distribution of anal HPV. Furthermore, some risk factors may have been underestimated in the statistical analyses.

Supporting information
S1 Data. Excel data sheet containing raw data on socio-demographic, behavioral characteristics, as well as the results of different biological analyzes carried out on MSM living in Bangui included in the study.
(XLSX)

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References
1. Park JN, Papworth E, Kassegne S, Moukam L, Billong SC, Macauley I et al. HIV prevalence and factors associated with HIV infection among men who have sex with men in Cameroon. J Int AIDS Soc. 2013; 16 Suppl 3:18752.

2. Keshinro B, Crowell TA, Nowak RG, Adebajo S, Peel S, Gaydos CA et al. High prevalence of HIV, chlamydia and gonorrhoea among men who have sex with men and transgender women attending trusted community centres in Abuja and Lagos, Nigeria. J Int AIDS Soc. 2016; 19(1):21270. https://doi.org/10.7448/IAS.19.1.21270 PMID: 27931519

3. Mmbaga EJ, Moen K, Makyao N, Mpembeni R, Leshabari MT. HIV and STIs among men who have sex with men in Dodoma municipality, Tanzania: a cross-sectional study. Sex Transm Infect. 2017. pii: sex-trans-2016-052770.

4. Wirtz AL, Trapence G, Kamba D, Gama V, Chalera R, Jumbe V et al. Geographical disparities in HIV prevalence and care among men who have sex with men in Malawi: results from a multisite cross-sectional survey. Lancet HIV. 2017. pii: S2352-3018(17)30042-5.

5. Looker KJ, Magaret AS, Turner KM, Vickerman P, Gottlieb SL, Newman LM. Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012. PLoS One. 2015; 10(1):e114989. https://doi.org/10.1371/journal.pone.0114989 PMID: 25608026

6. Müller EE, Rebe K, Chinwa TF, Struthers H, McIntyre J, Lewis DA. The prevalence of human papillomavirus infections and associated risk factors in men-who-have-sex-with-men in Cape Town, South Africa. BMC Infect Dis. 2016; 16(1):440. https://doi.org/10.1186/s12879-016-1706-9 PMID: 27549219

7. Nowak RG, Gravitt PE, He X, Ketende S, Dauda W, Omuh H et al. Prevalence of Anal High-Risk Human Papillomavirus Infections Among HIV-Positive and HIV-Negative Men Who Have Sex With Men in Nigeria. Sex Transm Dis. 2016; 43(4):243–8. https://doi.org/10.1097/OLQ.0000000000000431 PMID: 26967301

8. Mbooumba Bouassa RS, Prazuck T, Lethu T, Meye JF, Bélec L. Cervical cancer in sub-Saharan Africa: an emerging and preventable disease associated with oncogenic human papillomavirus. Med Sante Trop. 2017; 27(1):16–22. https://doi.org/10.1684/mst.2017.0646 PMID: 28490646

9. Scheurer ME, Tortolero-Luna G, Adler-Storthz K. Human papillomavirus infection: biology, epidemiology, and prevention. Int J Gynecol Cancer. 2005; 15(5):727–46. https://doi.org/10.1111/j.1525-1438.2005.00246.x PMID: 16174218

10. Parkin DM. The global burden of infection-associated cancers in the year 2002. Int J Cancer 2006; 118: 3030–44. https://doi.org/10.1002/ijc.21731 PMID: 16404738
11. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of human papillomavirus-associated cancers among persons with AIDS. J Natl Cancer Inst. 2009 Aug 19;101(16):1120–30. https://doi.org/10.1093/jnci/djp205 PMID: 19648510

12. Garbuglia AR, Gentile M, Del Nonno F, Lorenzini P, Lapa D, Luzzi F et al. An anal cancer screening program for MSM in Italy: Prevalence of multiple HPV types and vaccine-targeted infections. J Clin Virol. 2015 Nov;72:49–54. https://doi.org/10.1016/j.jcv.2015.09.001 PMID: 26397204

13. Baggaley RF, White RG, Boily MC. HIV transmission risk through anal intercourse: systematic review, meta-analysis and implications for HIV prevention. Int J Epidemiol. 2010; 39(4):1048–63. https://doi.org/10.1093/ije/dyq057 PMID: 20406794

14. Mendez-Martinez R, Rivera-Martinez NE, Crabtree-Ramirez B, Sierra-Madero JG, Caro-Vega Y, Galvan SC et al. Multiple human papillomavirus infections are highly prevalent in the anal canal of human immunodeficiency virus-positive men who have sex with men. BMC Infect Dis. 2014; 14:671. https://doi.org/10.1186/s12879-014-0671-4 PMID: 25510243

15. Ong JJ, Chen M, Tabrizi SN, Cornall A, Garland SM, Jin F et al. Anal HPV detection in men who have sex with men living with HIV who report no recent anal sexual behaviours: baseline analysis of the Anal Cancer Examination (ACE) study. Sex Transm Infect. 2016; 92 (5):368–70. https://doi.org/10.1136/sextrans-2015-052121 PMID: 26472920

16. Skeate JG, Porras TB, Woodham AW, Jang JK, Taylor JR, Brand HE et al. Herpes simplex virus down-regulation of secretory leukocyte protease inhibitor enhances human papillomavirus type 16 infection. J Gen Virol. 2016; 97(2):422–34. https://doi.org/10.1099/jgv.0.003401 PMID: 26555939

17. Mbopi-Keou FX, Grese nguet G, Mavolomade EE, Mandeng MJ, Talarmin A. Advantages of an alternative strategy based on Estrade C, Sahli R. Comparison of SeegeneAnyplex II HPV28 with the PGMY-CHUV assay for human papillomavirus genotyping. J Clin Microbiol. 2014; 52(2):807–12.

18. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F et al. A review of human carcinogens—Part B: biological agents. Lancet Oncol. 2009; 10(4):321–2. PMID: 19390698

19. Kwon MJ, Roh KH, Park H, Woo HY. Comparison of the Anyplex II HPV28 assay with the Hybrid Capture 2 assay for the detection of HPV infection. J Clin Virol. 2014; 59(4):246–9. https://doi.org/10.1016/j.jcv.2014.01.015 PMID: 24568964

20. Lilsunde Larsson G, Carlsson J, Karlsson MG, Helenius G. Evaluation of HPV Genotyping Assays for Archival Clinical Samples. J Mol Diagn. 2015; 17(3):293–301. https://doi.org/10.1016/j.jmoldx.2014.12.004 PMID: 25791291
30. Marcuccilli F, Farchi F, Miranda W, Ciccozzi M, Paba P, Bonanno E et al. Performance evaluation of Anyplex™II HPV28 detection kit in a routine diagnostic setting: comparison with the HPV Sign® Genotyping Test. J Virol Methods. 2015 Jun 1; 217:8–13. https://doi.org/10.1016/j.jviromet.2015.02.018 PMID: 25724435

31. Latsuzbaia A, Tapp J, Nguyen T, Fischer M, Arbyn M, Weyers S et al. Analytical performance evaluation of Anyplex II HPV28 and Euroarray HPV for genotyping of cervical samples. Diagn Microbiol Infect Dis. 2016; 85(3):318–322. https://doi.org/10.1016/j.diagmicrobio.2016.04.011 PMID: 27156793

32. Pasquier C, Sauné K, Raymond S, Boisneau J, Courtade M, Izopet J. Comparison of Cobs® HPV and Anyplex™ II HPV28 assays for detecting and genotyping human papillomavirus. Diagn Microbiol Infect Dis. 2017; 87(1):25–27. https://doi.org/10.1016/j.diagmicrobio.2016.08.022 PMID: 28336133

33. Espy MJ, Uhl JR, Mitchell PS, Thorvilson JN, Svien KA, Wold AD et al. Diagnosis of herpes simplex virus infection in the clinical laboratory by LightCycler PCR. J ClinMicrobiol. 2000; 38(2):795–9.

34. LeGoff J, Pére H, Bélec L. Diagnosis of genital herpes simplex virus infection in the clinical laboratory. Virol J. 2014; 11:83. https://doi.org/10.1186/1743-422X-11-83 PMID: 24885431

35. Rindskopf D. Infinite Parameter Estimates in Logistic Regression: Opportunities, Not Problems”, J Educ Behav Stat 2002; 27:147–161. (Last accessed 15 April 2018). Available at http://www.jsstor.org/stable/3648130

36. World Health Organization (WHO). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach –2nd ed. 2016. Last accessed: December 2017. Available at: http://www.who.int/hiv/pub/arv-arv-2016/en/

37. Beyrer C, Wirtz AL, Walker D, Johns B, Sifakis F, and Baral SD. The Global HIV Epidemics among Men Who Have Sex with Men (MSM). The World Bank, Washington DC. 2011. (Last accessed 02 May 2017). Available at: http://elibrary.worldbank.org/doi/book/10.1596/978-0-8213-8726-9

38. Beyrer C, Sullivan P, Sanchez J, Baral SD, Collins C, Wirtz AL et al. The increase in global HIV epidemics in MSM. AIDS. 2013; 27(17):2665–78. https://doi.org/10.1097/01.aids.0000432449.30239.fe PMID: 23842129

39. Baral S, Trapence G, Motimedi F, Umar E, Iipinge S, Dausab F et al. HIV prevalence, risks for HIV infection, and high viral loads following seroconversion among MSM in Malawi, Namibia, and Botswana. PLoS One. 2009; 4(4):e4997. https://doi.org/10.1371/journal.pone.0004997 PMID: 19325707

40. Sanders EJ, Okuku HS, Smith AD, Mwangom e M, Wahome E, Fegan G et al. High HIV-1 incidence, correlates of HIV-1 acquisition, and high viral loads following seroconversion among MSM. AIDS. 2013; 27(3):437–46. https://doi.org/10.1097/QAD.0b013e32835b0f81 PMID: 23079811

41. Vuylysteke B, Semde G, Si ka L, Crucitti T, Etétique Traore V, Buve A et al. High prevalence of HIV and sexually transmitted infections among male sex workers in Abidjan, Cote d’Ivorie: need for services tailored to their needs. Sex Transm Infect. 2012; 88(4):288–93. https://doi.org/10.1136/sextrans-2011-050276 PMID: 22328644

42. Keglovitz K, Richardson AD, Lancki N, Walsh T, Schneider JA. Anal Squamous Intraepithelial Lesions and HPV Among Young Black Men Who Have Sex with Men. LGBT Health. 2017; 4(1):72–74. https://doi.org/10.1089/lgbt.2016.0049 PMID: 27673362

43. Donà MG, Palamara G, Di Carlo A, Latini A, Vocatur o A, Benevolo M et al. Prevalence, genotype diversity and determinants of anal HPV infection in HIV-uninfected men having sex with men. J ClinVirol. 2012; 54(2):185–9.

44. Hu Y, Qian HZ, Sun J, Gao L, Yin L, Li X et al. Anal human papillomavirus infection among HIV-infected and uninfected men who have sex with men in Beijing, China. J Acquir Immune DeficSyndr. 2013; 64(1):103–14.

45. Cranston RD, Althouse AD, van Griensven F, Janocko L, Curlin ME, Chaikummao S et al. Prevalence of Anal Human Papillomavirus Vaccine Types in the Bangkok Men Who Have Sex With Men Cohort Study. Sex Transm Dis. 2015; 42(12):671–6. https://doi.org/10.1097/OLQ.0000000000000372 PMID: 26562695

46. Ren X, Ke W, Zheng H, Yang L, Huang S, Qin X et al. Human Papillomavirus Positivity in the Anal Canal in HIV-Infected and HIV-Uninfected Men Who Have Anal Sex with Men in Guangzhou, China: Implication for Anal Exams and Early Vaccination. Biomed Res Int. 2017; 2017:2641259. https://doi.org/10.1155/2017/2641259 PMID: 28133605

47. Goldstone S, Palefsky JM, Giuliani AR, Moreira ED Jr, Aranda C, Jessen H et al. Prevalence of and risk factors for human papillomavirus (HPV) infection among HIV-seronegative men who have sex with men. J Infect Dis. 2011; 203(1):66–74. https://doi.org/10.1093/infdis/jiq016 PMID: 21149498

48. Nyitray AG, Carvalho da Silva RJ, Baggio ML, Lu B, Smith D, Abrahamson M et al. Age-specific prevalence of and risk factors for anal human papillomavirus (HPV) among men who have sex with women and men who have sex with men: the HPV in men (HIM) study. J Infect Dis. 2011; 203(1):49–57. https://doi.org/10.1093/infdis/jiq021 PMID: 21148496
49. Mboumba Bouassa RS, Prazuck T, Lethu T, Jenabian MA, Meye JF, Bélec L. Cervical cancer in sub-Saharan Africa: a preventable noncommunicable disease. Expert Rev Anti Infect Ther. 2017; 15(6):613–627. https://doi.org/10.1080/14787210.2017.1322902 PMID: 28440679

50. Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L et al. Global burden of human papillomavirus and related diseases. Vaccine. 2012; 30 Suppl5:F12–23.

51. Saraiya M, Unger ER, Thompson TD, Lynch CF, Hernandez BY, Lyu CW et al. US assessment of HPV types in cancers: implications for current and 9-valent HPV vaccines. J Natl Cancer Inst. 2015; 107(6): djv086. https://doi.org/10.1093/jnci/djv086 PMID: 25925419

52. Okolo C, Franceschi S, Adewole I, Thomas JO, Follen M, Snijders PJ et al. Human papillomavirus infection in women with and without cervical cancer in Ibadan, Nigeria. Infect Agent Cancer. 2010; 5(1):24. https://doi.org/10.1186/1750-9378-5-24 PMID: 21129194

53. Akarolo-Anthony SN, Famooto AO, Dareng EO, Olaniyan OB, Offiong R, Wheeler CM et al. Age-specific prevalence of human papilloma virus infection among Nigerian women. BMC Public Health. 2014; 14:656. https://doi.org/10.1186/1471-2458-14-656 PMID: 24972674

54. Centers for Disease Control and Prevention (CDC). Recommendations on the use of quadrivalent human papillomavirus vaccine in males—Advisory Committee on Immunization Practices (ACIP), 2011. MMWR Morb Mortal Wkly Rep. 2011; 60(50):1705–8. PMID: 22189893

55. Petrosky E, Bocchini JA Jr, Hariri S, Chesson H, Curtis CR, Saraiya M et al. Use of 9-valent human papillomavirus (HPV) vaccine: updated HPV vaccination recommendations of the advisory committee on immunization practices. MMWR MorbWkly Rep 2015; 64(11):300–4.

56. Chow EP, Tucker JD, Wong FY, Nehl EJ, Wang Y, Zhuang X et al. Disparities and risks of sexually transmissible infections among men who have sex with men in China: a meta-analysis and data synthesis. PLoS One. 2014; 9(2):e89959. https://doi.org/10.1371/journal.pone.0089959 PMID: 24587152

57. del Amo J, González C, Geskus RB, Torres M, Del Romero J, Viciana P et al. What drives the number of high-risk human papillomavirus types in the anal canal in HIV-positive men who have sex with men? J Infect Dis. 2013; 207(6):1235–41. https://doi.org/10.1093/infdis/jit028 PMID: 23325914

58. de Pokomandy A, Rouleau D, Ghattas G, Vézina S, Coté P, Macleod J et al. Prevalence, clearance, and incidence of anal human papillomavirus infection in HIV-infected men: the HPVIRG cohort study. J Infect Dis. 2009; 199(7):965–73. https://doi.org/10.1086/597207 PMID: 19239366

59. Supindham T, Chariyalertaks S, Utaipat U, Miura T, Ruanpeng D, Chotrosniramit N et al. High Prevalence and Genotype Diversity of Anal HPV Infection among MSM in Northern Thailand. PLoS One. 2014; 9(2):e89959. https://doi.org/10.1371/journal.pone.0089959 PMID: 24587152

60. Doekeun O, Fox J. An overview of the relative risks of different sexual behaviours on HIV transmission. CurrOpin HIV AIDS. 2010; 5(4):291–7. https://doi.org/10.1097/COH.0b013e32833a88a3 PMID: 20543603

61. Marks G, Millett GA, Bingham T, Lauby J, Murrill CS, Stueve A. Prevalence and protective value of sero-sorting and strategic positioning among Black and Latino men who have sex with men. SexTransm Dis. 2010; 37(5):325–7.

62. Videla S, Danwich L, Cañadas MP, Coll J, Piñol M, García-Cuyás F et al. Natural history of human papillomavirus infections involving anal, penile, and oral sites among HIV-positive men. Sex Transm Dis. 2013; 40(1):3–10. https://doi.org/10.1097/OLQ.0b013e31827e8b7d PMID: 23250297

63. Veldhuijzen NJ, Snijders PJ, Reiss P, Meijer CJ, van de Wijgert JH. Factors affecting transmission of mucosal human papillomavirus. Lancet Infect Dis. 2010; 10(12):862–74. https://doi.org/10.1016/S1473-3099(10)70190-0 PMID: 21059056

64. Tebit DM, Ndembni N, Weinberg A, Quiñones-Mateu ME. Mucosal transmission of human immunodeficiency virus. Curr HIV Res. 2012; 10(1):3–8. PMID: 22624040

65. Möller LM, Stolte IG, Geskus RB, Okuku HS, Wahome E, Price MA et al. Changes in sexual risk behavior among MSM participating in a research cohort in coastal Kenya. AIDS. 2015; 29 Suppl3:S211–9.

66. Herbst JH, Raiford JL, Carry MG, Wilkes AL, Ellington RD, Whittler DK. Adaptation and National Dissemination of a Brief, Evidence-Based, HIV Prevention Intervention for High-Risk Men Who Have Sex with Men. MMWR Suppl. 2016; 65(1):42–50. https://doi.org/10.15585/mmwr.su6501a7 PMID: 26916033

67. Eaton LA, Kalichman SC, Kalichman MO, Driffin DD, Baldwin R, Zohren L et al. Randomised controlled trial of a sexual risk reduction intervention for STI prevention among men who have sex with men in the USA. Sex Transm Infect. 2017. pii: sextrans-2016-052835.

68. Rhodes SD, Alonso J, Mann L, Song EY, Tanner AE, Arellano JE et al. Small-Group Randomized Controlled Trial to Increase Condom Use and HIV Testing Among Hispanic/Latino Gay, Bisexual, and Other Men Who Have Sex With Men. Am J Public Health. 2017; 107(6):969–976. https://doi.org/10.2105/AJPH.2017.303814 PMID: 28426301
69. Ong JJ, Chen M, grulich AE, Fairley CK. Regional and national recommendations for digital ano-rectal examination as a means for anal cancer screening in HIV positive men who have sex with men: a systematic review. BMC cancer. 2014; 14:557. https://doi.org/10.1186/1471-2407-14-557 PMID: 25081485

70. Taylor S, Bunge E, Bakker M, Castellsagué X. The incidence, clearance and persistence of non-cervical human papillomavirus infections: a systematic review of the literature. BMC Infect Dis. 2016; 16:293. https://doi.org/10.1186/s12879-016-1633-9 PMID: 27301867