Elevated pH levels and HPV among Senegalese women

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Abstract

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Our investigation aimed to explore the extent to which elevated vaginal pH is associated with prevalent HPV infection among a cohort of Senegalese women ages 18-84. Sub-Saharan Africa has the greatest global burden of vaginal dysbiosis and the highest prevalence of HPV infection among women (24%). Globally, Africa has the highest estimated age-standardized cervical cancer incidence and mortality rates at 27.6 and 17.5 per 100,000 cases respectively. While elevated levels of vaginal pH have been associated with vaginal inflammation and infection, evidence demonstrating the nature of the relationship between vaginal pH and HPV infection are not well characterized. Among a cohort of menopausal/post-menopausal Senegalese women, we observed a 3.01-fold increase in HPV in women with vaginal pH levels between 5.0-5.9 relative to women with vaginal pH levels between 4.0-4.9 (95% CI[1.24,7.30], p=0.02). For women with vaginal pH levels between 5-5.9 there was a 3.48-fold increase in the aOR of any HPV infection relative to women with vaginal ph levels between 4.0-4.9 (95% CI [1.38, 8.77], p=.01). Among women of vaginal pH levels ≥7 there was a 4.84-fold increase in the aOR of any HPV infection relative to individuals with vaginal pH levels between 4.0-4.9 (95% CI [1.84-12.71], p=0.001). Our findings suggest the presence of a trend in elevated vaginal pH and the OR of any HPV infection among a sample of women attending Fann and Pikine clinics in Dakar, Senegal, particularly among menopausal/post-menopausal women who are HIV positive.

Background

Sub-Saharan Africa has the greatest global burden of vaginal dysbiosis (1) and the highest prevalence of HPV infection among women (24%) (2-4). Globally, Africa has the highest estimated age-standardized cervical cancer incidence and mortality rates at 27.6 and 17.5 per 100,000 cases respectively (5). While elevated levels of vaginal pH have been associated with vaginal inflammation and infection, evidence demonstrating the nature of the relationship between vaginal pH and HPV infection are not well characterized (6-8). The predominance of lactic acid and hydrogen peroxide producing lactobacilli in the vaginal flora is associated with a healthy vaginal microbiome (9, 10). Normal pH is considered to range between 3.5-4.5 in premenopausal women and ≥4.7 in premenarchal and postmenopausal women (11). Changes in the composition of the vaginal flora driven by a decline in Lactobacillus species result in increased vaginal pH. Elevated vaginal pH can signal vaginal dysbiosis (12-14), infection, and inflammation (15) of the genital tract-- all risk factors associated with the acquisition and persistence of HPV infection (10, 16, 17).

Increased bacterial diversity in the vaginal microbiome has been associated with elevated vaginal pH (13), HPV infection (9-10, 18), *Trichomonas vaginalis* (TV) (19), HIV acquisition (13, 20), and *Bacterial vaginosis* (BV) (6, 21). Studies have documented BV (19) and TV (22, 23) are each associated with prevalent and incident HPV infection in addition to the persistence of HPV infection (24). Both TV and BV are marked by elevated pH levels with BV typically occurring at a vaginal pH levels >4.5 (25) and TV occurring between a vaginal pH 5-6 (11). BV has been characterized as a poly-microbial dysbiosis dysbiosis marked by a decrease in lactobacilli (10, 20-21, 26). The clinical features of TV can range from acute severe inflammation to an asymptomatic carrier state (25). A longitudinal study of TV in adolescent girls aged 14-17 found untreated infections remained asymptomatic for a minimum of 3 months (27). While the exact incubation period of TV is unknown, in vitro studies suggest a period of 4-28 days (28).

HPV infection has been identified as a necessary but not sufficient factor in the development of cervical cancer (10). Most cases of cervical cancer caused by HPV are attributable to persistent infection with carcinogenic or "high risk" HPV strains; these include: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 (29). In 2012, GLOBCAN estimates placed the annual incidence of cervical cancer at 528,000 cases/year with 84% of cases and 87% and deaths due to cervical cancer occurring in developing nations (5). Estimates of the prevalence of HPV infection in Dakar, Senegal range from 12% in women with normal cytology and 76% among those with high-grade squamous intraepithelial lesion (HSIL) in women aged >35 to 27.1% among HIV negative women in the same population (30). In a study estimating the overall prevalence of HPV in four regions across Senegal, the reported prevalence of HPV among women aged 18 years-80 years in Dakar was 20.1%, including 17.4% for high-risk HPV infections (31). In West Africa age standardized incidence rate of cervical cancer is 29.3 per 100,000 placing it just below the 30 per 100,000 thresholds for high-risk regions identified by GLOBOCAN 2012 (5, 32). Research on the composition of the vaginal microbiome in sub-Saharan African women as it relates to HPV is limited (33, 34). Clinicians have used elevated pH levels as a biomarker for vaginal dysbiosis. Elevated vaginal pH may serve as a biomarker in determining risk of HPV acquisition and persistence of HPV infections and may inform the underlying biological mechanisms driving the complex interplay between vaginal pH the composition of the vaginal microbiota and HPV types.

Methods

Study setting

Our study is a secondary analysis of data taken from the Cervical Hypermethylation study, a clinic-based longitudinal study of women aged 15-84 in Dakar, Senegal. During the 2006-2011 study period, a total of 1679 women were screened at two study clinics, 323 from the Fann University Hospital Infectious Disease Clinic and 1356 from the Pikine outpatient primary care clinic. Our sample features data collected from consenting women aged 18-84 enrolled into a

follow-up study. Demographic data, sexual and reproductive history, and behavioral data were collected during enrollment. Serological assays for HIV-1 and HIV-2, polymerase-chain-reaction (PCR) detection of HPV DNA, cytological screening, and vaginal pH were performed on blood and cervical cellular samples collected during clinical exams at baseline and every four months thereafter. A total of 1359 study visits are recorded from 462 participants, with each woman contributing between 1-11 data points.

Study Design

This study is a secondary data analysis of repeated cross-sectional measures in a cohort of Senegalese women ages 18-84.

HPV polymerase-chain-reaction (PCR) assays were conducted to detect the presence of HPV DNA with the use of highly specific MY09 and MY11 consensus primers (35, 36) targeting the L1 region. β-globin gene detection was performed to confirm the detection of viral DNA. Dot blot hybridization methods previously outlined (37) were used to genotype HPV strains located in positive samples. A liquid bead microarray assay identified a total of 37 HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-59, 61, 62, 64, 66-73, 81-84, 89[1], and HPV 82 sub-type IS39) (37).

Our primary exposure of interest is vaginal pH. Vaginal pH was assessed using non-bleeding colorpHast® pH indicator strips (EMD Chemicals Inc., Gibbstown, NJ USA) immersed in a weakly buffered solution for 1-10 minutes or until there is no apparent further color change. Manufacturer guidelines instruct study staff to record pH level while the pH strip is still moist. Increasing pH levels are represented by a yellow to blue color gradient with pH values

[1] Also known as CP6108

demarcated at 4.0, 4.4, 4.7, 5.0, 5.3, 5.5, 5.8, 6.1, 6.5, 7.0, though pH readings maybe approximated for color levels between the indicated thresholds. (Figure 1)

Confounder selection

A Directed Acyclic Graph (DAG) was created to identify potential confounders and effect modifiers (Figure 2) using DAGitty 3.0 software (60 38) according to findings from published literature. Age (7, 54-59) 39-44, douching (45-53 43, 46-53), and multiple sex partners (50) were identified as the minimum set of confounders required to estimate the direct effect of vaginal pH on prevalent HPV infections. HIV is a potential mediator and collider of the association between elevated vaginal pH and HPV infection due to the unidirectional association between elevated vaginal pH and HIV acquisition and the bidirectional association between HIV and HPV (54-59 40-45). The DAG illustrates unidirectional association between elevated vaginal pH and HIV acquisition and the bidirectional association between HIV and HPV. This suggests HIV status is a potential mediator in the association of vaginal pH and HPV infection and the descendent of the outcome and exposure. Since we are interested in determining the total effect of vaginal pH on HPV infection, we will not attempt to decompose the direct and indirect effects of vaginal pH on HPV infection mediated through the HIV pathway. However, given HIV status is fixed at baseline we will assess HIV status at baseline as a potential confounder.

Statistical analysis

This study is a secondary data analysis of repeated cross-sectional measures in a cohort of Senegalese women ages 18-84. We performed a logistic regression using GEE and specifying an independent correlation structure to calculate the odds ratio of any HPV infection given vaginal pH levels. Potential confounders identified *a priori* include practicing douching, multiple sex partners, smoking, and age. While smoking was also a potential confounder the prevalence of smoking in the sample is exceeding very rare (6/453) and unlikely to confer a significant effect on

the association between vaginal pH and HPV infection. Variables were considered confounders if the difference between adjusted and crude ORs exceeds 10%. Statistical analysis were performed using STATA version 11 (STATA Corp., Texas, USA).

Results

Univariate and Bivariate analyses

A total of 1359 study visits are recorded from 462 participants, with each woman contributing between 1-11 data points. The majority of data in the sample comes from the first 5 clinic visits of each participant: the median number of data points contributed by each individual 2 (IQR: 1,4).

We performed a univariate analysis of demographic variables at baseline (Table 1). Nearly 95% of participants reported being born in Senegal; the remainder reported having been born in another African country (5.2%). The average age of women was 43.1 (SD=10) and 62% of women reported not having undergone menopause. Very few participants reported smoking (1.3%) or drinking (3.3%), respectively. The majority of observations came from women who reported having a primary education (51.9%) or no formal education (30.9%). Just under 39% percent of participants reported practicing douching and the median number of times these women douched over 30 days was 28 (IQR=4.0, 30.0). The majority of participants in the sample reported having a single sexual partner over the course of their life (59.1%). Overall, most of the women reported being widowed (39.0%) or in a polygamous marriage (32.9%). The vast majority of women reported not using any form of contraception (82.8%).

We performed a bivariate analysis of demographic variables and factors associated with vaginal pH and HPV, respectively (Tables 2). The median vaginal pH for women who reported three or more partners was 6.1 compared to 5.8 for women who reported having either a single or just two sexual partners over their lifetime, respectively. The median vaginal pH of women who attended Fann clinic was 6.1 and 5.8 in Pikine, respectively. The distribution of higher vaginal pH levels

was skewed towards older women. Vaginal pH appears to increase modestly with age. This is consistent with the observation that higher vaginal pH levels appear occur more frequently among women who reported they had undergone menopause at baseline. There is no apparent difference in the distribution of vaginal pH levels by marital status. The distribution of individuals who reported practicing douching appears balanced across levels of vaginal pH. While birthplace was similarly well balanced across levels of vaginal pH,

We also performed a bivariate analysis of demographic variables and factors associated with HPV (Tables 3). The majority of women who screened positive for HPV at baseline (n=250) also reported having either 2 (35%) and 3 or more (19%) sexual partners over their lifetime, respectively. A greater proportion of widows, concubines, and women in monogamous relationships were HPV positive. At baseline, 67% of individuals attending Fann clinic tested positive for any HPV infection compared to 40% of women attending Pikine. Prevalent HIV infections were more common among women who were positive for HPV at baseline (57% vs 16%). The majority of women with HIV had vaginal pH levels between 5.0-6.8. The distribution of women who reported undergoing menopause is balanced among women who screened positive for any HPV infection at baseline. Douching was also more common among women who screened positive for HPV at baseline. Nineteen of the 24 participants who reported being born outside of Senegal screened positive for HPV. Transition probability tables revealed that though the majority of women who screened positive for HPV at one clinic visit remained positive at their next visit, only 19% of women attending Fann clinic cleared an HPV infection at a subsequent visit compared to 39% of women attending Pikine. Among women without HIV, 30% cleared a HPV infection at a subsequent visit after having screened HPV positive during their last visit, while only 17% of women with HIV were able to did so. (data not shown).

Simple and multivariate logistic regression analysis

The data did not demonstrate evidence of significant confounding by age, menopausal status, or lifetime sexual partners at any level of vaginal pH (Table 4). However HIV was a significant confounder for individuals with the highest and lowest vaginal pH levels. Individuals whose vaginal pH levels ranging between 5.0-5.9 had a 1.55–fold increase in the odds of HPV infection relative to individuals with vaginal pH levels between 4.0-4.9 95% CI[1.05,2.29],) suggesting an attenuation of the association towards the null. Alternatively, after controlling for HIV status, the odds of HPV infection were 2.14 greater among individuals with vaginal pH levels ≥7 than that of individuals with vaginal pH levels between 4.0-4.9 (95% CI[1.27,3.60]).

There was no presence of significant statistical interaction in the association between vaginal pH and odds of HPV infection by either HIV status, clinic attended, or the practice of douching, respectively (Table 5). There was a significant interaction present by estimated menopausal status for individuals whose vaginal pH was ≥7, however lower levels of vaginal pH demonstrated no evidence of statistical interaction. Holding vaginal pH level constant the odds of HPV infection was 5.04 times greater in individuals who were estimated to have gone through menopause (95% CI [1.50,16.9]).

A logistic regression of vaginal pH on the odds of HPV infection revealed a statistically significant but modest effect of increasing vaginal pH on the odds of prevalent HPV infection (Table 6). The unadjusted OR of any HPV infection when vaginal pH ranges between 5-5.9 was estimated to be 1.73 (95% CI [1.20, 2.50]) times that of individuals with vaginal pH levels between 4.0-4.9. Individuals with vaginal pH levels between 5.0-5.9 had a 1.77-fold increase in the odds of HPV infection relative to individuals with vaginal pH levels between 4.0-4.9 (95% CI[1.24, 2.51]). When vaginal pH are at the highest level of vaginal pH (≥7) the odds ratio was 1.85 (95% CI[1.10, 3.10]) times that of individuals with vaginal pH levels between 4.0-4.9. We

calculated the odds of HPV infection controlling for HIV status and stratified by the estimated menopausal status using two separate logistic models (Table 6). The aOR among premenopausal women was attenuated towards the null for and not significant for each level of vaginal pH. Relative to individuals with vaginal pH levels between 4.0-4.9 the aOR was: 1.35 for vaginal pH levels between 5-5.9 (95% CI[0.87-2.09]); 1.38 for individuals with vaginal pH levels between 6-6.9 (95% CI[0.92-2.08]); and 0.96 among individuals with vaginal pH levels ≥7 (95% CI[0.43-2.14]). Among menopausal/post-menopausal women, the we observed a 3.01-fold increased in women with vaginal pH levels between 5.0-5.9 relative to women with vaginal pH levels between 4.0-4.9 (95% CI[1.24,7.30]). For women with vaginal pH levels between 5-5.9 there was a 3.48-fold increase in the aOR relative to women with vaginal ph levels between 4.0-4.9 (95% CI [1.38, 8.77]) after controlling for HIV status and stratifying by menopausal status. Among women of vaginal pH levels ≥7 there was a 4.84-fold increase in the aOR relative to individuals with vaginal pH levels between 4.0-4.9 (95% CI [1.84-12.71]) after controlling for HIV status and stratifying by menopausal status.

Limitations

Our study has a number of limitations. First, given the cross-sectional design of our study we are unable to infer the temporal relationship between vaginal pH and HPV. Our study is predicated on the assumption that elevated vaginal pH signals dysbiosis and facilitates opportunistic infections, which have been associated with a higher risk of progressing HPV infections (10). The distribution of vaginal pH in the sample was heavily weighted towards women with vaginal pH levels between 5.0-6.9, leaving a small sample size in our reference group (n=47) and in individuals with vaginal pH levels ≥7 (n=18). Persistent elevated vaginal pH levels may signal prevalent disruptions in the composition of the vaginal microbiome and may also facilitate the recurrence of bacterial infection, which may, in part, account for the increased vaginal pH levels observed across the sample. While observations within an individual are likely to be correlated with one another, the lack of multiple data points for participants coupled with wide and

unequally spaced intervals for those with multiple visits precludes the use of an autoregressive correlation structure. In an exploratory analysis of our covariates we found reporting of multiple sex partners, HIV status, and vaginal douching remained constant for observations within an individual. Similarly age categories remained fixed across observations within an individual, though this may be due to the relative length of participation in the study and the 10-year bands created for each age category. There are no apparent time-varying effects of confounders represented in our analysis as these values are assessed at baseline and remained fixed for individuals during the duration of their participation in the study. Approximately 36% of our participants contributed just a single observation point and 46% percent of participants have at least 3 observations. While most participants contributed 2 observations the average number of days elapsed between 1st and 2nd clinic visit is 273 (std dev=262). The number of cumulative days from between the first and second visits ranges from 1 to 1267 days. However our use of a sandwich estimator produces standard errors robust to the misspecification of the correlation structure for observations over time (60). We also verified our time varying-exposure did not appear to correlate with lagged vaginal pH, this may be due to the fact observations on average are far apart in time and vaginal pH can varies with respect to age and cycle of menstruation. While our data include some missing data, missingness does not exceed 10% in any variable.

Finally, while vaginal pH may serve as a proxy measure of vaginal dysbiosis, our data do not include microbiological samples to confirm the composition and diversity of bacteria present in the vaginal flora. In addition to vaginal pH, vaginal discharge is also a clinical feature used by clinicians to inform diagnoses of vaginitis. Clinicians in the study documented the presence of abnormal vaginal discharge and TV following inspection and the assessment of symptoms reported by participants. Our estimates may still be subject to some residual confounding by asymptomatic imbalances in the vaginal microbiome.

Discussion

Our findings suggest the presence of a trend in elevated vaginal pH and the OR of any HPV infection among a sample of women attending Fann and Pikine clincs in Dakar, Senegal, particularly among menopausal and post-menopausal women who are HIV positive. Future research may explore the effect of recurrent vaginal dysbiosis and elevated vaginal pH on the HPV acquisition and persistence.

A study of women ages 20-49 participating in the 2003-04 US National Health and Nutrition Examination Survey investigators found douching was positively associated with all HPV infections (RR = 1.26; 95% [1.03-1.54]) and HPV high-risk types (RR = 1.40 95% [1.09-1.80]) (43), though authors note residual confounding by STIs and microbiome changes may attenuate the observed association towards the null. While research has documented the association between vaginal douching and increased diversity (46) the direct effect of douching on HPV and indirect effect of vaginal douching on HPV via Bacterial Vaginosis (BV) and Trichomoniasis Vaginitis (TV) is not well established. Insofar as elevated vaginal pH is indicative imbalances in the vaginal microbiome we did not observe a significant confounding effect of vaginal douching on the odds of HPV infection at increasing levels of vaginal pH.

Clarke et al. (7) found age was a significant effect modifier in the association between vaginal pH and HPV. The vaginal pH of premenarchal and postmenopausal women is known to be greater on average than women who are between those life stages due to lower levels of estrogen production in premenarchal and postmenopausal women. The distribution of age in our sample is truncated due to the exclusion of women aged less than 18 yrs. While this prevented us from evaluating the effect of age at all relevant life stages we were able to evaluate the presence of effect modification by menopausal status on the association of interest. We discovered a significant effect among menopausal/post-menopausal women and no effect among

premenopausal women. This suggests menopausal status is an appropriate alternative to the use of a continuous or categorical parameterization of age when examining biologic factors related to HPV.

Individuals whose vaginal pH levels are more neutral or alkaline are at a significantly increased risk of acquiring HIV infection (45, 46). Moreover in a 2013 study conducted by Aldunate et al (2013) investigators reported a vaginal concentration of 1% lactobacillus lactic acid demonstrated potent HIV virucidal activity (59). These studies illustrate a predominance of lactobacillius in the vaginal microbiota may be associated with reduced susceptibility to HIV acquisition which may suggest HIV infection may be more common among women whose elevated vaginal pH levels stem from low levels of lactobacillus in the vaginal flora. Research has documented a bidirectional interaction between HPV infection and HIV. HIV infected individuals are immunosuppressed and as a result may have more difficulty clearing HPV infections. Studies conducted in both male and female populations report HPV infections are more common in HIV-infected individuals (54-56). In a study of 468 heterosexual south African couples Mbulawa et al. found incident HPV infections and the inability to clear HPV infections occurred more frequently among HIV-infected individuals (55). While HIV acquisition is also associated with HPV infection the mechanisms driving this association remain unclear. Summary evidence from a systematic review and meta-analysis conducted by Lissouba et al detected a 2.0-fold increase in risk of HIV acquisition among individuals infected with any HPV (OR: 2.0 95% CI 1.6-2.5) (38). A summary of evidence from longitudinal data in women found an increased hazard of HIV acquisition with any prevalent HPV (HR = 2.06 95% CI = 1.44–2.94) (61). After including an interaction term for pH and HIV status we found no evidence to support a significant change in the odds of HPV infection (Vaginal pH levels 5.0-5.9: OR=0.72 95% CI [0.32, 1.62]; Vaginal pH levels 6.0-6.9= OR=0.75 95% CI [0.34, 1.67]; and Vaginal pH levels ≥7 OR=1.13 95% CI [0.36, 3.56]). While in the current data the association between vaginal pH levels and HPV detection did not appear to vary among individuals HIV+ and HIV- individuals, the association has been

documented in the same population in prior studies (62-64). Future research may consider exploring mediation of the association between vaginal pH and HPV acquisition and recurrence by HIV with data more suitable to longitudinal analysis.

Figure 1 Vaginal pH strips used to measure vaginal pH at each clinic visit

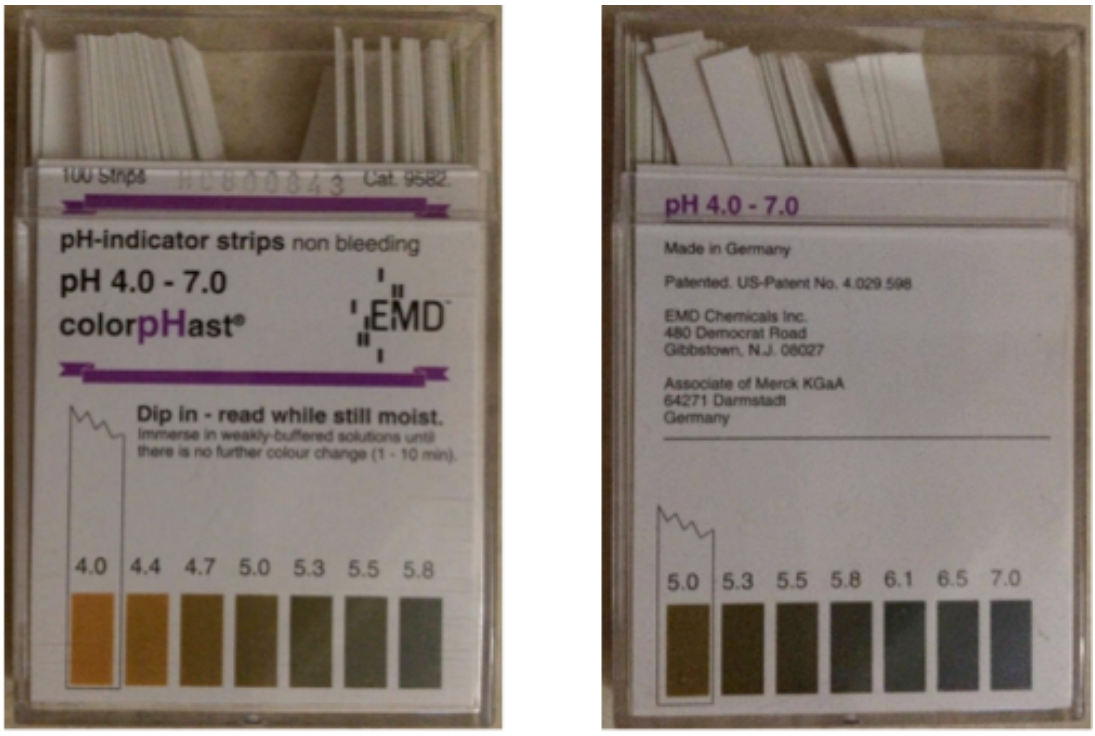
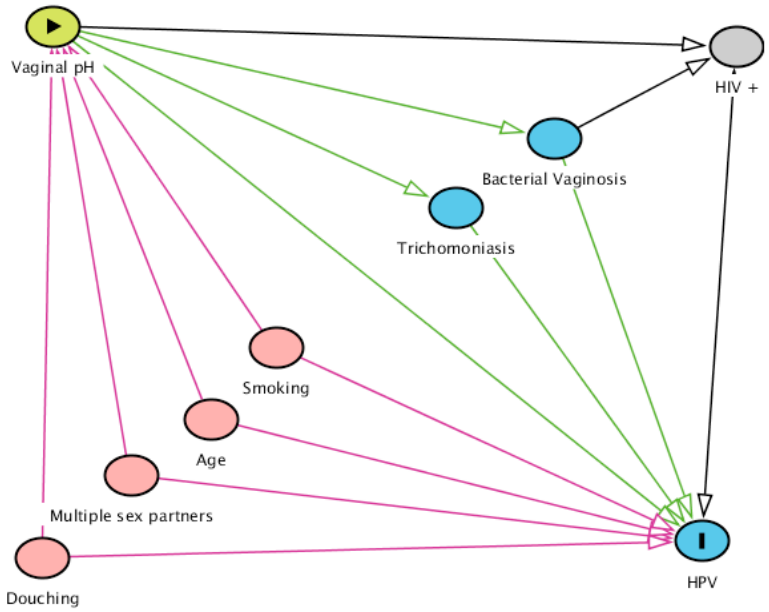


Figure 2 Directed Acyclic Graph illustrating association between vaginal pH and HPV infection



1. Bacterial Vaginosis (BV) is an unobserved latent variable that exists along the vaginal pH and HPV pathway and is characterized by high vaginal pH. Trichomoniasis Vaginitis (TV) is similarly associated with increased vaginal pH. HIV is a potential mediator and confounder. Douching, multiple sex partners, age and smoking are potential confounders. Red nodes indicate

a variable is a ancestor of exposure and outcome, gray indicates irrelevant nodes as they are not in the direct causal pathway, blue nodes are ancestors of outcome, green nodes are ancestors of exposure. The green node with an arrow is the exposure and the Blue node with a line is the outcome.

Table 1 Demographic variables and risk factors at baseline in Senegalese women aged 18-82

Factor	Level	n (%)
N		462
Vaginal pH, mean (SD)		5.8 (0.7)
HPV (+)		250 (54.1%)
age, mean (SD)		43.1 (10.0)
Age categories	< 25	18 (3.9%)
	25-34	69 (15.0%)
	35-44	160 (34.8%)
	45-54	155 (33.7%)
	55-64	51 (11.1%)
	65+	7 (1.5%)
Lifetime number of sexual partners	1	264 (59.1%)
	2	121 (27.1%)
	3+	62 (13.9%)
Clinic	Pikine	223 (48.4%)
	Fann	238 (51.6%)
Current smoker		6 (1.3%)
Alcohol use		15 (3.3%)
HIV (+)		171 (37.9%)
Marital Status	Monogamous	19 (4.2%)
	Polygamous	150 (32.9%)
	Widowed	178 (39.0%)
	Concubine	49 (10.7%)
	Unmarried	60 (13.2%)
Menopausal Status	Premenopausal	283 (62.5%)
	Menopausal/post-menopausal	111 (24.5%)
	Uncertain	59 (13.0%)
Practice douching		177 (38.7%)
Frequency of douching over the last 30 days, median (IQR)		28.0 (4.0, 30.0)
Birthplace	Senegal	435 (94.8%)
	Other African	27 (5.2%)
Education	None	237 (51.9%)
	Primary	141 (30.9%)
	Secondary	67 (14.7%)

	≥University/ College	12 (2.6%)
Current method of contraception	None	217 (82.8%)
	Oral Contraception Pills	12 (4.6%)
	Injections	11 (4.2%)
	Female condom	7 (2.7%)
	Spermicide	1 (0.4%)
	IUD	8 (3.1%)
	Norplant	5 (1.9%)
	Tubal ligation	1 (0.4%)
Vaginal pH level	4.0-4.9	47 (10.2%)
	5.0-5.9	194 (42.0%)
	6.0-6.9	203 (43.9%)
	7.0+	18 (3.9%)

Table 2 Factors associated with vaginal pH level at baseline

Factor	Level	4.0-4.7	5.0-5.8	6.0-6.8	7.0+	p-value
N		47	194	203	18	
HPV		13 (27.7%)	110 (56.7%)	118 (58.1%)	9 (50.0%)	0.002
HRhpv		8 (17.0%)	79 (40.7%)	89 (43.8%)	7 (38.9%)	0.009
Age, mean (SD)		42.2 (7.8)	43.8 (8.9)	41.9 (10.9)	51.3 (11.3)	<0.001
Lifetime sexual partners	1	36 (78.3%)	100 (52.6%)	115 (59.6%)	13 (72.2%)	0.015
	2	7 (15.2%)	63 (33.2%)	46 (23.8%)	5 (27.8%)	
	3+	3 (6.5%)	27 (14.2%)	32 (16.6%)	0 (0.0%)	
Age categories	< 25	2 (4.3%)	5 (2.6%)	11 (5.5%)	0 (0.0%)	<0.001
	25-34	6 (12.8%)	21 (10.8%)	41 (20.4%)	1 (5.6%)	
	35-44	16 (34.0%)	75 (38.7%)	65 (32.3%)	4 (22.2%)	
	45-54	21 (44.7%)	73 (37.6%)	56 (27.9%)	5 (27.8%)	
	55-64	2 (4.3%)	18 (9.3%)	25 (12.4%)	6 (33.3%)	
	65+	0 (0.0%)	2 (1.0%)	3 (1.5%)	2 (11.1%)	
Clinic	Pikine	33 (70.2%)	101 (52.1%)	74 (36.6%)	15 (83.3%)	<0.001
	Fann	14 (29.8%)	93 (47.9%)	128 (63.4%)	3 (16.7%)	
Current smoker		0 (0.0%)	4 (2.1%)	2 (1.0%)	0 (0.0%)	0.59
HIV (+)		5 (10.6%)	88 (46.1%)	76 (39.0%)	2 (11.1%)	<0.001
Marital Status	Monogamous	1 (2.2%)	5 (2.6%)	13 (6.5%)	0 (0.0%)	0.37
	Polygamous	18 (39.1%)	65 (33.9%)	62 (31.0%)	5 (27.8%)	
	Widowed	19 (41.3%)	78 (40.6%)	72 (36.0%)	9 (50.0%)	
	Concubine	5 (10.9%)	22 (11.5%)	22 (11.0%)	0 (0.0%)	
	Celibate	3 (6.5%)	22 (11.5%)	31 (15.5%)	4 (22.2%)	
Menopausal Status	Premenopausal	40 (85.1%)	116 (61.4%)	123 (61.8%)	4 (22.2%)	<0.001
	Menopausal/post-menopausal	3 (6.4%)	49 (25.9%)	49 (24.6%)	10 (55.6%)	
	Uncertain	4 (8.5%)	24 (12.7%)	27 (13.6%)	4 (22.2%)	
Estimated Menopausal Status	Menopausal/post-menopausal	6 (12.8%)	69 (36.5%)	72 (36.2%)	14 (77.8%)	<0.001
Practice douching		18 (38.3%)	83 (43.2%)	72 (36.0%)	4 (22.2%)	0.23
Frequency of douching over last 30 days, median (IQR)		20.0 (5.0, 30.0)	15.0 (4.0, 30.0)	30.0 (8.0, 30.0)	10.0 (4.5, 21.0)	0.23
Birthplace	Senegal	47 (100.0%)	186 (96.4%)	186 (92.5%)	16 (88.9%)	0.23
	Other African	0 (0.0%)	7 (3.6%)	15 (750%)	2 (11.1%)	

Table 3 Factors associated with HPV status at baseline

Factor	Level	HPV(-)	HPV(+)	p-value
N		212	250	
Vaginal pH, mean (SD)		5.7 (0.8)	5.9 (0.6)	0.003
Age, mean (SD)		44.0 (10.2)	42.3 (9.7)	0.073
Lifetime number of sexual partners	1	153 (73.2%)	111 (46.6%)	<0.001
	2	39 (18.7%)	82 (34.5%)	
	3+	17 (8.1%)	45 (18.9%)	
Age categories	< 25	8 (3.8%)	10 (4.0%)	0.39
	25-34	31 (14.6%)	38 (15.3%)	
	35-44	64 (30.2%)	96 (38.7%)	
	45-54	78 (36.8%)	77 (31.0%)	
	55-64	28 (13.2%)	23 (9.3%)	
	65+	3 (1.4%)	4 (1.6%)	
Clinic	Pikine	134 (63.2%)	89 (35.7%)	<0.001
	Fann	78 (36.8%)	160 (64.3%)	
Current smoker		2 (0.9%)	4 (1.6%)	0.52
HIV (+)		34 (16.0%)	137 (57.3%)	<0.001
Marital Status	Monogamous	6 (2.8%)	13 (5.3%)	<0.001
	Polygamous	82 (38.9%)	68 (27.8%)	
	Widowed	91 (43.1%)	87 (35.5%)	
	Concubine	13 (6.2%)	36 (14.7%)	
	Celibate	19 (9.0%)	41 (16.7%)	
Menopausal Status	Premenopausal	129 (61.4%)	154 (63.4%)	0.38
	Menopausal/post-menopausal	57 (27.1%)	54 (22.2%)	
	Uncertain	24 (11.4%)	35 (14.4%)	
Estimated Menopausal Status	Menopausal/post-menopausal	77 (36.7%)	84 (34.6%)	0.64
Practice douching		71 (33.5%)	106 (43.3%)	0.032
Frequency of douching over the last 30 days, mean (IQR)		12.0 (4.0, 30.0)	30.0 (4.5, 30.0)	0.26
Birthplace	Senegal	207 (97.6%)	228 (92.3%)	0.042
	Other African	5 (2.4%)	19 (7.7%)	
Vaginal pH	4.0-4.9	34 (16.0%)	13 (5.2%)	0.002
	5.0-5.9	84 (39.6%)	110 (44.0%)	
	6.0-6.9	85 (40.1%)	118 (47.2%)	
	7.0+	9 (4.2%)	9 (3.6%)	

Table 4 Evaluating effect of confounding by multiple sex partners, age, douching, estimated menopausal status and HIV status at baseline on association between vaginal pH and HPV

Odds Ratio adjusted for multiple lifetime partners, age, douching, and HIV+												
Crude			**Adjusted**									
			Multiple sex partners		Age		Vaginal douching		Estimated Menopausal Status		HIV (+)	
Vag pH	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
4.0 – 4.9	1	REF	1	REF	1	REF	1	REF	1	REF	1	REF
5.0 – 5.9	1.73	1.20-2.50	1.6	1.09-2.34	1.77	1.23-2.55	1.77	1.23-2.55	1.73	1.20-2.50	1.55	1.05-2.29
6.0 – 6.9	1.77	1.24-2.52	1.7	1.18-2.44	1.84	1.30-2.62	1.84	1.30-2.62	1.73	1.22-2.46	1.69	1.16-2.47
7.0 – 7.9	1.85	1.11-3.10	1.7	0.98-2.92	1.99	1.18-3.35	1.99	1.18-3.35	1.79	1.08-2.97	2.14	1.27-3.60

Table 4 Evaluation of potential effect modification of the association between vaginal pH and HPV

Odds Ratio by estimated menopausal status, HIV status, clinic, and douching								
Vaginal pH Level	4.0-4.9		5.0-5.9		6.0-6.9		7.0+	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Statistical Interaction OR								
Menopausal x Vaginal pH	1	REF	2.24	0.88-5.70	2.16	0.85-5.46	5.04	1.50-16.9
HIV (+) x Vaginal pH	1	REF	0.72	0.32-1.62	0.75	0.34-1.67	1.13	0.36-3.56
Fann clinic x Vaginal pH	1	REF	1.17	0.54-2.54	0.69	0.33-1.46	1.31	0.46-3.72
Practice douching x Vaginal pH	1	REF	0.89	0.42-1.86	0.78	0.38-1.60	0.46	0.15-1.40
Stratified Analysis OR								
Menopausal/post-menopausal	1	REF	3.38	1.46-7.82	3.3	1.42-7.66	4.38	1.75-10.9
Premenopausal	1	REF	1.51	1.00-2.27	1.53	1.04-2.26	0.87	0.39-1.92
HIV (+)	1	REF	1.29	0.70-2.38	1.43	0.77-2.67	2.41	0.97-6.01
HIV (-)	1	REF	1.78	1.06-3.02	1.9	1.16-3.12	2.12	1.07-4.23
Pikine	1	REF	1.61	0.88-2.93	2.25	1.23-4.12	1.9	0.90-4.03
Fann	1	REF	1.88	1.16-3.05	1.56	1.00-2.42	2.5	1.21-5.15
Practice douching	1	REF	1.65	0.93-2.93	1.59	0.92-2.74	1.15	0.46-2.89
Do not practice douching	1	REF	1.86	1.16-3.00	2.03	1.28- 3.24	2.5	1.33-4.68

Table 5 Comparison of crude and adjusted odds of HPV infection given vaginal pH level

Odds Ratio adjusted for HIV+ by Estimated Menopausal status						
Crude			**Adjusted**			
			Premenopausal		Menopausal/post-menopausal	
Vag pH	OR	95% CI	OR	95% CI	OR	95% CI
4.0 – 4.9	1	REF	1	REF	1	REF
5.0 – 5.9	1.73	1.20-2.50	1.35	0.87-2.09	3.01	1.24-7.30
6.0 – 6.9	1.77	1.24-2.52	1.38	0.92-2.08	3.48	1.38-8.77
7.0 – 7.9	1.85	1.11-3.10	0.96	0.43-2.14	4.84	1.84-12.7

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