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Screening for abnormal vaginal microflora by self-assessed vaginal pH does not enable detection of STI's in Ugandan women

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Short title "Screening for Abnormal Vaginal Flora and STI in Ugandan Women"

Abstract

Objective

Is self-assessed vaginal pH measurement to detect abnormal vaginal bacterial microflora (AVF) an adequate pre-screening method for detection of genital sexually transmitted infections (STI's)?

Material and methods

360 Ugandan women tested themselves with a gloved finger and a pH color strip. PCR for Bacterial vaginosis (BV)-associated bacteria were tested by PCR for *Mycoplasma hominis*, *Ureaplasma urealyticum* and/or *Atopobium vaginae*, while the STI's were diagnosed by positive PCR for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and/or *Trichomonas vaginalis*.

Results

A strong correlation was found between self-assessed pH values and BV-associated bacteria ($p < 0.0001$), but not with STI's, not as single infections, nor in general.

Conclusion

Self-measured vaginal pH correlated well with markers of high risk microflora types such as BV or aerobic vaginitis, but not with STI's. Hence, in a screening program addressing AVF in low resource countries, extra specific tests are required to exclude STI's.

(Word count: 144)

Keywords: bacterial vaginosis; aerobic vaginitis; screening; abnormal vaginal flora; lactobacillary grades; PCR; M genitalium; C trachomatis; N gonorrhoeae; T vaginalis

Introduction

Vaginal infections, like bacterial vaginosis (BV), *Candida* vaginitis (CV) and aerobic vaginitis (AV) occur frequently in all classes of women, worldwide, and are a frequent source of recurrent or recalcitrant complaints. They pose an increased risk of pelvic infection, post-abortion infection, postoperative wound infections, and adverse pregnancy outcomes^{1-3 4-18}. Acquisition of HIV, HSV-2 and *Chlamydia* is increased in women with abnormal vaginal flora (AVF) and BV^{19;20 21}. African women are more vulnerable, due to BV prevalence rates surpassing 30 to 50%^{22;23}

In former communications we conclude self-screening for increased vaginal pH is a feasible, acceptable and efficient method to detect high risk vaginal flora types in Ugandan women²⁴. Besides AVF, AV and BV, which can be pre-screened by vaginal pH measurements, also other genital infections like *Chlamydia*, gonorrhea, *Mycoplasma genitalium* and *Trichomonas vaginalis* also have a serious impact on the burden of acute disease, long term sequelae and pregnancy complications. Hence, it would be ideal if increased pH would also be a marker for these infections, and predict their potential presence. However, although AVF and BV predispose to STI acquisition, it is currently unknown whether the substitute screening with pH self-measurement also enables an increased risk of detecting the actual presence of such STIs.

Material and methods

Subjects

360 consecutive women, of whom 148 pregnant (41%) presenting at the outpatient clinics at Mulago and Mbuike Hospitals in Kampala, Uganda examined themselves by introducing one gloved finger in the vagina and spread the obtained vaginal fluid on a glass slide, on which a pH strip was fixed (PH-Fix, range pH 3.6 – 6.1 Macherey-Nagel GmbH & Co. KG, Düren, Germany). After this, patients had to rinse their gloved index finger into a vial filled with ethanol-based BD SurePath™ Preservative medium (BD SurePath™; BD Diagnostics –

TriPath, Burlington, NC, USA), which was kept for later PCR analysis. After 1 minute, the patients had to interpret the color of the pH strip as yellow (pH range ≤ 4.4), orange (pH range 4.5-4.7) or red (pH range > 4.7). In this study, pH ≤ 4.4 was defined as 'low pH', pH between 4.5 and 4.7 as 'intermediate' and pH > 4.7 as 'high'. As pH tends to be higher in African women, a pH > 4.7 was considered 'abnormal'. The slide was kept for later microscopy after rehydration with saline by a blinded investigator at Femicare, Tienen, Belgium, according to a standardized protocol²⁵. Demographic data like age, living area, medical, sexual and obstetrical history were shown in a former paper assessing the acceptance rates of the self-sampling technique²⁴. The study was approved by the ethical committee of the Kampala University Hospital and all patients signed informed consent before entering the study.

Laboratory testing

At AML laboratories in Antwerp, Belgium, samples were processed on the Abbott m2000sp instrument (600 μ l) according to the manufacturer's instruction. Final extracted DNA elution was done in 120 μ L, followed by detection of *C. trachomatis* (CT) and *N. gonorrhoeae* (NG) on the Abbott m200rt instrument using the Abbott RealTime CT/NG assay. Remaining DNA was used in singleplex qPCR for *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU), *Atopobium vaginae* (AtV) and *Mycoplasma genitalium* (MG)²⁶, and *Trichomonas vaginalis* (TV)²⁷.

Statistics

Chi² test, Chi² for trend or in case expected cells were < 5 , Fisher test, were used to compare the findings of the different self-assessment pH groups. A p value was < 0.05 was considered significant.

Funding

The study was funded by Femicare, a non-profit organisation based in Tienen, Belgium, devoted to perform, promote and guide research for women.

Results

In 338(93.9%) women with proper pH assessments and rinsing vials present, test results could be analysed. Women had a mean age of 28.3 years, 72% had an intermediate to low education, and three quarters were living in an urbanized environment.

By self-assessment, 106 (31.4%) women had a low, 145 (42.9%) an intermediate and 87 (25.7%) a high vaginal pH, and 84 (24.7%) had BV, by wet mount as well as by gram stain.

In the high pH group 38 (43.7%) were diagnosed with BV.

There was a strong association with vaginal pH and the presence of BV associated organisms as detected by PCR (**Table 1**): AtV ($p<0.001$), UU ($p<0.01$), MH ($p<0.001$) were all gradually more frequently encountered in the intermediate pH group and the high pH group, as compared to the low pH group. In the high pH group, roughly half presented BV-associated microorganisms.

Prevalences of TV, CT, NG, or MG were 7.1%, 1.5%, 1.8% and 0.6%, respectively. Of these STIs none was correlated with any self-assessed pH class. On the contrary, in the normal pH group, STI rates were similar or even slightly higher than in the intermediate pH group, and no different from the high pH group. Similarly, women in the high pH group did not harbour more STIs (12.6%) than the other women (8.8%).

Discussion

STI prevalence rates in this population of women presenting at outpatient clinics were rather low compared to other areas in Africa. In Tanzania, Zambia, and Malawi, 47.8% had BV, 18.8% TV, 2.6% CT and 1.7% NG but these were obtained in an HIV-infected population²⁸. In a neighbouring area in Uganda (Entebbe), the prevalence of infections were even higher in non-HIV infected women: BV 47.7% , TV 17.3%, NG 4.3% and CT 5.9%²⁹. Screening and treating STIs is a cornerstone in prevention of HIV transmission³⁰, according to a large prospective study³¹.

As about 40% of our population were antenatal patients, this may have influenced the prevalence of STI in this study. However, in neighbouring countries, the prevalence of BV and TV was not inferior in antenatal patients (38% and 17% respectively)³². Also the frequency of vaginal douching did not influence the pH (data not shown), and did not lower the infection rates with CT, NG and pelvic infections in another, large prospective study³³. Also other co-factors may have influenced the lower rate, for instance the urban environment, where access to medical care, education about STI prevention measures, and Western style, monogamous life style may more evident. The low prevalence of CT in this rather young population came as a surprise, but in neighbouring areas and countries the prevalence of CT was also rather low (2.6-5.9%) as compared to the prevalence in other parts of the world. It looks like *Chlamydia* may be competitive with the presence of TV and NG which are low in most countries with high CT rates.

In order to reduce HIV acquisition and other complications in low resource countries, self-sampling is a promising way to reduce risk factors like AVF/BV, CT and NG^{34 31;35}. During previous work, we had noted that AVF in African antenatal patients was associated with the presence of NG^{36;37}, TV³⁶, syphilis^{36 37} and CT^{36 38}. As microscopy is not commonly used as a screening tool in African countries, we tested whether self assessment of vaginal pH could be as a simple and cheap substitute test for both high risk vaginal flora and the presence of STIs.

As expected, the respective pH types correlated well with high risk flora types and BV-associated bacteria. *A. vaginae* is an excellent marker for BV and its concentration correlates well with the Nugent score³⁹. In the low pH group, only 13% harbored *A. vaginae*, while in the high pH group, more than half did. In the latter group, 44% were found to have BV, confirming the correlation, but at the same time demonstrates that not all cases of abnormal pH can be explained by the presence of BV. In former work we emphasized that other types of abnormal flora, such as AV and TV also have to be taken into consideration^{40;40-42}.

The use of pH assessments, however, failed to demonstrate a similar correlation with STIs. Therefore, as pH test is not reliable to exclude STIs, it is still obligatory to test for NG, CT TV and other STIs using separate tests.

This finding was a surprise, as most of these STIs are frequently associated with abnormal vaginal flora patterns, like lactobacillus deficient flora³⁶, BV or AV⁴³. We hypothesize that several other factors than AVF (AV/BV) can cause the pH to raise, and that in women with a normal pH STI's can colonize the cervix without disrupting the microflora of the vagina.

In order to improve the general health of a population in a low resource setting, high risk conditions, leading to multiple adverse outcomes and to which efficient treatment methods are available should be targeted. One such condition is the presence of BV, a condition in which the normal lactobacillary dominant microflora of the vagina is replaced by high numbers of anaerobes, and other conditions that disrupt the vaginal flora, like aerobic vaginitis⁴⁴, as these aberrations are associated with increased risk of acquiring genital infections, PID, post-operative complications, cervical cancer and adverse outcomes during pregnancy^{45;46}. Screening for high risk flora is ideally performed using microscopy of fresh wet mount, or of Gram's stained specimens of vaginal fluid. However, as these techniques and expertise are not readily available in low-resource settings, a simple, cheap, reliable and accepted method for self-screening for high risk microflora would be ideal. The method we tested, self-assessment of vaginal pH, correlated well with AVF and BV, but not with STIs. Extra specific tests to detect CT, NG, TV and MG are needed in these populations.

In conclusion, self-assessment of vaginal pH can be used as a triage tool for detection of high risk vaginal microflora patterns, but is not a good screening tool for STIs. For the latter, appropriate extra tests have to be used.

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Table 1. PCR findings of selected BV-associated bacteria and sexually transmitted infections (STI) in vaginal rinsing fluid of 338 Ugandan women, compared to self tested vaginal pH assessment

PCR	pH < 4,4 n=106	pH 4,5 – 4,7 n=145	pH > 4,7 n=87	P value
Bacterial vaginosis (BV) associated micro-organisms				
<i>A. vaginae</i>	12 (13.8%)	46 (31.8%)	45 (51%)	<0.0001
<i>U. urealyticum</i>	5 (4.7%)	7 (4.8%)	11 (12.6%)	<0.01
<i>M. hominis</i>	4 (3.8%)	6 (4.1%)	34 (39%)	<0.0001
<i>One or more of above BV bacteria present</i>	17 (16%)	54 (37.2%)	57 (65.5%)	<0.0001
Sexually transmitted infections (STI)				
<i>C. trachomatis</i>	2 (1.9%)	3 (2%)	0	n.s.
<i>N. gonorrhoeae</i>	3 (2.8%)	1 (0.6%)	2 (2.3%)	n.s.
<i>T. vaginalis</i>	8 (7.5%)	8 (5.5%)	8 (9.2%)	n.s.
<i>Mycoplasma genitalium</i>	0	1 (0.7%)	1 (1.1%)	n.s.
<i>One or more of the above STI positive</i>	11 (10.4%)	11 (7.5%)	11 (12.6%)	n.s.