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Test performance and correlates of bacterial vaginosis among women in Western Kenya

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Bacterial vaginosis (BV), a common lower genital tract infection among women, is associated with adverse birth outcomes and increased risk of HIV/STD. BV, is by far most common and yet under-diagnosed among women in sub-Saharan Africa. With poor laboratory infrastructure and lack of skilled personnel, evaluating BV associated factors and point-of-care diagnostic tests are important for prevention and management in Kenya. Vaginal swabs from 227 women (18 years or older) attending Kakamega County Referral Hospital (KCRH) in Western Kenya were tested for BV using Quickvue Advance pH and Amines test, Amsel's and Nugent's criteria as the gold standard. Structured interviews gathered information on factors associated with BV among this population. Sensitivity, specificity, positive and negative predictive values of tests in this population with a high prevalence of BV (39%; 95% CI 32.5 – 45.9 by Nugent) were Amsel: 69.2%, 87.7%, 79.8% and 78.3% respectively, and Quickvue: 56.4%, 86.1%, 75.5% and 72.1% respectively. Evaluating the performance of QuickVue test against Amsel criteria, the standard method for clinical diagnosis; offered no improvement in sensitivity 55.1%, specificity 82.4% and NPV 62.3% but improved the PPV 77.7% over when compared to Nugent's score. Occupation, condom use, lower abdominal pain, milky vaginal discharge and yeast infection were associated with BV infection. Sexual behavior contributes to high prevalence of BV among women of Western Kenya. The QuickVue test could not be recommended as either a stand-alone assay or as a confirmatory test for BV in this population.

Key words: Bacterial vaginosis, test performance, correlates, women of reproductive age, Western Kenya

INTRODUCTION

Bacterial vaginosis (BV) is the most prevalent lower genital tract infection causing vaginal discharge among women of reproductive age (Klebanoff et al., 2004). It is associated with neonatal morbidity (Laxmi et al., 2012), preterm delivery (Das et al., 2011), and low birth weight infants (Thorsen et al., 2006). BV is further associated with a 60% increased risk of HIV-1 acquisition in women (Coleman et al., 2007), and a 3·62-fold increased risk of female-to-male HIV-1 transmission (Cohen et al., 2012). Unfortunately, BV is considerably more common among women in sub-Saharan Africa and other resource poor countries, affecting up to 55% of these women (Bukusi et al., 2006). Young age at coitarche, life time number of sex partners, a recent history of multiple sex partners, and recent history of a new sex partner, poor condom use and vaginal douching are some of the important factors for BV infection (Verstaelen et al., 2010). Understanding the contributions of these factors to the

pathogenesis of BV is therefore integral in preventing and managing BV and its associated complications.

Generally, BV is diagnosed using Amsel's criteria (Amsel et al., 1983) or Nugent criteria (Nugent et al., 1991) both of which require expertise and laboratory infrastructure. These are often lacking in resource-limited settings, necessitating syndromic management of vaginal symptoms caused by BV (Romoren et al., 2007). Point-of-care testing approaches could be suitable for consistent rapid diagnosis of BV allowing accurate management. Assays detecting the presence of proline amino peptidase, trimethylamine and high vaginal pH have been developed with varying performance (Posner et al., 2005). QuickVue Advance pH and Amines test is a layered thin film chromogenic technology that contains two colorimetric tests for use in the characterization of a vaginal fluid sample: a pH test and a test that detects alkali volatilizable amines in vaginal fluid produced by anaerobic flora including *Bacteroides*, *Prevotella*, and *Gardnerella* species (Wiggins et al., 2000). Studies have shown high sensitivity and specificity of QuickVue Advance pH and Amines test compared to Amsel criteria (Charonis and Larsson, 2006). Data are lacking on the utility of this rapid test to detect BV in Kenya.

BV standard treatment with antibiotics tends to be ineffective in the long run (Verstaelen and Verhelst, 2009), more efforts may be directed to preventive measures to reduce the incidence of BV. Availability of effective and cheaper BV diagnostic method(s) as well as understanding factors associated with infection, are vital in preventing and providing improved management strategies. These were the focus of this study among women attending various reproductive health clinics in Kakamega County Referral Hospital (KCRH) located in Western Kenya.

METHODOLOGY

Study design and sample collection

This cross sectional study enrolled consenting non pregnant women of reproductive age (18 years and older) attending various clinics (family planning, post natal and STI) within KCRH in western part of Kenya. A total of 227 eligible women were conveniently sampled between January and December 2014. High vaginal swabs were collected by a trained clinician during the women hospital visits to test for BV, *Trichomonas vaginalis*, and vaginal candidiasis. Signs of vaginal discharge including amount, odor, color, and consistency were noted. Three vaginal swabs were collected simultaneously and immediately used as follows: one for Amsel scoring, the second for QuickVue Advance pH and Amines rapid testing while the third one was smeared onto clean microscope slide and taken to the laboratory where they were stained for Nugent scoring. This study was approved by Ethical Review Committee of Kenya Medical Research Institute (KEMRI/SSC No. 2545).

BV scoring using Amsel criteria

BV was assessed using Amsel criteria (Amsel et al., 1983) and was considered to be present on the basis of at least 3 of the following 4 signs: vaginal pH > 4.5, presence of amine odor on addition of 10% potassium hydroxide (whiff test), presence of 3–5 clue cells per high power field on wet-mount microscopy, and homogenous vaginal discharge.

BV scoring by Nugent's Criteria

Nugent scoring involved methanol fixing the third vaginal smear slides followed by Gram-staining and examination under oil immersion objective (1000x magnification). Grading was done using methods described by Nugent et al., (Nugent et al., 1991). Briefly, the method involved assigning a score between 0 and 10 based on the quantitative assessment of the Gram-stain for three different bacterial morphotypes:

(i) large Gram-positive rods (indicative of *Lactobacillus* spp),

(ii) small Gram-negative or variable rods (indicative of *Gardnerella*, *Bacteroides* and other anaerobic bacteria),

(iii) curved, Gram-variable rods (indicative of *Mobiluncus* spp).

Scores between 0 and 3 represented 'normal vaginal flora', between 4 and 6 'intermediate vaginal flora', and scores between 7 and 10 were considered diagnostic for 'BV'. In this study, microbiological definition of BV was a score of 7–10 by Nugent's method. Quality control of the readings was checked by rereading 10% of all slides by a second experienced microbiologist for Nugent's score.

The QuickVue Advance pH and Amines test

The QuickVue Advance pH and Amines Test (Quidel Corporation, San Diego, USA) was performed according to manufacturer's instruction. Briefly, the pH test was done first. Using a circular motion, the entire surface of pH test was rubbed with a moistened swab until completely wet. The formation of a blue PLUS sign (+) within the pH test circle indicated a positive pH test (elevated vaginal fluid pH (pH> 4.7). The amines test was done second using the same swab as described above. The formation of a blue PLUS sign (+) within the amines test circle indicated a positive amines test (indicative of the presence of alkali volatilized amines of concentrations above 0.50 millimolar).

Trichomonas vaginalis

After smearing the slide for Nugent score, this swab was then used to inoculate InPouchTV culture kit (Biomed Diagnostic, White City, OR, USA), for detection of *T. vaginalis* infection according to manufactures

Table 1: Test performance in two scenario (i) Amsel criteria and QuickVue Advance pH and Amines rapid test against Nugent score and (ii) QuickVue Advance pH and Amines rapid test against Amsel criteria.

		Bacterial vaginosis (Nugent score 7–10)				
Test	N	Concordant results (%) 95% CI	Sensitivity (%) 95% CI	Specificity (%) 95% CI	NPV (%) 95% CI	PPV (%) 95% CI
Amsel score	200	80.5(74.6 - 85.6)	69.2(58.6 - 78.7)	87.7(81.1 - 92.7)	79.8(72.5 - 86.1)	78.3(67.7 - 86.8)
Quickvue rapid test	200	74.5(68.2 - 80.2)	56.4(45.4 - 66.9)	86.1(79.2 - 91.4)	75.5(68.1 - 82.1)	72.1(60.3 - 82.2)
Amsel and Quickvue combined	200	64(57.1 - 70.3)	43.5(33.1 - 54.7)	77.1(68.8 - 83.3)	67.6(59.4 - 74.8)	55.7(43.2 - 67.5)
		Bacterial vaginosis (Amsel score)				
Quickvue rapid test	200	73(66.2 - 78.9)	55.1(42.6 - 66.9)	82.4(74.6 - 88.3)	62.3(48.9 - 74.1)	77.7(69.7 - 84.1)

Nugent score of 0–6 were considered negative, and 7–10 considered positive. PPV: positive predictive value; NPV: negative predictive value. Amsel's criteria defined as presence of any three of the four characteristics: vaginal pH > 4.5, presence of amine odor on addition of 10% potassium hydroxide (whiff test), presence of 3–5 clue cells per high power field on wet-mount microscopy, and homogenous vaginal discharge. Quickvue rapid results was used when definitive color change was clearly visible both for pH and amines

instructions. The pouches were incubated at 37◦C incubator for five days or until trichomonads were detected. The pouches were microscopic examined at 10x and 40x magnification.

Factors associated with BV

Factors associated with BV infection among this population were gathered through a face to face interviews using structured questionnaire.

Data analysis

Using Nugent criteria as the gold standard, Amsel scoring and the QuickVue rapid test performance were calculated in terms of sensitivity, specificity, and predictive values. Descriptive statistics (proportion and frequency) was used to describe the population. The bivariate and multivariate analysis were done to assess the association of selected variables with BV infection at the significance level of $p \leq 0.05$. All statistical analyses were performed using STATA v 13 (StataCorp LP, Texas, USA).

RESULTS

Population characteristic

Out of the 227 women recruited, all data were available for 200 of them who were included in this analysis. The mean age of the participants was 35years with a range of 41 (18-58 years). Nearly three quarters (78%) of these women had age of sexual debut below 18 years. Almost all participants' sexual partners (93.5%) were circumcised with 57% reported using condoms during their last sexual encounter. During this study, about 12% of them were experiencing vaginal irritation while 21% had lower abdominal pain. Only 6% of the women reported practicing douching (washing vagina using medicinal or cleansing agents) (Table 2).

Burden of BV

Prevalence in this high risk cohort varied widely depending on the test used. Using Nugent score, 129 (64.5%; 95% CI 57.7 – 70.8) women had abnormal vaginal flora with Nugent Score (NS) of 4–10, while 78 (39%) were diagnosed with BV (NS 7–10). Using Amsel's criteria, 69 (34% 95% CI 32.4 – 45.8) women had BV and 61 (30.5%; 95% CI 24.4 – 37.1) by the Quickvue rapid test. About 4 (2%) women had *T. vaginalis,* while 19 (9.5%) women had yeast infection

Performance of BV Diagnostic tests

Performance of BV tests evaluated against Nugent score is summarized in table 1. Data were used for performance analyses only if the results were definitive. Results concordant with those of Nugent score were obtained in 161 (80.5%) of 200 swabs by Amsel criteria and 149 (74.5%) of 200 swabs by QuickVue Advance pH and Amines rapid test. The test sensitivities were as follows: 54 (69.2%) Amsel criteria of the 78 true positive swab by Nugent score and 44 (56.4%) by QuickVue Advance pH and Amines rapid test of the 78 true positive

swab by Nugent score. The specificities of each test were: Amsel criteria 107 (87.7%) out of the 122 true negative swabs by Nugent score and 105 (86.1%) by QuickVue Advance pH and Amines rapid test out of 122 true negative score by Nugent. The positive predictive values (PPV) of the two tests ranged from 72.1%by QuickVue Advance pH and Amines rapid test to 78.3%by Amsel criteria. The negative predictive values (NPV) ranged from 75.5% by QuickVue Advance pH and Amines rapid test to 79.8%by Amsel score.

Because Nugent score is almost exclusively used in research settings and not used in clinical settings. We evaluated the performance of QuickVue Advance pH and Amines rapid test against Amsel's criteria which is the standard method for clinical diagnosis. The sensitivity of QuickVue rapid test was 55.1% and a specificity of 82.4%. The NPV was 77.7% and a PPV of 62.3%. Comparing the performance of QuickVue Advance pH and Amines rapid test against Amsel criteria, reduces its performance as opposed to when compared to Nugent criteria in terms of: sensitivity (55.1% versus 56.4%), specificity (82.4% versus 86.1%) and PPV (62.3% versus 72.1%). The NPV however increased from 75.5% to 77.7% (Table 1).

Effect of test combination

In this section we evaluated the effect of combining the scores of both Amsel and QuickVue Advance pH and Amines rapid test to evaluate if this criteria improves the overall performance of the combined test against the gold standard Nugent criteria. In this model, all the swabs were tested by Amsel criteria first then followed by Quickvue rapid test. Only swabs positive by the two tests were counted as positive while those positive by Amsel criteria but negative by QuickVue Advance pH and Amines rapid test were counted as negative. The sensitivity and specificity of this test combination were 43.5% (34/78) and 77.1% (94/122) respectively. The PPV was 54.1% (33/66) and NPV was 67.6% (94/122). Attempting to perform a test combination analysis does not improve concordance, sensitivity, specificity, PPV and NPV over that of either Amsel or Quickvue test as the sole test (Table 1).

Factors associated with BV infection

Using Nugent criteria as the gold standard, in the bivariate analyses, participants who were employed or business women, were more likely to be infected with BV than those women who were unemployed (PR 1.6, 95% CI 1.03 to 2.5). Women whose partner used condoms during the last sexual encounter were less likely to be infected with BV compared to women whose partners did not use condoms (PR 0.6, 95% CI 0.39 to 0.95). Women who experienced abdominal pains were more likely to be infected with BV compared to women who did not experience abdominal pain (PR 1.6, 95% CI 1.03 to 2.7). Women who had milky vaginal discharge were more likely to be infected with BV compared to women with clear vaginal discharge (PR 1.66, 95% CI 1.03 to 2.67). Lastly, women who had yeast infection were more likely to be BV infected than those who had no yeast infection (PR 2.1, 95% CI 1.1 to 4.1). In multivariate analysis women whose partner used condoms in the last sexual encounter were 50% less likely to be infected with BV (PR 0.5, 95% CI 0.26 to 0.71) (Table 2).

DISCUSSION AND CONCLUSION

This study constituted to a buildup of growing data highlighting the importance of BV among women of reproductive age in Kenya. In our view, this study which is among the first; provided additional data on the prevalence and factors associated with BV infection among women of reproductive age in Western Kenya. Further, the study provided data on the utility of point of care test "the QuickVue Advance pH and Amines rapid test" as a suitable diagnostic test for BV compared to Nugent criteria (almost exclusively used in research settings) and Amsel's criteria, a standard method for clinical diagnosis of BV in Kenya. . Using Nugent criteria, this study had high (64.5%) proportion of women with abnormal vaginal flora (NS 4–10) with 39% of them being diagnosed with BV (NS 7–10). This BV prevalence is higher than other studies that showed different rates ranging from 11% to 37% such as in Nigeria (Ibrahim et al., 2014), in India (Bhalla et al., 2007), other sub-Saharan Africa (Jespers et al., 2014) and in other industrialized countries (Holzman et al., 2001). Our observations were, however, consistent with previous reports from Kenya, Tanzania and India. Cohen et al., (2007) reported BV prevalence rates of 41% in Kenya, while Rao et al., (2004) and Baisley et al., (2009) reported high prevalence rates of 48.5% and 63% in India and Tanzania, respectively. This intra and inter BV variation between the current study and others from Kenya and other regions may be due to environmental, behavioral, socioeconomic status and stressor differences in the geographical variation.

Our results showed that QuickVue Advance pH and Amines rapid test had poor sensitivity in detecting BV (56.4% versus 74.5% by Amsel) but slightly highly specific (86.1% versus 87.7% by Amsel). In previous report, QuickVue Advance pH and Amines has been shown to vary in performance often poorly compared with conventional diagnostic methods for the diagnosis of BV in populations in Canada, and USA (Quidel Corporation, 2005; Charanis and Larsson, 2006). Sensitivity ranged from 53% to 92% and specificity from 95% to 97% using Nugent Gram stain as a gold standard. On the other hand Amsel criteria has been widely used and have been shown in some cases to perform with good concordance

Table 2: Characteristics and factors associated with BV infection

Participants characteristics	Sample size	BV infection (Nugent criteria)		Bivariate	Multivariate
		N	%	PR (95% CI)	PR (95% CI)
Age Group					
< 20	3	2	66.6	2.8(0.6 - 12.7)	
21-25	25	11	44	1.8(0.8 - 4.23)	
26-30	45	21	46.7	1.9(0.97 - 4.03)	
31-40	76	32	42.1	1.7(0.92 - 3.4)	
>41	51	12	23.5	Referent	
Marital status					
Single	15	3	20	0.7(0.2 - 2.6)	
Married	157	67	42.7	1.4(0.7 - 3.1)	
Separeted/Divorced/Widowed	28	8	28.60	Referent	
Occupation					
Employed/Bussiness	94	46	48.90	1.6(1.03 - 2.5)	
Unemployed	106	32	30.20	Referent	
Education Level					
< Grade 12 complet	83	40	48.2	1.5(0.9 - 2.31)	
≥ Grade 12 complete	117	38	32.5	Referent	
Parity					
Nongravid	5	2	40.00	Referent	
Primigravidia	30	13	43.30	1.1(0.2 - 4.8)	
Multigravidia	165	63	38.20	0.9(0.2 - 3.9)	
Age of sexual debut					
<18	156	62	39.7	1.1(0.6 - 1.8)	
>18	44	16	36.4	Referent	
Current sexual partners					
None	24	7	29.2	Referent	
1	174	69	39.7	1.3(0.6 - 2.9)	
>1	2	2	100	3.4(0.7 - 16.5)	
Partner circumcised					
Yes	187	75	40.1	1.6(0.5 - 5.1)	
No	13	3	25	Referent	
Condom use (Last sexual act)					
Yes	114	35	30.7	0.6(0.39 - 0.95)	0.5(0.26 - 0.71)
No	86	43	50	Referent	
HIV status					
Positive	98	33	33.6	0.76(0.48 - 1.19)	
Negative	102	45	44.1	Referent	
Vaginal irritation					
Yes	24	14	58.3	1.6(0.89 - 2.86)	
No	176	64	36.4	Referent	
Lower abdominal pains					
Yes	42	24	57.1	1.6(1.03 - 2.7)	
No	158	54	34.2	Referent	
Yeast infection on microscopy					
Yes	12	9	75	2.1(1.1 - 4.1)	
No	188	69	36.7	Referent	
Current vaginal discharge					
Milky	112	53	47.3	1.6(1.1 - 2.6)	
Clear	88	25	28.4	Referent	
Practice douching					
Yes	12	3	25	0.6(0.2 - 1.98)	
No	188	75	39.9	Referent	

N - Number; % - Percentage; PR - Prevalence ratio; CI - confidence interval

with Nugent score (Helmalatha et al., 2013). Women who were economically stable (employed or in

business) were more likely to be infected with BV. This is contrary to other studies which have associated higher incidences of BV among women with low socioeconomic status (Allsworth and Peipert, 2007). The economic status has been linked to women's health, sexual behavior and hygiene; factors which play an even bigger role in BV infection (Allsworth and Peipert, 2007).

Condom use during sexual encounters was protective against BV. In sub-Saharan Africa, the association between BV and male condom use is inconsistent; perhaps reflecting the heterogeneity of the formulations. In Burkina Faso, condom use was not associated with BV (Hay et al., 2001). Further, in Zimbabwe and Uganda, condom use was not associated with BV (Miller et al., 2005). In other regions studies have yielded contradicting results. Some studies have however showed the beneficial effect of condom use vis-à-vis BV acquisition (Hutchinson et al., 2007; Yotebieng et al., 2009). Women who experienced abdominal pains and those with milky vaginal discharge were more likely to be infected with BV. Mengistie et al., (2009) also showed that the presence of abnormal vaginal discharge and unpleasant smell were associated with BV.

Other factors that we did not find associated with BV in this study include: education level, marital status, women's parity, age of sexual debut, abortion, and number of sexual partners, contraceptive use, and partner's circumcision status. Other factors included STI and reproductive tract infection, HIV status and the presence of vaginal irritations. The cross sectional nature of this study, relatively small sample size, inadequate assessment of sexual behaviors and condom use could partly explain the observed lack of association between BV infection and the above listed independent factors.

In conclusion, this study has shown a significantly high prevalence of BV infection among women from Western Kenya which were associated with certain socio-demographic and sexual behavior and hygienic practices. Hence, enhanced awareness of these factors associated with BV infection could be important for BV infection prevention in this community. Further, QuickVue Advance pH and Amines test performed slightly poorly compared to Amsel criteria limiting its usefulness as a point of care screening test in our population. Additional evaluation of this rapid kit in other setting will be beneficial.

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