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Diagnosis of Bacterial Vaginosis and evaluation of associated factors: Comparable findings using Hay/ Ison's and Nugent's Scoring System

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ABSTRACT
Although Nugent's criterion is considered as the gold standard for the diagnosis of bacterial vaginosis (BV), the method requires an experienced slide reader and considerable time and skill. We compared Hay/ Ison and Amsel with Nugent's scoring criteria to determine the prevalence and corelates for BV among women of reproductive age attending reproductive health related clinics (family planning, post-natal and sexually transmitted Infection - STI) at Mbagathi County Referral Hospital in Nairobi. Vaginal specimens were collected from 201 consenting women, presenting with or without the vaginal symptoms of vaginitis. BV was diagnosed using Hay/ Ison, Amsel and Nugent's method while factors corelates were gathered using structured interviews. Sensitivity, specificity, and predictive values for positive and negative test were calculated for both Hay/ Ison and Amsel methods using Nugent criteria as the gold standard. Corelates for BV in this population were evaluated against the three methods. There were 66 cases (32.8%) of BV by Amsel's method, 79 cases (39.3%) of BV by Hay/ Ison's Criteria and 72 (35.8%) cases by the Nugent's method. Using Nugent criteria as the gold standard, the concordant, sensitivity, specificity, positive and negative predictive values of tests were. Amsel: 77.1%, 63.9%, 84.5%, 80.7% and 69.7% respectively, and Hay/ Ison: 96.5%, 100%, 94.6%, 100% and 91.1% respectively. Evaluating the performance of Hay/ Ison test against Amsel criteria, the standard method for clinical diagnosis; offered no improvement in sensitivity 72.7%, specificity 77.1%, NPV 85.3% and PPV 60.8% when compared to Nugent's score. Factors independently associated with BV infection included. For all the three tests: Presence of clue cells; Nugent and Hay/ Ison methods: education level, marital status and positive for whiff tests: For Nugent and Amsel criteria: Presence of vaginal discharge and for Hay/ Ison test: condom use. Attributes of socio-demographic and sexual hygiene and behavior contributes to high prevalence of BV among women in the capital city of Kenya. The Hay/ Ison's method shows good agreement with the Nugent criteria and can be recommended as a stand-alone alternative assay to Nugent's criteria or as a confirmatory test for BV in this population.
Key words: Nugent, Amsel and Hay/ Ison method Bacterial Vaginosis, Test performance, Correlates, Women of Reproductive Age, Western Kenya

BACKGROUND

Vaginal discharge resulting from diverse physiological and pathological circumstances, is by far the most prevalent and uncomfortable condition for women of different age groups in any society whether they are sexually active or not (Modak et al., 2011). Bacterial vaginosis (BV) is by far the most common cause of abnormal vaginal discharge among women (Allsworth and Peipert, 2007). BV is characterized mainly by change in the complex vaginal flora, marked by a replacement in the predominant *Lactobacillus* by mixed microbial flora consisting of anaerobes and *Gardnerella vaginalis* (Pirotta et al., 2009). Among pregnant women, diagnosis and immediate treatment of BV is recommended when symptomatic to curb associated complications such as low birth weight infants (Thorsen et al., 2006), preterm births (Das et al., 2011), pelvic inflammatory disease (Peipert et al., 1998), postpartum endometritis (Wolrath et al., 2001), and infertility (Mania-Pramanik et al., 2009. BV is further associated with a 60% increased risk of HIV-1 acquisition in women and a 3·62-fold increased risk of female-to-male HIV-1 transmission (Bukusi et al., 2006; Verstraelen et al., 2010) and also several other sexually transmitted infections (STIs), including herpes simplex virus, gonorrhea, trichomoniasis, and chlamydia trachomatis infection (Cherpes et al., 2003; Wiesenfeld et al., 2003).

By far, BV is still prevalent more common among women in sub-Saharan Africa reported in over half of this population (Klebanoff et al., 2004). In this setting BV infection has been associated with multiple and life time number of sex partners, poor condom uptake and vaginal douching (Okuku et al., 2016). Determining the contributioning factors to the prevalence of BV infection is essential in designing preventative and management measures.

Several methods have been developed and used to dragonize BV including; Amsel's criteria (Amsel et al 1983), Nugent criteria (Nugent et al., 1991) and rapid point-of-care tests such as QuickVue Advance pH and Amines test (Charonis and Larsson, 2006). While QuickVue Advance pH and Amines test is no longer in the market, both Amsel and Nugent methods score the smears by quantification of the different vaginal morphotypes, making the evaluation of smears very subjective that requires an experienced slide reader and also considerable time and skill. Consequently, in 2002 Ison and Hay (2002) described a simpler version of the two methods in which vaginal flora is divided into three different categories namely: normal, intermediate, and BV depending on the relative amount of *Lactobacillus* morphotypes to *Gardnerella* morphotypes. The majority of Kenyan hospitals do not diagnosis BV while those that does have been using Amsel method while Nugent mainly used for research purposes. Data are currently lacking on the utility of Hay/ Ison test to detect BV in Kenya. This study therefore evaluated the performance of simple reading scheme of Hay/Ison and Amsel method against the scoring method of Nugent in diagnosing BV in our setting.

METHODOLOGY

Study design and sample collection

This cross-sectional study enrolled consenting women of reproductive age (≥18 years) attending reproductive health related clinics (family planning, post-natal and sexually transmitted Infection - STI) in Mbagathi County Referral Hospital located in the capital city of Kenya. This study conducted between March and December, 2016 conveniently enrolled a total of 201 eligible women. These participants underwent through a face to face interview and also provided vaginal swabs. High vaginal swabs were collected by a trained clinician which were used for diagnosing BV, *Trichomonas vaginalis*, and vaginal candidiasis. Signs of vaginal discharge including amount, odor, color, and consistency were noted. Two vaginal swabs were collected simultaneously and immediately used as follows: one for Amsel scoring, the second smear air-dried, heat- fixed, and Gram-stained for Nugent and Hay/ Ison scoring. This study was approved by Ethical Review Committee of Kenya Medical Research Institute (KEMRI/SSC No. 2905).

Amsel criteria

BV was assessed using Amsel criteria (Amsel et al., 1983) and was considered positive 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. The presence of discharge was recorded by clinician while vaginal pH > 4.5, whiff test and the presence of 3–5 clue cells per high power field on wet-mount microscopy were done by two independent trained researchers in the Laboratory.

Nugent and Hay/ Ison's Criteria

The air-dried and heat- fixed glass smears were Gram-stained as follows. Briefly, the fixed smear was covered with crystal violet for 1 minute, washed with water, flooded with Gram's iodine for 1 minute, washed with water, and then decolorized with acetone for 2-3 seconds. The smears were rinsed quickly under running water to stop the decolorization and then counterstained with safranin for 1 minute. The smear was rinsed with running water and blot-dried. The Nugent and Hay/ Ison's grading were undertaken by two independent trained researchers as follows

Nugent scoring: For diagnosis of bacterial vaginosis, Gram-stained vaginal smear was examined under oil immersion objective (1000x magnification) and graded as per standardized, quantitative, morphological classification developed by Nugent. Composite score was categorized into three categories, scores 0–3 being normal, 4–6 being intermediate, and 7–10 being definite bacterial vaginosis (Nugent et al., 1991).

Hay/ Ison scoring: For diagnosis of bacterial vaginosis by Hay/ Ison's method, Gram-stained vaginal smear was examined under oil immersion objective (1000x magnification) and graded in the following manner: grade I (normal flora), *Lactobacillus* morphotype only; grade II (intermediate flora), reduced *Lactobacillus* morphotype with mixed bacterial morphotypes; grade III (bacterial vaginosis), mixed bacterial morphotypes with few or absent *Lactobacillus* morphotypes (Ison and Hay, 2002).

Clue cells: The presence of clue cells was recorded from Gram-stained vaginal smear examined under oil immersion objective (1000x magnification)

***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 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.

Yeast cells: The yeast cells and hyphae were observed and recorded both from wet mount slides as well as from Gram-stained vaginal smear examined under oil immersion objective (1000x magnification)

Corelates for BV

Sociodemographic, sexual behavior, HIV status and reproductive tract infections and hygienic related factors associated with BV infection among this population were gathered during the face to face interviews using structured questionnaire.

Data analysis

Descriptive statistics (proportion and frequency) was used to describe the population. The performance (concordance, sensitivity, specificity, and predictive values) were calculated for two scenarios: 1. For Amsel and Hay/ Ison's method against Nugent criteria as the gold standard and 2. For Hay/ Ison using Amsel as gold standard for routine clinical diagnosis. The bivariate and multivariate analysis were done to assess the association of selected variables with BV infection separately for the three tests (Nugent, Hay/ Ison and Amsel). All statistical analyses were performed using STATA v 13 (StataCorp LP, Texas, USA) at the significance level of $p \leq 0.05$.

RESULTS

Population characteristic

Table 1 summarizes the characteristics of the study population. Analyzable data were available for all the 201 women recruited. The mean age of the participant was 26.05 years ranging from 37 (18-55 years). The majority of the participants 36.3% were aged 21- 25 years, 51.2% were unemployed, 44.8% had secondary education, 79.6% were married. Further, the majority of participants 86.1% had not procured an abortion, 95.5% had single sexual partner, 58.2% had age of sexual debut >18 years, 49.7% were using either regular pill or injection or coil for their contraceptive, 75.6% had circumcised partners and 43.3% were using condoms. There were 10.4% of the participants who were HIV positive, 92% had no previously STD infections, 38.3% reported vaginal irritation while 48.8% had lower abdominal pain. The majority of the participants 63.7% reported taking bath seven times in a week, 76.6% reported ever washing their vagina apart from when bathing and 66.7% washed their vagina immediately after sex.

Table 1: Baseline characteristics of study population

Variables (N = 201)	Unit	Frequency	Percentage
Age	21 - 25 Years	73	36.3
Occupation	Unemployed	103	51.2
Education Level	Secondary level	90	44.8
Marital status	Married	160	79.6
Number of children	>1	68	33.8
Somking	Yes	4	2
Previous abortion	Yes	28	13.9
Number of sexual partners	1	192	95.5
Lifetime sexual partner	1	184	91.5
Age of sexual debut	<18	117	58.2
Contraceptive types used	Regular pill/Injection/ Coil	100	49.7
Partner circumcised	Yes	152	75.6
Condom use	Yes	114	56.7
HIV status	Positive	15	7.5
Previous STI infection	Yes	16	8
Frequency of STI infection	Once	16	8
Vaginal Irritation	Yes	77	38.3
Frequency of Vaginal irritation	>1	43	21.4
Abdominal pain	Yes	98	48.8
Vaginal Discharge	Yes	65	32.3
Yeast cell	Positive	36	17.9
Trichomonas	Positive	7	3.5
Whiff Test	Positive	66	32.8
Clue cells	Positive	25	12.4
Weekly bathing times	7 times	128	63.7
Items used for bathing	Soap and water	125	62.2
Number of times washed pants in a week	7 times	92	54.2
Washed vagina other than during bathing	Yes	154	76.6
Viginal washing immediately after sex	Yes	134	66.7
Practised douching	Yes	15	7.5
Douching items	Soap/detergent/disinfectant	10	5

Prevalence of BV

The prevalence of BV was as follows: using Nugent criteria (Score of 7–10), 72 (35.8%; 95% CI 29.5 – 42.7) were diagnosed with BV, 66 (32.8%; 95% CI 26.7 – 39.6) using Amsel's method and 79 (39.3%; 95% CI 32.8 – 46.2) by the Hay/lson criteria.

Performance of BV Diagnostic tests

Performance of BV tests evaluated against Nugent score is summarized in Table 2. Data were used for performance analyses only if the results were definitive. Results concordant with those of Nugent score were obtained in 155 of 201 (77.1%; 95% CI 70.8 – 82.4) swabs by Amsel criteria and 194 of 201 (96.5%; 95% CI 92.9 - 98.3) swabs by Nugent score. Based on Nugent score as the gold standard, the test sensitivities were as follows: Amsel criteria 46 of the 72 true positive (63.9%; 95% CI 52.4 – 74.1) swab by Nugent score and by Hay/lson criteria 72 of the 72 true positive (100%; 95% CI 94.9 – 100) swab by Nugent score. The specificities of each test were: Amsel criteria 109out of the 129 true negative (84.5%; 95% CI 77.3 – 89.7) swabs by Nugent score and 122 (94.6%; 95% CI 89.2 – 97.3) by Hay/lson criteria out of 129 true negative score by Nugent criteria. The positive predictive values (PPV) of the two tests ranged from 46 (69.7%) out of 66 by Amsel criteria to 72 (91.1%) out of 79 by Hay/lson criteria. The negative predictive values (NPV) ranged from 109 (80.7%) out of 135 by Amsel criteria to 122 (100%) out of 122 by Hay/lson criteria.

Amsel's criteria is the standard method for clinical diagnosis while Nugent method generally used in research settings, evaluating the performance of Hay/ Ison criteria against Amsel's criteria the performance was as follows. Test concordance of Hay/ Ison was 75.4%, sensitivity of 72.7% and a specificity of 77.1%. The NPV

was 85.3% and a PPV of 60.8%. Comparing the performance of Hay/ Ison method against Amsel criteria, reduces its performance as opposed to when compared to Nugent criteria in terms of: concordance (75.4% versus 96.6%), sensitivity (72.7% versus 100%), specificity (77.1% versus 94.6%), NPV (85.3% versus 100%) and PPV (60.8% versus 91.1%).

Table 2: Test performance in two scenario (i) Amsel's criteria and Hay method against Nugent score and (ii) Hay's Method 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	**Cohen's kappa** coefficient (κ)	P
Amsel criteria	201	77.1(70.8 - 82.4)	63.9(52.4 - 74.1)	84.5(77.3 - 89.7)	80.7(73.3 - 86.5)	69.7(57.8 - 79.5)	0.126	0.001
Hay/Ison criteria	201	96.5(92.9 - 98.3)	100(94.9 - 100)	94.6(89.2 - 97.3)	100(96.9 - 100)	91.1(82.8 - 95.6)	0.926	0.001
		Bacterial vaginosis (Amsel score)						
Hay/Ison criteria	201	75.4(68.9 - 81.3)	72.7(60.2 - 82.6)	77.1(68.9 - 83.7)	85.3(77.4 - 90.8)	60.8(49.1 - 71.4)	0.126	0.001

% - Percentage; CI - Confidence Interval; NPV -Negative Predictive Value; PPV - Positive Predictive Value; κ - Cohen's kappa coefficient

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 odour 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. Hay's criteria grade I (*Lactobacillus* morphotype only) was referred to as normal vaginal flora while grade III (absent *Lactobacillus* morphotypes) was considered bacterial vaginosis

Factors associated with BV infection

Table 3 shows Socio-demographic and Sexual behavior-specific prevalence of BV infection and bivariate prevalence ratios for BV infection (vs no infection).

Socio-demographic related factors: In the bivariate analyses, using the Nugent scoring criteria participants who had primary level of education were less likely to be infected with BV than those women who had tertiary level of education (OR 0.5, 95% CI 0.3 to 0.9). Similarly, using the Hay/Ison scoring criteria participants who had primary level of education were less likely to be infected with BV than those women who had tertiary level of education (OR 0.6, 95% CI 0.3 to 0.9).

Sexual behavior related factors: In the bivariate analyses, Hay/Ison scoring criteria, women whose partner used condoms during sexual encounters were more likely to be infected with BV compared to women who did not use condoms (OR 1.6, 95% CI 1.1 to 2.5).

Table 4 shows reproductive tract infection including HIV and personal hygiene-specific prevalence of BV infection and bivariate prevalence ratios for BV infection (vs no infection).

HIV status and reproductive tract infection factors: In the bivariate analyses, using Nugent scoring criteria, women who had vaginal discharge were more likely to be infected with BV compared to women who reported no vaginal discharge (OR 1.6, 95% CI 1.1 to 2.5). Similarly, women whose whiff test turned positive were more likely to be infected with BV compared to women had negative whiff test (OR 3.6, 95% CI 2.2 to 5.8). Further, women who had clue cells were more likely to be BV infected than those who had no clue cells (OR 3.5, 95% CI 2.2 to 5.7). Using Hay/Ison scoring criteria, women who had *Trichomonas vaginalis* infection were more likely to be infected with BV compared to women who had no T. vaginalis infection (OR 2.3, 95% CI 1.1 to 5.2). Similarly, women whose whiff test turned positive were more likely to be infected with BV compared to women had negative whiff test (OR 3.2, 95% CI 2.0 to 4.9). Further, women who had clue cells were more likely to be BV infected than those who had no clue cells (OR 3.1, 95% CI 1.9 to 4.9). Using Amsel scoring criteria, women who had vaginal discharge were more likely to be infected with BV compared to women who reported no vaginal discharge (OR 1.6, 95% CI 1.1 to 2.7). Further, women who had clue cells were more likely to be BV infected than those who had no clue cells (OR 3.1, 95% CI 1.8 to 5.2).

Personal hygiene: In the bivariate analyses for the three tests, none of the personal hygiene attributes such as number of times women bathed in a week, items used during this bathing, number of times washed their

underpants, washing of vagina other than during bathing, washing vagina immediately after sex, douching detergent and items used for douching were associated with BV infection.

As summarized in Table 5 the following variables in multivariate analysis remained significantly associated with BV infection across the three tests. Women who were single (OR 1.8, 95% CI 1.1 to 3.1) by Nugent criteria and (OR 1.6, 95% CI 1.0 to 2.7) by Hay/Ison criteria, those who had primary level of education (OR 0.5, 95% CI 0.2 to 0.9) by Nugent criteria and (OR 0.5, 95% CI 0.3 to 0.9) by Hay/Ison criteria, those who tested whiff positive (OR 3.3, 95% CI 2.1 to 5.5) by Nugent criteria and (OR 2.4, 95% CI 1.5 to 4.1) by Hay/Ison criteria, those who clue cells (OR 2.4, 95% CI 1.4 to 4.3) by Nugent criteria (OR 2.1, 95% CI 1.2 to 3.7) by Hay/Ison criteria and (OR 2.1, 95% CI 1.1 to 3.3) by Amsel criteria. Further, women who had vaginal discharge (OR 1.6, 95% CI 1.1 to 2.7) by Nugent criteria and (OR 1.7, 95% CI 1.1 to 2.8) by Amsel criteria and lastly, women whose partner used condoms during sexual encounter (OR 1.6, 95% CI 1.1 to 2.5) by Hay/Ison criteria were independently associated with BV infection

Table 3. Characteristic-specific prevalence of BV infection and bivariate prevalence ratios for BV infection (vs no infection)

Variables	Frequency	BV positive using Nugent criteria		P - value	Bivariate	BV positive using Hay/Ison criteria		P - value	Bivariate	BV positive Amsel criteria		P - value	Bivariate
		No	%		uOR (95% CI)	No	%		uOR (95% CI)	No	%		uOR (95% CI)
Age													
18 - 20	32	14	43.8	0.744	1.2(0.4 - 3.7)	15	46.9	0.652	1.3(0.4 - 3.9)	12	37.5	0.622	1.4(0.4 - 4.9)
21 - 25	73	25	34.2	0.911	0.9(0.3 - 2.7)	26	35.6	0.969	0.9(0.3 - 2.8)	25	34.2	0.709	1.3(0.4 - 4.2)
26 - 30	56	21	37.5	0.955	1.1(0.4 - 3.1)	24	42.9	0.761	1.2(0.4 - 3.4)	17	30.4	0.864	1.1(0.3 - 3.8)
31 - 35	29	8	27.6	0.652	0.7(0.2 - 2.5)	10	34.5	0.928	0.9(0.3 - 3.1)	9	31	0.846	1.1(0.3 - 4.2)
>35	11	4	36.4	Referent	Referent	4	36.4	Referent	Referent	3	27.3	Referent	Referent
Occupation													
Employed	58	21	36.2	0.898	1.1(0.6 - 1.8)	23	39.7	0.936	1.1(0.6 - 1.7)	18	31	0.832	0.9(0.5 - 1.7)
Business	40	15	37.5	0.819	1.1(0.5 - 1.9)	16	40	0.92	1.1(0.6 - 1.8)	14	35	0.854	1.1(0.6 - 1.9)
Unemployed	103	36	35	Referent	Referent	40	38.8	Referent	Referent	34	33	Referent	Referent
Education Level													
Primary	85	26	30.6	**0.049**	**0.5(0.3 - 0.9)**	29	34.1	**0.048**	**0.6(0.3 - 0.9)**	28	32.9	0.482	0.8(0.4 - 1.6)
Secondary	90	31	34.4	0.101	0.6(0.3 - 1.1)	34	37.8	0.108	0.6(0.3 - 1.1)	27	30	0.337	0.7(0.4 - 1.4)
College/university	26	15	57.7	Referent	Referent	16	61.5	Referent	Referent	11	42.3	Referent	Referent
Marital status													
Single	39	22	56.4	**0.021**	**1.8(1.1 - 2.9)**	24	61.5	**0.035**	**1.7(1.1 - 2.7)**	16	41.2	0.343	1.3(0.7 - 2.3)
Married	160	50	31.3	Referent	Referent	56	35	Referent	Referent	50	31.3	Referent	Referent
Divorced/Separated/Widowed	2	0	0	ND	ND	0	0	0.987	ND	0	0	0.991	ND
Previous abortion													
Yes	28	7	25	0.306	0.7(0.3 - 1.5)	8	28.6	0.332	0.7(0.3 - 1.4)	6	21.4	0.261	0.6(0.3 - 1.4)
No	173	65	37.6	Referent	Referent	71	41	Referent	Referent	60	34.7	Referent	Referent
Number of sexual partners													
1	192	2	28.6	0.148	0.4(0.09 - 1.5)	75	39.1	0.19	0.4(0.09 - 1.6)	64	33.3	0.687	0.7(0.09 - 4.8)
>1	7	68	35.4	0.21	0.3(0.04 - 2.1)	2	28.6	0.21	0.3(0.04 - 2.1)	1	14.3	0.376	0.3(0.02 - 4.6)
None	2	2	100	Referent	Referent	2	100	Referent	Referent	1	50	Referent	Referent
Lifetime sexual partner													
1	184	66	35.9	0.97	1.1(0.5 - 2.3)	72	39.1	0.898	0.9(0.4 - 2.1)	60	32.6	0.853	0.9(0.4 - 2.1)
>1	17	6	35.3	Referent	Referent	7	412.2	Referent	Referent	6	35.3	Referent	Referent
Age of sexual debut													
<18	80	28	35	0.839	0.9(0.7 - 1.6)	32	40	0.942	1.1(0.6 - 1.7)	25	46.7	0.815	0.9(0.5 - 1.6)
18	37	13	35.1	0.882	0.9(0.5 - 1.8)	14	37.8	0.906	0.9(0.5 - 1.8)	53	32.1	0.875	1.1(0.5 - 2.1)
>18	84	31	36.9	Referent	Referent	33	39.3	Referent	Referent	6	28.6	Referent	Referent
Contraceptive types used													
Condoms	12	3	25	0.427	0.6(0.2 - 2.1)	3	25	0.347	0.6(0.2 - 1.9)	3	25	0.582	0.7(0.2 - 2.4)
Regular pill/Injection/ Coil	100	33	33	0.444	0.8(0.5 - 1.4)	37	3	0.484	0.8(0.5 - 1.4)	30	30	0.587	0.9(0.5 - 1.5)
Emergency contraceptive	35	14	40	0.957	0.9(0.5 - 1.9)	15	42.9	0.912	0.9(0.5 - 1.8)	14	40	0.716	1.1(0.6 - 2.3)
None	54	22	40.7	Referent	Referent	24	44.4	Referent	Referent	19	35.2	Referent	Referent
Partner circumcised													
Yes	152	55	36.2	0.88	1.1(0.6 - 1.8)	60	39.5	0.946	1.1(0.6 - 1.7)	51	33.6	0.755	1.1(0.6 - 1.9)
No	49	17	34.7	Referent	Referent	19	38.8	Referent	Referent	15	30.6	Referent	Referent
Condom use													
Yes	114	34	29.8	0.096	1.5(0.9 - 2.4)	36	31.6	**0.042**	**1.6(1.1 - 2.5)**	36	31.6	0.687	1.1(0.7 - 1.8)
No	87	38	43.7	Referent	Referent	43	49.4	Referent	Referent	30	34.5	Referent	Referent

No - Number; % - Percentage; OR - Odds ratio; CI - confidence interval; u - Unadjusted odds ratio; a - adjusted OR; ND - Not done; Bold - Significant association

Table 4. Characteristic-specific prevalence of BV infection and bivariate prevalence ratios for BV infection (vs no infection)

Variables	Frequency	BV positive Nugent criteria		P - value	Bivariate	BV positive using Hay/Ison criteria		P - value	Bivariate	BV positive Amsel criteria		P - value	Bivariate
		No	%		uOR (95% CI)	No	%		uOR (95% CI)	No	%		uOR (95% CI)
HIV status													
Positive	15	8	53.3	0.248	1.9(0.6 - 5.4)	9	60	0.243	1.8(0.7 - 4.8)	7	46.7	0.378	1.6(0.5 - 4.8)
Negative	165	58	35.2	0.629	1.2(0.5 - 2.9)	63	38.2	0.733	1.1(0.5 - 2.5)	53	32.1	0.786	1.1(0.5 - 2.6)
Not tested	21	6	28.6	Referent	Referent	7	33.3	Referent	Referent	6	28.6	Referent	Referent
Previous STI infection													
Yes	16	6	37.5	0.907	1.1(0.5 - 2.4)	9	56.3	0.263	1.4(0.7 - 2.9)	6	37.5	0.735	1.2(0.5 - 2.6)
No	185	66	35.7	Referent	Referent	70	37.8	Referent	Referent	60	32.4	Referent	Referent
Vaginal Irritation													
Yes	77	33	42.9	0.191	1.3(0.9 - 2.2)	37	48.1	0.121	1.4(0.9 - 2.2)	28	36.4	0.492	1.2(0.7 - 1.9)
No	124	39	31.5	Referent	Referent	42	33.9	Referent	Referent	38	30.6	Referent	Referent
Abdominal pain													
Yes	98	32	47.7	0.465	0.8(0.5 - 1.3)	35	35.7	0.429	0.8(0.5 - 1.3)	32	32.7	0.965	0.9(0.6 - 1.60
No	103	40	38.8	Referent	Referent	44	42.7	Referent	Referent	34	33	Referent	Referent
Vaginal Discharge													
Yes	65	31	47.7	**0.044**	**1.6(1.1 - 2.5)**	33	50.8	0.075	1.5(0.9 - 2.3)	29	44.6	**0.046**	**1.6(1.1 - 2.7)**
No	135	41	30.4	Referent	Referent	46	34.1	Referent	Referent	36	26.7	Referent	Referent
Yeast cell													
Positive	36	13	36.1	0.61	1.1(0.7 - 1.9)	25	44.6	0.454	1.2(0.7 - 1.9)	23	41.1	0.207	1.4(0.8 - 2.3)
Negative	165	59	35.8	Referent	Referent	54	37.2	Referent	Referent	43	29.7	Referent	Referent
Trichomonas vaginosis													
Positive	7	4	57.1	0.342	1.6(0.6 - 4.5)	25	44.6	**0.049**	**2.3(1.1 - 5.2)**	4	57.1	0.26	1.8(0.7 - 4.9)
Negative	194	68	35.1	Referent	Referent	54	37.2	Referent	Referent	62	32	Referent	Referent
Whiff Test													
Positive	66	46	69.7	**0.001**	**3.6(2.2 - 5.8)**	48	72.7	**0.001**	**3.2(2.0 - 4.9)**	66	100	0.985	ND
Negative	135	26	19.3	Referent	Referent	31	23	Referent	Referent	0	0	Referent	Referent
Clue cells													
Positive	25	24	96	**0.0001**	**3.5(2.2 - 5.7)**	48	72.7	**0.001**	**3.1(1.9 - 4.9)**	20	80	**0.001**	**3.1(1.8 - 5.2)**
Negative	176	48	27.3	Referent	Referent	31	23	Referent	Referent	46	26.1	Referent	Referent
Weekly bathing times													
> 7	24	7	29.2	0.803	0.9(0.4 - 2.2)	7	29.2	0.699	0.8(0.3 - 2.1)	6	25	0.49	0.7(0.3 - 1.8)
7	128	49	38.3	0.581	1.2(0.7 - 2.1)	55	43	0.441	1.2(0.7 - 2.1)	43	33.6	0.91	0.9(0.6 - 1.7)
< 7	49	16	32.7	Referent	Referent	17	34.7	Referent	Referent	17	34.7	Referent	Referent
Washed vagina other than during bathing													
Yes	154	56	36.4	0.816	1.1(0.6 - 1.9)	61	39.6	0.9	1.1(0.6 - 1.7)	49	31.8	0.649	0.9(0.5 - 1.5)
No	47	16	34	Referent	Referent	18	38.3	Referent	Referent	17	36.2	Referent	Referent
Viginal washing immediately after sex													
Yes	134	51	38.1	0.855	0.9(0.5 - 1.8)	58	43.3	0.836	1.1(0.6 - 2.1)	43	32.1	0.821	1.1(0.5 - 2.3)
No	67	21	31.1	Referent	Referent	21	31.3	Referent	Referent	23	34.3	Referent	Referent
Practised douching													
Yes	15	5	33.3	0.867	0.9(0.4 - 2.3)	6	40	0.964	1.0(0.4 - 2.3)	3	20	0.373	0.6(0.2 - 1.9)
No	186	67	36	Referent	Referent	73	39.2	Referent	Referent	63	33.9	Referent	Referent

No - Number; % - Percentage; OR - Odds ratio; CI - confidence interval; u - Unadjusted odds ratio; a - adjusted OR; ND - Not done; Bold - Significant association

Table 4. Factors independently associated with BV infection (vs no infection) across three tests

Variables	Frequency	BV positive using Nugent criteria		P - value	Multivariate	BV positive using Hay/Ison criteria		P - value	Multivariate	BV positive Amsel criteria		P - value	Multivariate
		No	%		aOR (95% CI)	No	%		aOR (95% CI)	No	%		aOR (95% CI)
Marital status													
Single	39	22	56.4	**0.044**	**1.8(1.1 - 3.1)**	24	61.5	**0.047**	**1.6(1.0 - 2.7)**	16	41.2	0.364	1.3(0.7 - 2.5)
Married	160	50	31.3	Referent	Referent	56	35	Referent	Referent	50	31.3	Referent	Referent
Divorced/Separated/Widowed	2	0	0	0.998	ND	0	0	0.987	ND	0	0	0.993	ND
Education Level													
Primary	85	26	30.6	**0.027**	**0.5(0.2 - 0.9)**	29	34.1	**0.036**	**0.5(0.3 - 0.9)**	28	32.9	0.434	0.7(0.4 - 1.5)
Secondary	90	31	34.4	**0.047**	**0.5(0.3 - 0.9)**	34	37.8	0.907	0.5(0.3 - 1.0)	27	30	0.255	0.7(0.3 - 1.4)
College/university	26	15	57.7	Referent	Referent	16	61.5	Referent	Referent	11	42.3	Referent	Referent
Whiff Test													
Positive	66	46	69.7	**0.001**	**3.3(2.1 - 5.5)**	48	72.7	**0.001**	**2.4(1.5 - 4.1)**	66	100	0.985	ND
Negative	135	26	19.3	Referent	Referent	31	23	Referent	Referent	0	0	Referent	Referent
Clue cells													
Positive	25	24	96	**0.002**	**2.4(1.4 - 4.3)**	48	72.7	**0.008**	**2.1(1.2 - 3.7)**	20	80	**0.001**	**2.1(1.1 - 3.4)**
Negative	176	48	27.3	Referent	Referent	31	23	Referent	Referent	46	26.1	Referent	Referent
Vaginal Discharge													
Yes	65	31	47.7	**0.048**	**1.6(1.1 - 2.7)**	33	50.8	0.122	1.5(0.9 - 2.4)	29	44.6	**0.049**	**1.7(1.1 - 2.8)**
No	135	41	30.4	Referent	Referent	46	34.1	Referent	Referent	36	26.7	Referent	Referent
Condom use													
Yes	114	34	29.8	0.096	1.5(0.9 - 2.4)	36	31.6	**0.049**	**1.6(1.1 - 2.5)**	36	31.6	0.617	1.1(0.7 - 1.9)
No	87	38	43.7	Referent	Referent	43	49.4	Referent	Referent	30	34.5	Referent	Referent

No - Number; % - Percentage; OR - Odds ratio; CI - confidence interval; a - adjusted Odds ratio; ND - Not done; Bold - Significant association

DISCUSSION

In order to prevent and manage any disease/condition it is imperative to understand its epidemiology. Consequently, this study was a buildup of growing need for data tackling one of the most common causes of vaginal discharge among women of reproductive age in Sub-Saharan Africa and other developing countries. This study provided additional data on the prevalence and factors associated with BV infection among women of

reproductive age attending one of the largest middle level public hospital in the Capital city of Kenya. Further, the study is the first of its kind to provided data on the utility of Hay/Ison criteria; simpler version of both Nugent criteria (exclusively used in research settings) and Amsel's criteria (a standard method for clinical diagnosis) as a suitable diagnostic test for BV.

The prevalence of BV according using Nugent criteria (Score of 7–10) as the gold standard was, 35.8%. In other settings varied BV prevalence have been reported including: 48.6% in Ethiopia (Bitew et al., 2017), 29.2% in US, 24.4% In Nepal (Ranjit et al., 2018). Other rates ranging from 11% to 37% have also been reported 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). Reports from other Kenya, Tanzania and India studies were consistent with our study. Okuku et al., (2016) reported a prevalence of 39% in Kenya, Cohen et al., (2012) 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. Environmental, behavioral, socioeconomic status and stressor differences across various geographical region have been implicated in the intra and inter regional variation in the prevalence of BV.

Hay/ Ison criteria marked by the following attributes; first, unlike the Nugent, the method does not rely in the estimate of the bacterial morphotypes quantity rather estimates the relationship between the amounts of bacteria, thus the field size of the microscope does not have an influence on the results (Forsum et al., 2002). Second, the method can be used on slides stained with different staining methods as well as on smears with no stains. Lastly, the method is simple and robust. These attributes call for evaluation of this method in different settings, populations and by different readers. To the best of our knowledge, this was the very first study to evaluate the utility of this method against both the Nugent and Amsel scoring methods in Kenya. Comparing the performance of Hay/ Ison's method verses Amsel's method using Nugent Gram stain as a gold standard were as follows: Sensitivity 100% verses 63.9% and specificity 94.6% verses 84.5% and Kappa of 0.926 verses 0.126. The comparable performance of Hay/ Ison to Nugent was also reported by (Ison and Hay, 2002) reporting kappa: 0.89 similar to study by Chawla et al., 2013 with a kappa 0.906 and by Larsson et al., (2004) in Sweden. From our study and concurrence with previous studies Hay's method is very similar to Nugent scoring criteria. We can conclude therefore the when there is a lack of time or expertise, Hay/ Ison's scoring method can be used as an alternative method of diagnosis of BV.

In this study women who were single were more likely to be infected with BV compared to their married counterparts in agreement to studies by Koumans et al., (2007) and Yen et al. (2003). We postulate that single women were likely to engage in sexual relationship including with multiple partners than the married women who were likely to be monogamous, which has been linked with increased chances of BV infection (Esber et al., 2015). We reported lower prevalence of BV among women who had primary level of education. On the contrary, Achondou et al., (2016) reported a higher prevalence of BV among participants who attained only primary education or no education at all. These findings raise the need for public awareness and education on vaginal infections in general. Emphasis should be laid on proper hygienic practices as well as the bad sides of early sex, multiple sex partners, change of sex partners, use of unprescribed drugs and antiseptics amongst others.

Condom use during sexual encounters was not protective against BV in our study contrary to others which have reported otherwise (Bukusi et al., 2006; Okuku et al., 2016). In sub-Saharan Africa, the association between BV and male condom use is inconsistent; perhaps reflecting the heterogeneity of the formulations. Studies in Burkina Faso, Zimbabwe and Uganda, condom use was not associated with BV (Miller at el., 2005; Hutchinson et al., 2007). In other regions and studies the beneficial effect of condom use vis-à-vis BV acquisition have been reported (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 in agreement with Mengistie et al., (2014) who reported association between the presence of abnormal vaginal discharge and unpleasant smell. Positive whiff test as well as the presence of clue cells are key markers of BV positivity by Amsel's criteria. It is not surprising that the positivity of whiff test and the presence of clue cells in our study were associated with BV. This agrees with several studies in different settings (Amsel et al., 1983; Chawla et al., 2013).

Other factors such as age, occupation, parity and cigarette smoking, age of sexual debut, abortion, and number of sexual partners, contraceptive use, partner's circumcision status, HIV/STD infection, vaginal irritations, douching were not found associated with BV infection in this study. The relatively small sampled population, the cross-sectional nature and variation in the testing method could account for the lack of this association.

Given the above stated limitations, we can conclude the following: That in this geographical defined population, the prevalence of BV infection is significantly high. Similar to other studies, certain socio-demographic and sexual behavior and hygienic practices are important predictors of BV infection. The strong agreement in the performance of Hay/ Ison's and Nugent's scoring criteria "considered the gold standard" implies that Hay/ Ison's method can be used as an alternative to Nugent's scoring method. Further Hay/ Ison's method seems more applicable for use in large busy hospitals covering a large population.

Authors' contributions

FK, EAB and VM conceived the study. FK collected and tested the samples. FK analyzed the data and prepared the draft manuscript. EAB and VM provided guidance and mentorship during the implementation of the study. All authors reviewed and approved the final manuscript

Competing interests

The authors declare that they have no competing interest.

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REFERENCE

1. Achondou AE, Fumoloh F, Aseneck A, Awah A, Utokoro A. Prevalence of bacterial vaginosis among sexually active women attending the CDC central clinic Tiko, South West region, Cameroon. Afr J Infect Dis. 2016; 10(2): 96–101.
2. Allsworth JE and J. F. Peipert, "Prevalence of bacterial vagi- nosis: 2001–2004 National Health and Nutrition Examination Survey data," *Obstetrics and Gynecology*, vol. 109, no. 1, pp. 114– 120, 2007.
3. Amsel R, Totten PR, Spiegel CA. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. The American Journal of Medicine. 1983; 74: 14–22.
4. Baisley K, Changalucha J, Weiss HA et al. Bacterial vaginosis in female facility workers in north-western Tanzania: prevalence and risk factors. Sex Transm Infect. 2009; 85: 370–375
5. Bhalla P, Chawla R, Garg S et al. Prevalence of bacterial vaginosis among women in Delhi, India. Indian J Med Res. 2007; 125 (2):167-72.
6. Bitew A, Abebaw Y, Bekele D, Mihret A. Prevalence of Bacterial Vaginosis and Associated Risk Factors among Women Complaining of Genital Tract Infection. *International Journal of Microbiology*. 2017:4919404.
7. Bukusi EA, Cohen CR, Meier AS et al. Bacterial Vaginosis: Risk Factors among Kenyan Women and Their Male Partners. Sex Tran Dis. 2006; 33: 361-367
8. Charonis G, Larsson PG. Use of pH/whiff test or QuickVue Advanced pH and Amines test for the diagnosis of bacterial vaginosis and prevention of postabortion pelvic inflammatory disease. Acta Obstet Gynecol Scand. 2006; 85(7):837-43.
9. Chawla R, Bhalla P, Chadha S, Grover S, Garg S. Comparison of Hay's Criteria with Nugent's Scoring System for Diagnosis of Bacterial Vaginosis. BioMed Research International. 2013. 1-5. http://dx.doi.org/10.1155/2013/365194
10. Cherpes TL., L. A. Meyn, M. A. Krohn, J. G. Lurie, and S. L. Hillier, "Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis," *Clinical Infectious Diseases*, vol. 37, no. 3, pp. 319–325, 2003.
11. Cohen C, Lingappa JR, Baeten JM et al. Bacterial vaginosis increases the risk of female-to-male HIV-1 transmission: a prospective cohort analysis among African couples. PLoS Med. 2012; 9(6):e1001251. doi: 10.1371/journal.pmed.1001251
12. Das TR, Jahan S, Begum SR et al. Association between bacterial vaginosis and preterm delivery. Mymensingh Medical Journal. 2011*;* 20: 115–120.
13. Emilia K, Maya S, Carol B, Geraldine M, Juliette K, Madeline S, Lauri M. The Prevalence of Bacterial Vaginosis in the United States, 2001–2004; Associations with Symptoms, Sexual Behaviors, and Reproductive Health. Sexually Transmitted Diseases: 2007. 34. 864-869

14. Esber A, Miguel RD, Cherpes TL, Klebanoff MA, Gallo MF, Turner AN. Risk of Bacterial Vaginosis Among Women with Herpes Simplex Virus Type 2 Infection: A Systematic Review and Meta-analysis, *The Journal of Infectious Diseases*. 2015. 212. 8–17.
15. Forsum U, Jakobsson T, Larsson PG et al., "An international study of the interobserver variation between interpretations of vaginal smear criteria of bacterial vaginosis," *APMIS*, vol. 110, no. 11, pp. 811–818, 2002.
16. Holzman C, Leventhal JM, Qiu H, et al. Factors linked to bacterial vaginosis in nonpregnant women. Am J Public Health. 2001; 91:1664–70
17. Hutchinson KB, Kip KE, Ness RB. Condom use and its association with bacterial vaginosis and bacterial vaginosis-associated vaginal microflora. Epidemiology. 2007; 18:702-8
18. Ibrahim SM, Bukar M, Galadima GB et al. Prevalence of bacterial vaginosis in pregnant women in Maiduguri, North-Eastern Nigeria. Nigerian Journal of Clinical Practice. 2014; 17-2
19. Ison CA, Hay PE. Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. Sex Transm Infect 2002; 78:413–415
20. Jespers V, Crucitti T, Menten J et al. Prevalence and Correlates of Bacterial Vaginosis in Different Sub-Populations of Women in Sub-Saharan Africa: A Cross-Sectional Study. PLoS ONE. 2014; Dol. 10.1371/journal.pone.0109670
21. Klebanoff MA, Schwebke JR, Zhang J, et al. Vulvovaginal symptoms in women with bacterial vaginosis. Obstet Gynecol. 2004; 104: 267–272
22. Koumans E. H., Sternberg M., Bruce C., et al. The prevalence of bacterial vaginosis in the United States, 2001-2004; associations with symptoms, sexual behaviors, and reproductive health. *Sexually Transmitted Diseases*. 2007;34(11):864–869.
23. Larsson PG, B. Carlsson, L. Fa ̈hraeus, T. Jakobsson, and U. Forsum, "Diagnosis of bacterial vaginosis: need for validation of microscopic image area used for scoring bacterial morpho- types," *Sexually Transmitted Infections*, vol. 80, no. 1, pp. 63–67, 2004.
24. Mania-Pramanik H, S. C. Kerkar, and V. S. Salvi, "Bacterial vaginosis: a cause of infertility?" *International Journal of STD and AIDS*, vol. 20, no. 11, pp. 778–781, 2009.
25. Mengistie Z, Woldeamanuel Y, Asrat D and Adera A. Prevalence of bacterial vaginosis among pregnant women attending antenatal care in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. BMC Research Notes. 2014, 7 :822
26. Miller C, Swygard H Hobbs M. The Prevalence of Trichomoniasis in Young Adults in the United States. Sexually Transmitted Disease. 2005; 32: 593- 598.
27. Modak T, P. Arora, C. Agnes et al., "Diagnosis of bacterial vaginosis in cases of abnormal vaginal discharge: comparison of clinical and microbiological criteria," *Journal of Infection in Developing Countries*, vol. 5, no. 5, pp. 353–360, 2011.
28. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. Journal of Clinical Microbiology. 1991; 29: 297–301
29. Okuku RA, Bii C, Makohka E, Gikunji J, Ngayo MNTest performance and correlates of bacterial vaginosis among women in Western Kenya. Prime Journal of Microbiology Research. 2016. 4. 183-189,
30. Peipert J. F., A. B. Montagno, A. S. Cooper, and C. J. Sung, "Bacterial vaginosis as a risk factor for upper genital tract infection," *American Journal of Obstetrics and Gynecology*, vol. 177, no. 5, pp. 1184–1187, 1997.
31. Pirotta M, K. A. Fethers, and C. S. Bradshaw, "Bacterial vagino- sis. More questions than answers," *Australian Family Physician*, vol. 38, no. 6, pp. 394–397, 2009.
32. Ranjit E, Raghubanshi R, Maskey S, Parajuli P. Prevalence of Bacterial Vaginosis and Its Association with Risk Factors among Nonpregnant Women: A Hospital Based Study. International Journal of Microbiology, vol. 2018, Article ID 8349601, 9 pages, 2018
33. Rao PS, Devi S, Shriyan A et al. Diagnosis of bacterial vaginosis in a rural setup: Comparison of clinical algorithm, smear scoring and culture by semi-quantitative technique. Indian J Med Microbiol. 2004; 22 (1):47-50.
34. Thorsen P, Vogel I, Olsen J et al. Bacterial vaginosis in early pregnancy is associated with low birth weight and small for gestational age, but not with spontaneous preterm birth: a population-based study on Danish women. Journal of Maternal-Fetal and Neonatal Medicine. 2006; 19: 1–7.
35. Verstraelen H, Verhelst R, Vaneechoutte M et al. The epidemiology of bacterial vaginosis in relation to sexual behavior. BMC Infectious Diseases. 2010; 10:81
36. Wiesenfeld H. C, S. L. Hillier, M. A. Krohn, D. V. Landers, and R. L. Sweet, "Bacterial vaginosis is a strong predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infection," *Clinical Infectious Diseases*, vol. 36, no. 5, pp. 663–668, 2003.

37. Wolrath H, U. Forsum, P. G. Larsson, and H. Bore´n, “Analysis of bacterial vaginosis-related amines in vaginal fluid by gas chromatography and mass spectrometry,” *Journal of Clinical Microbiology*, vol. 39, no. 11, pp. 4026–4031, 2001.
38. Yen S., Shafer M.-A., Moncada J., Campbell C. J., Flinn S. D., Boyer C. B. Bacterial vaginosis in sexually experienced and non-sexually experienced young women entering the military. *Obstetrics and Gynecology*. 2003;102(5):927–933.
39. Yotebieng M, Turner AN, Hoke TH, Van Damme K, Rasolofomanana JR, Behets F: Effect of consistent condom use on 6-month prevalence of bacterial vaginosis varies by baseline BV status. Trop Med Int Health. 2009; 14:480-6