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Simple and inexpensive point-of-care tests improve diagnosis of vaginal infections in resource constrained settings

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Summary

OBJECTIVE Bacterial vaginosis (BV) and *Trichomonas vaginalis* infection (TV) have been associated with adverse birth outcomes and increased risk for HIV. We compare the performance of simple inexpensive point-of-care (POC) tests to laboratory diagnosis and syndromic management of BV and TV in poor settings.

METHODS Between November 2005 and March 2006, 898 sexually active women attending two reproductive health clinics in Mysore, India were recruited into a cohort study investigating the relationship between vaginal flora and HSV-2 infection. Participants were interviewed and screened for reproductive tract infections. Laboratory tests included serology for HSV-2; cultures for TV, *Candida* sp., and *Neisseria gonorrhoeae*; Gram stains; and two POC tests: vaginal pH; and Whiff test.

RESULTS Of the 898 participants, 411 [45.7%, 95% confidence interval (95% CI): 42.4–49.0%] had any laboratory diagnosed vaginal infection. BV was detected in 165 women (19.1%, 95%CI: 16.5–21.9%) using Nugent score. TV was detected in 76 women (8.5%, 95%CI: 6.7–10.4%) using culture. Among the entire study population, POC correctly detected 82% of laboratory diagnosed BV cases, and 83% of laboratory diagnosed TV infections. Among women with complaints of vulval itching, burning, abnormal vaginal discharge, and/or sores (445/898), POC correctly detected 83% (60 of 72 cases) of laboratory diagnosed BV cases *vs.* 40% (29 of 72 cases) correctly managed using the syndromic approach ($P < 0.001$). Similarly, POC would have detected 82% (37 of 45 cases) of TV cases *vs.* 51% (23 of 45 cases) correctly managed using the syndromic approach ($P = 0.001$).

CONCLUSIONS In the absence of laboratory diagnostics, POC is not only inexpensive and practical, but also significantly more sensitive than the syndromic management approach, resulting in less over-treatment.

keywords bacterial vaginosis, India, point-of-care, vaginal discharge, *Trichomonas vaginalis,* resource constrained settings

Introduction

Bacterial vaginosis (BV) and *Trichomonas vaginalis* infection (TV) have been associated with adverse pregnancy and health outcomes (Watts *et al.* 1990; Germain *et al.* 1994; Hillier *et al.* 1995; Cotch *et al.* 1997). Being the most common vaginal infections among women, BV and TV also increase risk for acquisition of other sexually tranmitted infections (STI) and HIV (Wiesenfeld *et al.* 2003; Madhivanan *et al.* 2007; McClelland *et al.* 2007; Atashili *et al.* 2008). Given the high prevalence of BV and TV and the large burden of associated disease, there is a compelling public health need for simple and inexpensive point-of-care diagnostic tests for expanded screening of women in poor settings.

In many parts of the developing world, laboratory diagnosis of vaginal infections is not available outside urban areas. Even simple and inexpensive methods such as Gram stain of vaginal smears or microscopic examination for motile trichomonads are generally beyond the reach of most primary healthcare settings (Mullick *et al.* 2005). Healthcare workers typically manage vaginal infections using a syndromic approach which bases treatment on symptoms and signs (World Health Organization 2005). Unfortunately, studies have shown low sensitivity and low predictive values for diagnoses of reproductive tract

infections (RTI) using a syndromic approach among women (Vishwanath *et al.* 2000; Hylton-Kong *et al.* 2004). Such an approach not only misses asymptomatic infections but also results in substantial under-diagnosis and over-treatment of BV and TV (Tann *et al.* 2006; Romoren *et al.* 2007).

This paper describes the performance of two simple and inexpensive point-of-care tests (POC), the pH and Whiff test, for diagnosis of BV and TV among women attending a reproductive health clinic in Mysore, India.

Methods

Study population

Between November 2005 and March 2006, 898 young sexually active nonpregnant women were recruited from two reproductive health clinics in Mysore, India, into a prospective cohort study investigating the relationship between abnormal vaginal flora and Herpes Simplex Virus Type 2 infection. Detailed recruitment methods have been described by Krupp *et al.* (2007). To be included in the study, participants had to: be 15–30 years old; have had vaginal intercourse at least once in the previous three months; be willing to undergo a pelvic examination; and stay in the area for at least 6 months. The study was approved by the institutional review boards at the University of California, Berkeley, and Asha Kirana Hospital, Mysore.

Data collection

All participants provided informed consent at enrolment. Trained interviewers collected information on sociodemographics, reproductive and sexual health, and partner characteristics, using a standardized questionnaire in *Kannada* or *Urdu*.

Specimen collection

A trained study clinician performed a pelvic examination to collect three vaginal swabs, two vaginal smears, and an endocervical swab. In addition, clinical signs from external and internal examination were recorded. Vaginal discharge signs included amount, colour and consistency. Vaginal pH was measured by holding a pH strip against the lateral vaginal wall with a cotton tipped swab and comparing the colour to a pH chart (pH test) provided by the manufacturer (SD fine chemicals Ltd, Mumbai, India). Women were treated according to the US Centers for Disease Control and Prevention treatment guidelines (Centers for Disease Control and Prevention 2006). Women diagnosed with a sexually transmitted infection were given additional medication for their partners.

Laboratory assessment

Diagnostic testing was completed at Holdsworth Memorial Hospital and Vikram Hospital in Mysore, India. Saline wet-mount preparations of vaginal fluids were examined microscopically for motile trichomonads, clue cells and yeast cells. A cotton swab was used to collect vaginal fluid from the lateral vaginal wall. Specimens were placed in a test tube containing three drops of sterile saline at the time of pelvic examination by the clinician. After agitation, one drop of solution was placed on a glass slide, covered with a cover slip, and observed at 10 × and 40 × magnifications. The wet mount preparation was examined for the presence of clue cells and motile trichomonads. Another drop of saline solution was placed on a sterile glass slide with an added drop of KOH solution. The slide was brought close to the nose for detection of amine odour (Whiff test). After the Whiff test, a cover slip was placed on the slide and the slide was read for detection of budding yeast or hyphae.

Vaginal fluid specimens were cultured for *T. vaginalis* using InPouch TV culture kits (Biomed Diagnostic, White City, OR, USA) and read daily for 5 days, for the presence of trichomonads. Vaginal specimens were cultured for *Candida* species (Biomed Diagnostic, White City, OR, USA), and endocervical swabs were cultured for *Neisseria gonorrhoeae* (GC) in a modified Thayer-Martin medium (Biomed Diagnostic, White City, OR, USA). The GC culture system was validated for growth of GC with 3 ATCC strains (49226, 31426 and 19424). There was independent verification of 10% of tests for TV and *Candida* by an independent microbiologist whose results matched the study findings. Gram stain of vaginal smears were assessed for BV by two trained independent technicians using Nugent score (Nugent *et al.* 1991). Before the study started, technicians were trained in Nugent scoring at St John's Medical College, Department of Microbiology, Bangalore, India. Vaginal flora was defined 'normal' if the Nugent score was 0–3, 'intermediate' if the score was 4–6, and 'abnormal' if the score was ≥7.

Data analysis

Data were analysed using STATA 9.1 (Stata Corporation, College Station, TX, USA). Prevalence of infection and 95% confidence intervals were estimated. Women were defined to have BV if their Nugent score was ≥7. Women

were defined as positive for TV if they had positive wet-mount microscopy results and/or a positive *T. vaginalis* culture. Women were defined as negative for TV infection if they had negative wet-mount microscopy results and a negative *T. vaginalis* culture. Descriptive analyses were conducted for normally distributed variables using Pearson chi-square or Fisher-exact tests for categorical variables and *t-test* for continuous variables. Sensitivity, specificity, and predictive values were calculated using Nugent score for BV, and TV culture, as gold standards. Logistic regression was used to calculate the odds ratio (OR) and corresponding 95% confidence intervals (CI) for the association between BV and TV, and preselected variables.

Results

Of the 898 participants, 411 [45.7%, 95% confidence interval (95% CI): 42.4–49.0%] had any laboratory diagnosed vaginal infection. BV was detected in 165 women (19.1%, 95% CI: 16.5–21.9%) using Nugent score. TV was detected in 76 women (8.5%, 95% CI: 6.7–10.4%) using culture (Table 1). Of women who had BV or TV, 15.7% had concurrent infections. Among the entire study population, POC correctly detected 82% of laboratory diagnosed BV cases, and 83% of laboratory diagnosed TV infections. Among women with complaints of vulval itching, burning, abnormal vaginal discharge, and/or sores (445 of 898), POC correctly detected 83% (60 of 72 cases) of laboratory diagnosed BV cases *vs.* 40% (29 of 72 cases) correctly managed using the syndromic approach ($P < 0.001$). Similarly, POC would have detected 82% (37 of 45 cases) of TV cases *vs.* 51% (23 of 45 cases) correctly managed using the syndromic approach ($P = 0.001$).

The most frequently reported complaints include abnormal vaginal discharge (37%), followed by genital itching (18%), and burning sensation in the genitalia (16%). Among women with symptoms, BV was not found to be significantly associated with any symptoms or signs (Table 2). Similarly there was no significant difference in the diagnosis of TV among the women who complained of vaginal discharge as compared to women who did not ($P = 0.24$). However, there were more cases of TV (15.5% *vs.* 6.8%) detected among women who complained of vulval itching than among women who did not ($P < 0.001$). In addition, both excess vaginal discharge and vaginal erythema were significantly associated with TV infection. Table 3 describes the performance of each of the symptoms, signs and POC for diagnosis of BV and TV.

Table 1 Prevalence of reproductive tract infections (RTI) among 898 Indian women in Mysore

Characteristic	*n/N*	Prevalence (95% CI)
Sexually transmitted infections		
Trichomonas vaginalis (TV)	76/898	8.5% (6.7, 10.4)
Neisseria gonorrhoeae (NG)	0/898	0% (0, 0.4)
Endogenous infections		
Bacterial vaginosis (BV)	165/864	19.1% (16.5, 21.9)
Vaginal candidiasis* (VC)	277/898	30.9% (27.8, 33.9)
Any lower genital tract infection (BV, TV, VC)	411/898	45.7% (42.4 to 49.0)

Denominators differ because of missing data. Among women with BV and/or TV, 15.7% had both infections.
*Vaginal candidiasis was defined as complaint of vaginal discharge or vulval itching with clinical signs of curdy white discharge or erythema with laboratory confirmed culture positive for candida species.

Discussion

Prevalence of BV and TV infection, at 19% and 8.5%, respectively, was relatively low among this population of young reproductive age women attending two reproductive health clinics in Mysore, India. As other studies have demonstrated, use of syndromic management of vaginal discharge had low sensitivity and resulted in high levels of overtreatment. Among our study population, use of simple and inexpensive point-of-care tests as compared to the syndromic approach would have increased sensitivity for detection and appropriate treatment of BV or TV cases from 44 cases to 84 cases among 445 women attending the clinic with complaints. Alternatively, if as is often the case in primary healthcare settings in India, all women with complaints had been treated with metronidazole, POC testing would have reduced overtreatment by 41%.

In addition to improving accuracy of diagnosis, POC are easily adaptable to almost any locale. Both tests can be performed by healthcare workers with minimal training in nonlaboratory settings. There is no requirement for additional equipment, refrigeration or even electricity – common limitations in most resource constrained environments. Furthermore, POC can be performed using self-collected vaginal swabs further reducing clinician time (Shafer *et al.* 2003; Tanksale *et al.* 2003). The cost of using POC in this population was less than 0.04 USD per woman, a minor outlay compared to cost of overtreatment using a syndromic approach.

Our findings are restricted to vaginal infections since there were no cases of gonorrhea detected and we did not test for *Chlamydia* (CT). Other studies have shown low

Table 2 Reproductive and laboratory characteristics association with *T vaginalis* infections and Bacterial vaginosis among young reproductive age women in Mysore, India

	T vaginalis infection					Bacterial vaginosis†				
	Total (898)			Univariate		Total (864)			Univariate	
Characteristic	*N*	*n*	%	OR	95% CI	*N*	*n*	%	OR	95% CI
Complaint of vaginal discharge										
Yes	334	33	9.8	1.32	0.82, 2.13	319	55	17.2	0.82	0.57,1.17
No	564	43	7.6	1.00		545	110	20.2	1.00	
Complaint of vulval itching										
Yes	168	26	15.5	2.49***	1.49, 4.13	158	31	19.6	1.04	0.67,1.61
No	730	50	6.8	1.00		706	134	18.6	1.00	
Clinical signs of excess vaginal discharge‡										
Yes	261	34	13.0	2.12**	1.31, 3.42	250	48	19.2	1.01	0.69,1.46
No	637	42	6.6	1.00		614	117	19.1	1.00	
Clinical signs of vaginal erythema										
Yes	114	29	25.4	5.33***	3.19, 8.92	110	30	27.3	1.72*	1.08,2.72
No	782	47	6.0	1.00		754	135	81.8	1.00	
pH examination										
>4.5	480	59	12.3	2.39**	1.24, 4.62	621	148	23.8	4.17***	2.46,7.05
≤4.5	418	17	4.1	1.00		243	17	7.0	1.00	
Whiff test (amine test)										
Positive	102	29	28.4	6.33***	3.76, 10.66	98	40	40.8	3.53***	2.26,5.1
Negative	785	47	5.9	1.00		766	125	16.3	1.00	
Wet mount examination§										
Yes	44	44	100		–	45	23	51.1	4.98***	2.69,9.17
No	854	32	3.75			818	142	17.4	1.00	
Symptoms and signs¶										
Yes	198	29	14.7	2.38***	1.46, 3.9	190	33	17.4	0.86	0.56,1.31
No	700	47	6.7	1.00		673	132	19.6	1.00	
Positive Whiff test and/or pH > 4.5										
Yes	507	63	12.4	4.13***	2.23, 7.6	481	135	28.1	4.57***	3.00,6.98
No	391	13	3.3	1.00		382	30	7.8	1.00	

*P-value < 0.05, **P-value < 0.01, ***P-value < 0.001.

†Diagnosis for BV was available for 864 (96%) as 34 slides were unreadable as they were missing or over-stained or had inadequate material.

‡Vaginal discharge defined as yellow/green discharge for TV and milky white discharge for BV.

§Wet mount examination shows Trichomonads for TV and Clue cells for BV.

¶Symptoms & Signs defined as complaint of 'discharge & or itching' with signs of 'yellow/green discharge and/or vaginal erythema' for TV and complaint of 'discharge & or itching' with signs of 'white discharge' for BV.

prevalence of cervical infections in India among similar populations (Sowmya *et al.* 2001; Chawla *et al.* 2004; Joyee *et al.* 2004; Panchanadeswaran *et al.* 2006; Patel *et al.* 2006). While some research has shown that infection with CT does not change vaginal pH (Mania-Pramanik *et al.* 2008), additional research is needed to assess the performance of POC among populations with a high prevalence of cervical infections. In this study, TV was diagnosed by culture instead of molecular methods so it is possible that some TV infections were missed (Crucitti *et al.* 2003). Data (not shown) on performance of POC for diagnosis of candidiasis was comparable to the syndromic method for management of vaginal discharge. Because positive and negative predictive values depend on the prevalence of a disease in a community, predictive values reported here are specific to this population and others areas with similar prevalence. Despite these limitations, the study had several strengths: It had a large sample size, used standardized laboratory investigations for diagnosis of vaginal infections, and screened participants in a standardized manner using trained study personnel.

In conclusion, in the absence of laboratory diagnostics, POC is not only inexpensive and practical, but also

Table 3 Sensitivity, specificity, and predictive value of clinical diagnosis for *T vaginalis* infection and bacterial vaginosis among young reproductive age women presenting with symptoms in Mysore, India (N = 445)

	Bacterial vaginosis				*T. vaginalis* infection			
Variable	Sens %	Spec%	PPV%	NPV%	Sens %	Spec%	PPV%	NPV%
Complaint of abnormal vaginal discharge	76.4	25.6	17.2	84.3	73.3	24.7	9.9	89.2
Complaint of vulval itching	43.1	64.2	19.6	84.8	57.8	64.5	15.5	93.1
Complaint of burning sensation	23.6	66.5	12.5	81.1	37.8	68.7	11.9	90.8
Complaint of discharge and/or itching	95.8	12.7	18.2	93.7	91.1	11.5	10.4	92.0
Signs of excess vaginal discharge†	37.5	61.1	16.4	82.8	55.6	63.3	14.5	92.7
Signs of Vaginal erythema	20.8	83.6	20.5	83.9	42.2	85.7	25.0	93.0
pH > 4.5	79.2	50.1	24.4	92.2	77.8	46.5	14.1	94.5
Positive Whiff test (amine test)	23.6	89.9	32.1	83.3	35.6	90.3	29.1	92.6
Wet Mount examination‡	13.9	97.2	47.4	84.6	53.3	100	100	94.9
Symptoms and signs	40.3	70.1	21.4	85.7	51.1	70.7	16.4	92.8
Positive Whiff test and/or pH > 4.5	83.3	47.0	24.2	93.3	82.9	43.5	14.1	95.6

All variables are compared to *T. vaginalis* culture as the gold standard for TV and to Nugent score of ≥7 on Gram stain for BV. Sens: sensitivity, Spec: specificity, PPV: positive predictive value, NPV: negative predictive value.
†Vaginal discharge defined as yellow/green discharge for TV and milky white discharge for BV.
‡Wet mount examination shows Trichomonads for TV and Clue cells for BV.

significantly more sensitive than the syndromic management approach, resulting in less overtreatment.

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