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Epidemiologic Features of Vaginal Infections among Reproductive-age Women in South India

By

Sujit D Rathod

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Epidemiology

in the

Graduate Division

of the

University of California, Berkeley

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Professor Arthur L. Reingold, Chair

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Abstract

Epidemiologic Features of Vaginal Infections among Reproductive-age Women in South India

By

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Doctor of Philosophy in Epidemiology

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Background: Vaginal discharge syndrome is a clinical condition characterized by leucorrhoea, which can be caused by reproductive tract infections (RTIs). Three RTIs are referred to as vaginal infections: bacterial vaginosis (BV), trichomoniasis (TV), and vulvovaginal candidiasis (VVC). Vaginal infections are known to increase susceptibility to sexually transmitted infections, including HIV, and to be associated with low birth weight and premature birth. The prevalence and incidence of and risk factors for vaginal infections are not well-studied in many settings. Further, the aforementioned consequences of these infections are highly prevalent in many low-income settings, providing ample justification for additional inquiry into the diagnosis and risk factors of these vaginal infections.

Methods: The Prerana dataset was collected as part of a prospective cohort study of 898 non-pregnant, reproductive-aged women living in and around Mysore, India between 2005 and 2006. The primary study objective was to evaluate the relationship between abnormal vaginal flora and the incidence of Herpes Simplex Virus - type 2 infection. Participants completed three study visits – conducted at baseline, and at three and six months - each of which involved an interviewer-administered questionnaire in Urdu or Kannada; a pelvic examination; and collection of vaginal and blood specimens for laboratory testing for reproductive tract infections.

Analyses investigating separate research aims will be conducted over three papers, as follows: Paper 1: Bacterial vaginosis and risk of *Trichomonas vaginalis* infection, Paper 2: Epidemiologic features of vulvovaginal candidiasis, and Paper 3: Syndromic diagnosis of vaginal infections using logic regression.

Discussion and significance: The Prerana cohort dataset is well-suited to filling in multiple gaps in the research literature. The analyses are among the first to test (or re-test) specific hypotheses concerning vaginal infection using a community-based sample based in a low-income setting. Paper 1 estimates the risk of *Trichomonas vaginalis* infection associated with the presence of bacterial vaginosis, which fills a conspicuous gap in the research literature. If susceptibility to *Trichomonas vaginalis* infection is found to be heightened among women with bacterial vaginosis, the burden of sexually transmitted infections attributable to bacterial vaginosis will increase dramatically, given that both conditions are highly prevalent. Next, paper 2 identifies factors associated with increased prevalence of vulvovaginal candidiasis, particularly with

respect to the presence of vaginal *Lactobacillus*. If vulvovaginal candidiasis is associated with decreased presence of *Lactobacillus*, there will be support for identifying interventions to enhance the presence of *Lactobacillus*, such as with probiotics, after women are treated with antibiotics. Finally, paper 3 examines the sensitivity, specificity, positive predictive value and negative predictive value of vaginal infections in this population using a World Health Organization diagnosis algorithm. Separate syndromic diagnosis models for vaginal infections are developed using logic regression, a machine-learning procedure; the models are evaluated for their diagnostic performance against laboratory-confirmed diagnoses of vaginal infections. Logic regression models offer the potential to improve upon the performance of the WHO algorithm, using parsimonious models which are easy to develop, comprehend, and implement in clinical facilities in low-income settings.

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Dedication

To Mom, Dad, and Nimishabehn. For your support, trust and belief: this Ph.D is also yours.

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Introduction

Vaginal discharge syndrome is a clinical condition characterized by leucorrhea, which can be caused by reproductive tract infections (RTIs). Negative outcomes associated with RTIs include discomfort, mental anguish and loss of economic productivity.(1,2) Three RTIs are referred to as vaginal infections: bacterial vaginosis (BV), trichomoniasis (TV), and vulvovaginal candidiasis (VVC). In addition to the negative outcomes associated with RTIs, these vaginal infections are known to increase susceptibility to sexually transmitted infections (STI), including HIV, and to be associated with low birth weight and preterm birth.(3–5) The prevalence and incidence of, and risk factors for, vaginal infections are not well-studied in many settings, precluding establishment of effective public health programs for their prevention. Further, diagnosis of vaginal infections is compromised by the absence of state-of-the-art diagnostic capability in low-income settings, leading to the use of syndromic diagnosis algorithms.

Bacterial vaginosis: Bacterial vaginosis is a complex polymicrobial syndrome that lacks an identified etiological agent. Symptomatic bacterial vaginosis is characterized by malodor and grey, thin, homogenous vaginal discharge. Development of bacterial vaginosis is associated with loss of *Lactobacillus* species - the dominant bacteria in healthy vaginal flora - and in particular hydrogen peroxide (H₂O₂)-producing *Lactobacillus* species. The vaginal flora of women with bacterial vaginosis is often dominated by various species of anaerobic bacteria, such as *Gardnerella*, *Bacteroides* and *Mobiluncus*. Clinical or laboratory criteria are used to differentiate between women with normal vaginal flora, intermediate vaginal flora, and bacterial vaginosis. Risk factors for bacterial vaginosis include having a new sexual partner, sex with another woman, black race, non-use of condoms, douching, and smoking.(6,7) Bacterial vaginosis is associated with "all major adverse pregnancy outcomes" among pregnant women, including preterm birth, premature ruptures of membrane and low birth weight, as well as acquisition of sexually transmitted infections, including HIV.(6)

Trichomonas vaginalis: *Trichomonas vaginalis* is a sexually transmitted protozoan, which can cause vaginal irritation, pruritis, and malodorous discharge, though asymptomatic carriage is common.(8) *Trichomonas vaginalis* is the most common curable sexually transmitted infection, with an estimated 170 million new cases worldwide each year.(9) *Trichomonas vaginalis* infection is associated with preterm delivery and low birth weight(9,10) and with an increased risk of HIV acquisition.(8,9,11)

Vulvovaginal candidiasis: Vulvovaginal candidiasis is caused by overgrowth of *Candida* yeast species, most often *Candida albicans*. The overgrowth causes vaginal pruritis, erythema and a curd-like vaginal discharge. Vulvovaginal candidiasis is more likely to occur with increases in estrogen levels, such as those seen in pregnancy, with use of high-dose hormonal contraception, and immediately prior to menstruation; uncontrolled diabetes; certain genetic factors relating to race and blood type; sexual behaviors such oro-vaginal contact; and possibly as a consequence of antibiotic use.(12,13) Colonization of the vagina by *Candida* is common - 20 to 30% of women are asymptotically colonized, although colonization is not considered clinically relevant in the absence of symptoms. Vulvovaginal candidiasis has been associated with considerable direct and indirect economic costs (1) and susceptibility to HIV infection(4) and is hypothesized to increase the risk of preterm birth.(14)

Paper 1: Bacterial vaginosis and risk for *Trichomonas vaginalis* infection: Several studies support a role for bacterial vaginosis in enhancing a woman's susceptibility to sexually transmitted infections. These processes have been described by Thurman and Doncel as involving: "initiation of a clinical or subclinical inflammatory response, alteration of innate mucosal immunity, alteration of normal vaginal microflora and pH, and weakening or breach of the cervicovaginal mucosa." Several in vitro studies have demonstrated *Trichomonas vaginalis* adhesion and growth is enhanced in the elevated pH range found among women with bacterial vaginosis.(15–18)

Though bacterial vaginosis and trichomoniasis are frequently diagnosed together, few studies have investigated the temporal relationship between the two conditions. Evidence that bacterial vaginosis increases susceptibility to *Trichomonas vaginalis* infection comes from only two studies: A cohort of 657 HIV-negative female sex workers in Kenya was followed monthly for a median of 6.4 months. Abnormal vaginal flora was associated with two times the hazard of subsequent *Trichomonas vaginalis* infection in a univariable proportional hazards-style model (HR 2.1, 95% CI 1.5, 2.9), as well as in a multivariable proportional hazards-style model.(19) Another cohort of 3620 reproductive-age women was enrolled from primary care clinics in the United States and followed quarterly for one year. Using univariable pooled logistic regression, women with either abnormal vaginal flora or bacterial vaginosis had an increased hazard of incident *Trichomonas vaginalis* infection (HR 1.65, 95% CI 1.20, 2.27 and HR 2.95 95% CI 2.27, 3.81, respectively).(20)

In vitro studies and limited empirical evidence support a role for bacterial vaginosis - and more broadly abnormal vaginal flora - in increasing a woman's susceptibility to *Trichomonas vaginalis* infection. However, only one of the two studies mentioned above was conducted among women not at high risk for sexually transmitted infections, and only one was conducted among women in a low-income setting. It remains to be confirmed, then, whether diagnosis and treatment of bacterial vaginosis might be a means of lowering the incidence of *Trichomonas vaginalis* infection.

Paper 1, which aims to estimate the risk of *Trichomonas vaginalis* infection associated with the presence of bacterial vaginosis, will fill a conspicuous gap in the research literature. While the incidence of several other sexually transmitted infections - such as HIV, *Neisseria gonorrhoeae* and HSV2 - has been shown to be increased among women with bacterial vaginosis, *Trichomonas vaginalis* has often not been included among those sexually transmitted infections examined. The absence is notable, as trichomoniasis is the most common curable sexually transmitted infection in the world. If susceptibility to *Trichomonas vaginalis* infection is found to be heightened among women with bacterial vaginosis, the burden of sexually transmitted infections attributable to bacterial vaginosis will increase dramatically, given that both conditions are highly prevalent.

Bacterial vaginosis is not a reportable condition, and it is given lower priority as a public health problem than the sexually transmitted infections with which it is associated. If bacterial vaginosis is found to enhance risk of infection by *Trichomonas vaginalis*, there will be greater impetus to evaluate women for bacterial vaginosis and for that screening to be conducted using gold-standard diagnostic tests, such as the laboratory criteria identified by Nugent et al. Additionally, because safe, effective and inexpensive treatments for bacterial vaginosis are available, it is reasonable to posit that there will be a favorable impact on negative birth outcomes from

diagnosis and treatment of bacterial vaginosis through two routes of intervention: directly, by reducing the prevalence of bacterial vaginosis, and indirectly, by reducing the incidence of the bacterial vaginosis-associated sexually transmitted infections.

Specific aims:

Women with bacterial vaginosis or abnormal vaginal flora have been shown to be at increased risk of sexually transmitted infections, including HIV. Few studies have evaluated a similar relationship between abnormal vaginal flora and *Trichomonas vaginalis* infection, though *Trichomonas vaginalis* is the most common curable sexually transmitted infections globally. The incidence and prevalence of bacterial vaginosis and *Trichomonas vaginalis* infection have not been measured among reproductive age women in Mysore district, India. Thus, paper 1 aims to:

1. Estimate the risk of *Trichomonas vaginalis* infection associated with changes in vagina flora, relative to the risk of *Trichomonas vaginalis* infection in reproductive age women in Mysore, India with unchanged, normal flora.

Hypothesis: Women with abnormal vaginal flora detected over a three-month period will have an increased risk of a diagnosis of *Trichomonas vaginalis* infection at the end of three months, relative to women with normal vaginal flora over the three-month period.

2. Describe the incidence, prevalence, frequency of recurrence, and resolution of a) abnormal vaginal flora, and b) *Trichomonas vaginalis* infection, over a six-month period, among reproductive age women in Mysore, India.

Paper 2: Epidemiologic features of vulvovaginal candidiasis: Bacterial vaginosis is an established risk factor for sexually transmitted infections. Yet, a similar relationship between the presence of bacterial vaginosis with the development of vulvovaginal candidiasis has not been established. Specifically, there is interest in the relationship between the presence of vaginal Lactobacillus - which is diminished among women with bacterial vaginosis - and the development of vulvovaginal candidiasis. Lactobacillus species predominate in normal vaginal flora and are thought to protect against infection by other species. The potential mechanisms for this protection have been identified as: 1) direct competition for nutrients, 2) blocking adhesion to the epithelial cell receptors, 3) production of bacteriocins, which can kill bacterial pathogens,(21,22) and production of lactic acid as a metabolite, which helps maintains a protective, acidic vaginal environment.(23) Additionally, several Lactobacillus species produce H₂O₂, which has antibacterial properties.(24)

With regard to the relationship between Lactobacillus and sexually transmitted infections, there are notable limitations to extrapolating that relationship to vulvovaginal candidiasis. First, *Candida* is not spread primarily through sexual contact. Second, *Candida* is considered a commensal organism in the genital tract, whereas no sexually transmitted infection is considered to be a commensal organism. The presence of *Candida* in the vagina is not inconsistent with healthy vaginal flora, is not considered to be of clinical importance, and is only of concern when women develop vulvovaginal candidiasis. Third, risk factors for *Candida* colonization, many of which have been investigated,(19,25,26) may not be risk factors for vulvovaginal candidiasis. In contrast, the literature around sexually transmitted infections risk focuses - by definition - on infection. Finally, *Candida* is a yeast species, as opposed to the bacterial, viral and protozoal agents that cause sexually transmitted diseases; any assumptions that overgrowth of vaginal

Candida is subject to the same pathogenesis mechanisms must be verified. For these reasons, it is essential to verify whether the presence of *Lactobacillus* protects against the development of vulvovaginal candidiasis, as it does for sexually transmitted infections.

Studies investigating the relationship between the presence of vaginal *Lactobacillus* and vulvovaginal candidiasis have not produced consistent results. A cross-sectional study of 340 women in Canada (the criteria employed in selecting these women were not described) found an inverse relationship, such that a smaller proportion of symptomatic women with detectable *Candida albicans* also had detectable *Lactobacillus*, compared to those without detectable *Candida albicans*.(27) Other studies have stratified vaginal *Lactobacillus* by its H₂O₂-production status, with conflicting conclusions: First, a cross-sectional study of 275 women recruited from prenatal clinics in the United States found that the prevalence of vulvovaginal candidiasis was higher among women with H₂O₂-negative *Lactobacillus* than among women with H₂O₂-positive *Lactobacillus*, or than among women who did not have detectable *Lactobacillus*.(28) Second, a prospective cohort study of 182 non-pregnant women presenting at a sexually transmitted disease clinic in the United States found no difference in the hazard of a subsequent diagnosis of vulvovaginal candidiasis between women with H₂O₂-positive *Lactobacillus*, H₂O₂-negative *Lactobacillus* and those without any detectable *Lactobacillus*.(29) A similar result was found in a cross-sectional study of 108 women recruited from outpatient clinics in Serbia and Montenegro.(30) Finally, a prospectus cohort study of 151 female sex workers in Kenya found that the presence H₂O₂-positive *Lactobacillus* and the presence of H₂O₂-negative *Lactobacillus* were both associated with increases in odds of a concurrent diagnosis of vulvovaginal candidiasis, relative to women who did not have detectable *Lactobacillus*.(31)

Paper 2 aims to identify factors associated with an increased prevalence vulvovaginal candidiasis. The examination of an association between vaginal *Lactobacillus* and prevalence of vulvovaginal candidiasis aims to address conflicting evidence in the literature: vulvovaginal candidiasis is thought to occur as a consequence of a reduction in the presence of vaginal *Lactobacillus*, possibly as a result of antibiotic use. Yet, the evidence for the widespread belief that a decrease in *Lactobacillus* is a mediator between antibiotic administration and incident vulvovaginal candidiasis is scant.(12,13,32) For this mediating role to be true, *Lactobacillus* must compete against or inhibit *Candida* colonization and growth, much as *Lactobacillus* does for other reproductive tract infections. If vulvovaginal candidiasis is, in fact, associated with decreased detection of *Lactobacillus*, there will be support for identifying interventions to enhance the presence of *Lactobacillus*, such as with probiotics, after women are treated with antibiotics.

Furthermore, identifying additional risk factors for vulvovaginal candidiasis remains an important goal, given the known economic and health repercussions of vulvovaginal candidiasis episodes. The literature frequently conflates risk factors identified for *Candida* colonization as being risk factors for vulvovaginal candidiasis, though only the latter is clinically relevant. Few modifiable risk factors for the development of vulvovaginal candidiasis have been identified, and research using laboratory-confirmed – rather than self-reported – detection of vulvovaginal candidiasis has largely been conducted in high-income settings. Less is known about the epidemiologic risk factors of vulvovaginal candidiasis in low-income settings, particularly among women at low risk of sexually transmitted infections. Identification of commonalities and

differences in risk factors across these settings may help elucidate the mechanism by which vulvovaginal candidiasis develop and thus aid in the search for potential interventions.

Specific aims:

The presence of vaginal *Lactobacillus* has been shown to be associated with reduced risk of sexually transmitted infection among women, although the same relationship has not been consistently demonstrated between the presence *Lactobacillus* and diagnosis of vulvovaginal candidiasis. The incidence and prevalence of, and other risk factors for, vulvovaginal candidiasis have not been well-characterized in low-income settings. Accordingly, paper 2 aims to:

1. Describe the incidence, prevalence, frequency of recurrence, and resolution of vulvovaginal candidiasis, over a six-month period, among reproductive-age women in Mysore, India.
2. Assess whether the prevalence of vulvovaginal candidiasis among reproductive-age women in Mysore, India, varies by the number of vaginal *Lactobacillus* morphotypes detected.

Hypothesis: The prevalence of vulvovaginal candidiasis will increase with increasing number of vaginal *Lactobacillus* morphotypes detected.

3. Describe the prevalence of vulvovaginal candidiasis by sociodemographic (i.e. age, education, religion, years with sex partner, socioeconomic status, parity, and religion), behavioral (i.e. age at first sexual intercourse, number of vaginal sex acts in three months, and ever had anal sex), clinical (i.e. tubal ligation, HSV2 infection, *Trichomonas vaginalis* infection, diagnosis of bacterial vaginosis, and vaginal pH ≥ 4.5) and partner (i.e. sex partner has other sex partners) characteristics among reproductive age women in Mysore, India.

Paper 3: Machine learning for syndromic diagnosis of vaginal infections: Syndromic diagnostic algorithms are commonly used to diagnose women presenting with vaginal discharge in low-income settings, where clinical facilities often lack laboratory capacity. The algorithms allow for diagnosis of reproductive tract infections based on the signs observed by a clinician and symptoms reported by women. While the World Health Organization (WHO) offers a diagnostic algorithm for women presenting with vaginal discharge, individual countries have made modifications to the algorithm, allowing for the possibility that some clinics will supplement clinical findings with simple laboratory tests and/or a pelvic examination.(33,34)

The use of these algorithms has enabled clinicians to offer women a diagnosis and treatment when they present with vaginal discharge. However, validation studies in low-income settings have shown the specificity of syndromic diagnosis of bacterial vaginosis, trichomoniasis and vulvovaginal candidiasis to be below 50%, and perhaps as low as 0%.(35–39) The correspondingly low positive predictive values of syndromic diagnosis indicate that use of these algorithms leads to substantial misdiagnosis and overtreatment of women, and an increase in the average cost per true case treated.(38)

Machine-learning statistical procedures offer opportunities to increase the potential for measurements collected in a clinical examination to predict the presence of vaginal infections. Logic regression is a machine-learning procedure that generates highly interpretable output, consisting of a Boolean combination of predictor variables which are evaluated as TRUE or FALSE for the presence of a given vaginal infection. Thus, with laboratory-confirmed diagnoses of vaginal infections as the gold-standard, logic regression can be used to develop models to

predict the presence of vaginal infections, whereby the potential predictors for the models can be a locally-appropriate set of measures collected in a clinical interview and examination. The most predictive diagnostic algorithm can be evaluated for its sensitivity, specificity, positive predictive value and negative predictive value, and can then be considered for validation by future research.

Paper 3 will be an evaluation of a WHO syndromic diagnosis algorithm of vaginal infection and comparison to logic regression-derived models. This paper will be the first to establish the specificity and positive predictive values for the syndromic diagnosis of vaginal infections in this population. On an individual level, if the specificities for syndromic diagnoses of vaginal infections from these algorithms are found to be poor, clinicians will have a rationale to conduct additional tests to confirm positive diagnoses. On a population level, poor specificities and low positive predictive values for syndromic diagnoses of vaginal infections will justify additional investigation into the cost per true case treated and consideration of whether these algorithms are the most effective tools to reduce the prevalence of vaginal infections.

The logic regression models (one for the presence of bacterial vaginosis or trichomoniasis, another for the presence of vulvovaginal candidiasis) can be compared to an existing WHO algorithm with regard to their diagnostic performance. If the logic regression-derived models have sufficiently high sensitivities and specificities for diagnoses of vaginal infections, the algorithms can be validated in separate populations of reproductive age, non-pregnant women, either prospectively or through another publicly available dataset. Moreover, the process by which logic regression derives models for diagnostic prediction could be implemented for other populations, using freely available software.

Specific aims:

Syndromic diagnosis of vaginal infections remains in common use in clinical facilities and in epidemiologic studies of disease burden in low-income settings. Though recommended by the WHO for use in these settings, the accuracy of syndromic diagnosis algorithms has been shown to be insufficient for individual treatment or to achieve a population-level reduction in the prevalence of vaginal infections. The accuracy of syndromic diagnosis of vaginal infections has not been evaluated in Mysore district, India, and the accuracy of the syndromic diagnosis algorithm has not been compared to the predictive models that can be developed through the logic regression procedure. In light of this, paper 3 aims to:

1. Measure the proportions of women with symptoms associated with vaginal infection (i.e. vaginal itching, burning or discharge) who have a laboratory-confirmed diagnosis of bacterial vaginosis, trichomoniasis, or vulvovaginal candidiasis.
2. Describe the performance characteristics (i.e. sensitivity, specificity, positive predictive value, negative predictive value) of a WHO algorithm for syndromic diagnosis of vaginal infection, among women reporting symptoms associated with vaginal infection.
3. Identify syndromic diagnostic algorithms for laboratory-confirmed bacterial vaginosis, trichomoniasis, or vulvovaginal candidiasis using logic regression, and describe their performance characteristics (i.e. sensitivity, specificity, positive predictive value, negative predictive value).

Hypothesis: The sensitivity, specificity, positive predictive value, and negative predictive value of the logic regression-derived diagnostic algorithms will be higher than those of the WHO algorithm for the laboratory-confirmed diagnoses of bacterial vaginosis, trichomoniasis, or vulvovaginal candidiasis.

The Prerana cohort dataset is well-suited to filling in multiple gaps in the research literature concerning the diagnosis and prevalence of, and risk factors for, vaginal infections in a low-income setting. With a cohort of nearly 900 women with three study visits, the effective sample size for both cross-sectional and longitudinal analyses is quite large, and provides substantial statistical power for investigating the multiple research aims described above. As the primary analysis also concerned reproductive tract infections, this prospective cohort study offers high-quality data for the secondary analyses. The study visits were completed by a trained staff using a standardized protocol for the interview, clinical examination and laboratory testing. Though the cohort was not randomly selected from the study population, the use of consecutive, rather than convenience, sampling, increases the likelihood results can be generalized to the population from which the cohort was recruited. That this population of reproductive age women in Mysore is relatively homogenous with regard to several factors (eg smoking, *Neisseria gonorrhoeae* infection, hormonal contraceptive use, multiple sex partners, and vaginal douching) that could confound hypothesized relationships may reduce confounding bias in estimating measures of association. The Prerana cohort allows for the opportunity to not only describe the prevalence of vaginal infection and its risk factors in a medically underserved population, but also to be one of the first studies to test (or re-test) hypotheses using a community-based sample based in a low-income setting.

There are important limitations to consider with these secondary analyses. Inclusion criteria led to enrollment of reproductive aged, sexually active, non-pregnant women in peri-urban and rural Mysore, so there are limits to the generalizability of results generated from these analyses beyond this population. Though laboratory testing was conducted using a standardized protocol, there is high variability of the bacterial flora in the vaginal environment,(45) which leads to an unquantifiable error in determining a woman's true number of Lactobacillus or her bacterial vaginosis status. It is not possible to determine whether the resulting misclassification of vaginal flora status is non-differential with regard to certain other measurements, and would thus conservatively bias estimation of the corresponding measures of association. Furthermore, as clinical diagnoses of bacterial vaginosis and vulvovaginal candidiasis both entail clinical assessment of discharge, each condition may be misdiagnosed as the other. For this reason laboratory - not clinical - diagnosis of bacterial vaginosis is used for analytic purposes. Additionally, the validity of self-reported vaginal discharge as a manifestation of reproductive tract infections in south Asian populations has come into question; reports of vaginal discharge may often be of psychosomatic, rather than infectious, origin.(46,47)

Due to the time interval between study visits, it was not possible to verify that treatment led to cure among women diagnosed with a treatable reproductive tract infection. As a result, estimation of persistence of a given vaginal infection includes women who were not cured, along with those with resolution and re-development of the condition between study visits. Similarly, we did not assess whether participants self-treated for vaginal discharge between visits. Self-treatment, given correct self-diagnosis of vaginal infection, would lead to a reduction in the study's measurements of incidence of vaginal infection. It is also important to note that due to

funding limitations, this study did not test participants for infection by *Chlamydia trachomatis*, *Treponema pallidum*, or HIV. This study cannot estimate the prevalence and incidence of these infections, or assess whether they have a relationship to the vaginal infections. This is unlikely to be a source of confounding bias for the analyses described above, as a large population-based study conducted in Mysore in 2005-2006 found low prevalences of these sexually transmitted infections among adult women (1.0%, 1.0% and 0.6%, respectively).(48) Yet, the results generated from the analyses described above may not apply to populations where these other infections are more prevalent. The Prerana cohort did not enroll womens' sexual partners, so many partner characteristics, behaviors, or sexually transmitted infections status could not be ascertained or verified. Any associations relating to partner characteristics should be interpreted with caution. Finally, as with all observational studies, unmeasured confounding limits the extent to which any associations detected can be interpreted causally.

Despite these limitations, a number of findings from these analyses are expected to fill gaps in knowledge. Of primary importance, the prevalence and incidence of bacterial vaginosis, trichomoniasis and vulvovaginal candidiasis will be determined in a sample of reproductive age non-pregnant women, which is an important first step to public health planning in this population. With estimates of the prevalence of vaginal infections, it is possible to develop and target programs to diagnose and treat the infections. By successfully diagnosing and treating these infections in this population, it is plausible that the risk of adverse birth outcomes and acquisition of sexually transmitted infections will also decline.

In sum, epidemiological studies - largely conducted in high-income settings - have identified many risk factors for bacterial vaginosis, trichomoniasis and vulvovaginal candidiasis. Less is known about the risk factors for these vaginal infections in low-income countries such as India, where gynecologic morbidity is extremely common.(40–44) Further, consequences of these conditions - namely adverse birth outcomes and sexually transmitted infections - are highly prevalent in many low-income settings, providing ample justification for additional inquiry into the diagnosis of and risk factors for these vaginal infections.

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Bacterial vaginosis and risk for *Trichomonas vaginalis* infection: a longitudinal analysis

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Abstract

Background: Bacterial vaginosis (BV) and *Trichomonas vaginalis* (TV), have been estimated to affect one-quarter to one-third of sexually active women worldwide, and are often found concurrently. Few studies have examined this relationship longitudinally to better understand the direction and temporality of this association.

Methods: Between 2005 and 2006, a cohort of 853 young, sexually active women was followed in Mysore, India; participants were interviewed and tested for BV and TV at baseline, and at three- and six-month visits. Generalized estimating equations were used to estimate how changes in vaginal flora between consecutive visits – as defined by Nugent diagnostic criteria for BV - were related to the risk of TV infection at the latter visit, adjusted for sociodemographic and behavioral covariates. Treatment was offered to women with TV and/or symptomatic BV.

Results: After adjustment for covariates, participants with abnormal flora at two consecutive visits had nine times higher risk of TV (95% CI 4.1, 20.0) at the latter visit, relative to those with persistently normal flora. An increased risk of TV was also observed for participants whose flora status changed from normal to abnormal (aRR 7.11, 95% CI 2.8, 18.2) and from abnormal to normal (aRR 4.50, 95% CI 1.7, 11.8).

Conclusions: Women experiencing abnormal flora during a three-month span appear to have significantly increased risk of acquiring TV infection. Reproductive-age women in low-resource settings found to have abnormal vaginal flora should be assessed for TV.

Introduction

Bacterial vaginosis (BV) and *Trichomonas vaginalis* (TV) infection have been estimated to affect as many as one-quarter to one-third of sexually active females worldwide,(1,2) and are often found concurrently.(3) Both BV and TV have been associated with adverse birth outcomes such as prematurity and low birth weight,(4,5) pelvic inflammatory disease,(6,7) infertility,(8,9) and acquisition of HIV and Herpes Simplex Virus type 2 (HSV-2) infections.(1,10-12) Surprisingly, given the well-described association between BV and TV, few data that would help clarify the temporality of the relationship between BV and TV infection are available.

Understanding the relationship between BV and TV has been problematic because most studies have been cross-sectional in nature.(13-17) Nevertheless, results of a number of studies suggest that TV colonization is increased in the presence of BV-defining phenomena such as elevated amine production, loss of facultative lactobacilli, and increased pH.(18-22) The present study describes the results of a secondary analysis of data originally collected in a prospective cohort study examining the relationship of abnormal vaginal flora and incident HSV-2 infections among young reproductive age women in Mysore, India.

Materials and Methods

Participant recruitment and laboratory methods have been described in detail elsewhere.(23-25) In brief, 15-30 year old, non-pregnant, sexually active women were recruited in 2005-2006, through health education camps offered in the rural and peri-urban communities around Mysore city, in south India. At baseline, and three- and six-month visits, participants underwent an interviewer-administered questionnaire and a physical examination, during which biological specimens, including vaginal, high cervical swabs and venous blood, were collected. Of the 898 women who completed the baseline visit, 853 provided data from at least 2 consecutive study visits. These 853 participants comprise the study sample for this analysis.

Laboratory methods: TV infection was diagnosed based on a positive result from either wet-mount microscopy for detection of motile Trichomonads and/or culture (InPouch Culture Kit, BioMed Diagnostics, White City, OR, USA). All women had specimens collected for both TV tests. Women with TV infection were treated with a single dose of two grams of oral metronidazole, and the same treatment was given to participants to give to their sex partners. BV was diagnosed clinically using Amsel's criteria and treated with 400 mg oral metronidazole twice daily for one week.(26) For analytic purposes, BV was diagnosed by Nugent scoring of gram stained vaginal smears.(27) ELISA testing was done for HSV-2 IgG antibodies (Focus Technologies, Cypress, CA, USA). Institutional review boards at the University of California, Berkeley, and the Asha Kirana Hospital in Mysore, India, approved the study protocol. Informed consent was obtained from all study participants.

Statistical analysis: In a cross-sectional analysis of baseline data, increased prevalence of TV was found among those testing positive for BV (Nugent score 7-10) and among those with Intermediate scores (Nugent score 4-6), both of which indicate the presence of abnormal vaginal flora.(24) Thus, for this analysis Nugent scores were categorized into a dichotomous measure of vaginal flora status, with 0-3 considered 'Normal' and 4-10 'Abnormal'.(27) To estimate the relationship between changing vaginal flora status and TV infection risk, log-linear generalized estimating equations (GEE) with an exchangeable covariance structure, and bootstrapped results with 5000 repetitions for robust standard errors, were used.(28) Four exposure patterns were

defined based on participant vaginal flora status on a given visit (Abnormal or Normal) and status at the previous visit (Abnormal or Normal). The four patterns represent different exposure periods of abnormal vaginal flora between visits. With three study visits, participants could contribute two exposure patterns (for 0 to 3 months, and 3 to 6 months) to the GEE model, to estimate the risk of TV at 3 and 6 months, respectively. The GEE model provides covariate-adjusted estimates of risk ratios (aRR) for TV by different vaginal flora exposure patterns between visits, compared to those whose Nugent tests scores were Normal at both visits. (Figure 1) Eight hundred and twenty participants contributed at least one observation to the GEE model; unreadable vaginal smears accounted for all but three of the 33 participants not included in the analysis.

The selection of possible confounders and cut points for categorization of continuous variables was based on findings from the baseline cross-sectional analyses for BV and TV infections (12,24) and previously published literature. For the multivariable GEE model, variables with small strata (<10% of sample) were not controlled for. Strata from ordinal categorical measures were combined if the baseline TV analysis showed similar prevalences of TV across adjacent groups. For the religious identification measure, Hindu and Christian women were combined into a single 'Non-Muslim' category. In three observations where a participant's marital status was not recorded, her marital status at the previous visit was used, as there was minimal change in marital status over time in this cohort. For HSV-2 testing, 21 missing results were recoded to negative, as HSV-2 incidence was low in this cohort. A socioeconomic (SES) index was created using the first factor generated from a principal components analysis of assets owned, financial instruments used, and dummy-coded cooking stove type.(29) SES score was made into a dichotomous categorical measure to create balanced categories of Low and High SES. Two participants with missing asset data were coded into the Low SES category.

Two additional GEE models were run to examine the sensitivity of results due to the clinical definition of abnormal flora. In the first case, abnormal flora was defined by a Nugent score of seven or higher. In the second case, abnormal flora was defined by an Amsel score of three or higher. A third GEE model was run using our original definition of abnormal flora (Nugent score of four or higher), but using only incident TV infections. All three additional GEE models used the same covariate and bootstrapping specifications as the original model. Data were analyzed using Stata 11.1 (StataCorp, College Station, TX, USA).

Results

A description of the full cohort with baseline TV prevalence has previously been reported elsewhere;(12) additional baseline measures are described here for the analyzed cohort. A majority of women had been with their partners for 7-19 years (78.1%), and these women were at higher risk of a TV diagnosis (9.4% vs. 6.5% for women who had partners for less than seven years). Women were equally divided across SES categories, with a higher prevalence of TV found in the lower SES category (10.6% vs. 6.4% with higher SES). While a large majority (69.1%) of women reported at least 13 sex acts in the three months prior to the baseline visit, prevalence of TV did not vary across sexual activity categories. Very few women reported having a partner who had other sex partners, or using an intrauterine device for contraception, leading to unstable estimates of the prevalence of TV in these strata. No participants reported

smoking, using hormonal contraception or vaginal douching. These latter measures were thus not included as potential confounders in the multivariable GEE model.

Table 1 shows TV risk by change in vaginal flora status between the baseline and three-month visits. For ease of reporting, only the baseline- to three-month visit results are tabulated; results from the three- to six-month visits (data not shown) demonstrate a similar risk gradient. A majority of women had normal flora at both visits (54.9%), and few of these women (1.4%) tested positive for TV at three months. Fewer women had normal flora at baseline and abnormal flora at the three-month visit (12.4%), though a higher proportion tested positive for TV at three months (6.2%). Of the women who had abnormal flora at baseline and normal flora at the three-month visit (13.2%), a similar proportion (7.8%) tested positive for TV at three months. Approximately one in five women had abnormal vaginal flora at both the baseline and three-month visit (20.6%), and these women were at greatest risk for testing positive for TV at the three-month visit (13.0%).

Prevalence and incidence of TV infection and abnormal vaginal flora

Prevalence of TV declined by study visit, from 8.5% at baseline to 5.5% at three months and 3.0% at six months. Of the 775 women who were TV negative at baseline, 23 (3.0%) had TV diagnosed at their three month follow-up visit and eight (1.1%) had TV at the six-month visit; of those 8, 5 were first-time TV diagnoses. In comparison, of the 73 women who had TV at baseline, 24 (32.9%) were TV infected again at the three-month visit, and 10 had TV at all three visits, despite having received repeated metronidazole treatment. The prevalence of abnormal vaginal flora also decreased by visit, from 33.7% at baseline to 32.4% at three months and 28.7% at six months. Of the 526 women with normal vaginal flora at baseline, 35/526 (6.6%) were Amsel positive for BV, and 97/526 (18.4%) had abnormal flora at three months. In comparison, of the 264 women with abnormal flora at baseline, 60/264 (22.7%) were Amsel positive, and 161/264 (61.0%) had abnormal flora again at three months, and 94 participants had abnormal flora at all three visits. For all visits, Amsel clinical diagnosis resulted in treatment of 4.1% of those with normal flora (Nugent score 0-3), 18.3% with intermediate flora (Nugent score 4-6), and 34.7% with BV (Nugent score 7-10).

TV risk by multivariable GEE analysis (Table 2)

Participants with abnormal flora across any two consecutive visits had nine times higher risk (95% CI 4.1, 20.0) of TV infection at the latter visit relative to those women with persistently normal flora, adjusted for covariates. Women whose flora became abnormal after being normal at the previous visit had a higher risk of TV (aRR 7.11, 95% CI 2.7, 18.2), as did those whose flora became normal after being abnormal at the previous visit (aRR 4.50, 95% CI 1.7, 11.7). Of the covariates examined, Muslim religion (aRR 0.44, 95% CI 0.2, 1.1), having a partner for at least seven years (aRR 1.94, 95% CI 0.9, 4.0) and HSV-2 infection (aRR 1.68, 95% CI 0.9, 3.0) each had weaker evidence for an association with TV infection.

Table 3 shows results from the alternate GEE models. The GEE models using different definitions for abnormal flora provided results that were all consistent in direction, though with reduced magnitude relative to the original GEE model. For the three longitudinal exposure categories Normal-Abnormal, Abnormal-Normal and Abnormal-Abnormal, the model using

Nugent scores of seven or higher to indicate abnormal flora resulted in aRR of 4.12 (95% CI 1.9, 8.9), 4.44 (95% CI 2.0, 10.1) and 3.15 (95% CI 1.4, 7.0) respectively and the model using Amsel score of three or higher resulted in aRRs of 2.81 (95% CI 1.5, 5.3), 1.94 (95% CI 0.7, 5.8; p=0.215) and 4.89 (95% CI 2.3, 10.5) respectively. The GEE model using our original abnormal flora definition of Nugent scores of four or higher but only including incident TV infection resulted in aRR of 8.59 (95% CI 3.2, 22.7), 3.15 (95% CI 0.9, 10.5; p=0.043) and 4.75 (95% CI 1.8, 12.6), respectively. Unless otherwise indicated, all p-values were less than 0.005.

Discussion

We found evidence for a 4- to 9-fold increased risk of TV infection among women who had abnormal vaginal flora within a three month span, with the highest risk among those women found to have abnormal flora at consecutive visits. Our alternate GEE models indicate the relationships are generally maintained when other widely accepted abnormal flora measurement criteria are used, and for incident TV infection. This finding is consistent with the findings of cross-sectional studies showing that a disturbed vaginal flora is associated with an increased risk of sexually transmitted infections, including HIV and TV.(13,30-33) It also confirms the findings from longitudinal studies in Kenya and the United States,(20,22) which show that abnormal vaginal flora is associated with subsequent acquisition of TV infection, and thus strengthens the evidence for a causal role. Thurman, Doncel and others have suggested a number of mechanisms whereby disturbed vaginal flora might increase the risk of HIV and other STI infections, including: initiation of a clinical or subclinical mucosal inflammatory response; alteration of innate mucosal immunity; alteration of normal vaginal microflora and pH; and weakening or breach of the cervico-vaginal mucosa.(22,34-38) As other studies have suggested, all of these mechanisms could also plausibly pertain to the relationship between abnormal vaginal flora and acquisition of TV infection.

The declining prevalence of TV infection and abnormal flora status over time in this cohort is unsurprising, given that the women were treated with metronidazole, which has been shown to be effective in treating both conditions.(39,40) As TV infection is typically self-limiting in men,(41) and reported extra-marital partnerships were low among participants, the reduction in prevalence from 8.5 to 3.0% after two rounds of diagnosis and treatment was expected, and demonstrates that control of this infection with metronidazole in resource-limited settings is possible. In contrast, the small reduction in the prevalence of abnormal flora is of concern, as is the high rate of repeat diagnosis. Treatment based on the Amsel diagnostic criteria identified only 35% of women who were found to have BV based on Nugent test (and 18% of women with Intermediate Nugent scores). In this setting, the Amsel criteria did not appear to be sufficiently a sufficiently sensitive method for diagnosis of BV. This is also a notable finding because BV has been found to increase the risk for STIs and adverse birth outcomes, which are commonly found in resource constrained settings.(42)

Consistent with other studies that found Muslim religion to be associated with a lower risk of HIV and STI, our study found that the risk of TV was lower among Muslim women, which could be due to cultural practices, such as male circumcision among their sex partners.(43,44) We also found some evidence of an increased risk of TV infection associated with HSV-2 infection, consistent with the findings of other studies of TV.(16,45) HSV-2 infection has been shown to

increase the risk of acquisition of STIs because of genital ulcers,(46) and may be a proxy of past sexual risk behavior.(47)

This study has a number of strengths, including: a large sample size, standardized laboratory testing for TV and BV, minimal loss to follow up, and the use of longitudinal measures. On the other hand, there were also several limitations: First, we did not use a population-based sample, so our findings may not be generalizable. Second, as vaginal flora are highly dynamic,(48) it is possible that we underestimated new abnormal flora episodes between visits. Third, because we did not do a ‘test of cure’ for women treated in the study, we do not know whether TV infections were new or persistent. Because metronidazole treatment is thought to be 95% effective,(49) it is unlikely that this limitation would significantly affect the findings. Furthermore, although all women diagnosed with TV were provided with treatment for their sex partners, we could not ascertain whether partners were offered or accepted the treatment. What appears to be repeated TV infection may be prevalent infections from the perspective of the sexual dyad, and as such the results from this analysis cannot fully rule out reverse causation. However, the final GEE model, using only incident TV infection, does provide additional evidence of abnormal flora preceding TV infection. Finally, since a minority of participants with abnormal vaginal flora was treated based on Amsel criteria, the aRR estimates are likely biased towards the null.

In conclusion, the findings of this study emphasize the need to screen for TV when women are found to have abnormal vaginal flora. More research is needed to help ascertain the mechanisms or co-factors involved in the relationship between BV and acquisition of TV including common exogenous factors, such as other sexually transmitted infections and host immune factors. It will be important to understand the mechanisms by which abnormal vaginal flora enhances susceptibility to TV.

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Figure 1: GEE model specification

For i = participants 1 to 853 and j = visits 2 to 3:

$$\begin{aligned} \log[\Pr(\text{TV})_{ij} = 1 \mid \text{covariates}] = & \beta_0 + \beta_1(\text{abnormal flora})_{ij} + \beta_2(\text{abnormal flora})_{ij-1} + \\ & \beta_3((\text{abnormal flora})_{ij} * (\text{abnormal flora})_{ij-1}) + \beta_4(\text{age of sexual debut})_{i1} + \\ & \beta_5(\text{years with partner})_{i1} + \beta_6(\text{years of education})_{i1} + \beta_7(\text{religion})_{i1} + \\ & \beta_8(\text{vaginal sex acts in past 3 months})_{ij} + \beta_9(\text{asset index score})_{i1} + \beta_{10}(\text{HSV2})_{ij} \end{aligned}$$

Table 1: Stratum-specific *Trichomonas vaginalis* infection by baseline to three-month visit vaginal flora status, Mysore, India 2005-2006

	Retained at three-month visit (n=790) *		Of which TV positive (n=41) at three-month visit	
	n	%	n	%
Vaginal flora status**				
Normal-Normal	429	54.9	6	1.4
Normal-Abnormal	97	12.4	6	6.2
Abnormal-Normal	103	13.2	8	7.8
Abnormal-Abnormal	161	20.6	21	13.0

* 58 women were missing one or both vaginal flora specimens, of which 6 (10.3%) were positive for TV at the three-month visit.

** Normal flora is defined as a Nugent score of 0-3, Abnormal flora is a Nugent score of 4-10.

Table 2: Log-linear multivariable GEE model results for *Trichomonas vaginalis* infection at three- or six-month visits, Mysore, India 2005-2006

	Adjusted RR	95% CI	p
Vaginal flora status change ⁺			
Normal-Normal	1.00		
Normal-Abnormal	7.11	2.77, 18.24	***
Abnormal-Normal	4.50	1.72, 11.77	***
Abnormal-Abnormal	9.00	4.05, 20.02	***
Age of sexual debut (years)			
7-14	1.00		
15-29	0.96	0.56, 1.64	
Years with partner			
0-6	1.00		
7-19	1.94	0.94, 4.00	*
Years of education			
0	1.00		
1-17	1.29	0.76, 2.18	
Religion			
Hindu/Christian	1.00		
Muslim	0.44	0.17, 1.13	*
Vaginal sex acts in past 3 months			
0-12	1.00		
13-120	0.80	0.47, 1.36	
SES			
Low	1.00		
High	0.72	0.43, 1.20	
HSV-2 positive			
No	1.00		
Yes	1.68	0.94, 3.03	*

* p < 0.10

*** p < 0.005

⁺ Normal flora is defined as a Nugent score of 0-3, Abnormal flora is a Nugent score of 4-10.

Table 3: Sensitivity of log-linear multivariable GEE model results for *Trichomonas vaginalis* infection at three- or six-month visits, Mysore, India 2005-2006

	Abnormal flora is Nugent ≥ 7 , all TV infection			Abnormal flora is Amsel ≥ 3 , all TV infection			Abnormal flora is Nugent ≥ 4 , incident TV infection		
	aRR	95% CI	p	aRR	95% CI	p	aRR	95% CI	p
Vaginal flora status change									
Normal-Normal	1.00			1.00			1.00		
Normal-Abnormal	4.12	1.91, 8.90	***	2.81	1.49, 5.26	***	8.59	3.24, 22.71	***
Abnormal-Normal	4.44	1.96, 10.07	***	1.94	0.65, 5.75		3.15	0.94, 10.50	*
Abnormal- Abnormal	3.15	1.41, 7.03	***	4.89	2.27, 10.54	***	4.75	1.79, 12.59	***

aRR = Adjusted Risk Ratio

* p < 0.10

*** p < 0.005

Epidemiologic features of vulvovaginal candidiasis among reproductive-age women in India

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Abstract

Objective

Vulvovaginal candidiasis is caused by overgrowth of *Candida* yeast species in the vagina, is characterized by curd-like vaginal discharge, itching and erythema, and is associated with considerable health and economic costs. Few studies have measured the incidence of confirmed episodes of vulvovaginal candidiasis or have examined potential risk factors for vulvovaginal candidiasis in a low-income setting.

Methods

We examined the incidence, prevalence, and risk factors for vulvovaginal candidiasis among a cohort of 898 women in south India. Women in the cohort completed three study visits over six months, comprised of a structured interview, a clinical examination, and collection of cervico-vaginal specimens for laboratory testing.

Results

The prevalence of vulvovaginal candidiasis declined over six months, from 9% to 5%. The positive predictive values for diagnosis of vulvovaginal candidiasis using individual signs or symptoms were low (<19%). In cross-sectional analysis, we did not find strong evidence for associations between sociodemographic characteristics and the prevalence of vulvovaginal candidiasis. Women clinically diagnosed with bacterial vaginosis had a higher prevalence of vulvovaginal candidiasis (Prevalence 12%, 95% CI 8.2, 15.8) compared to women assessed to be negative for bacterial vaginosis (Prevalence 6.5%, 95% 5.3, 7.6); however, differences in the prevalence of vulvovaginal candidiasis were not observed by the presence or absence of laboratory-confirmed bacterial vaginosis.

Conclusion

We found that syndromic diagnosis will result in substantial overdiagnosis and overtreatment of vulvovaginal candidiasis-negative women. For correct diagnosis of vulvovaginal candidiasis, laboratory confirmation of vaginal infection with *Candida* is necessary, as is a means of assessing whether the discharge has been caused by bacterial vaginosis. Studies of women infected with *Candida* yeast species are needed to determine the risk factors for the yeast's overgrowth in the vagina.

Introduction

Vulvovaginal candidiasis is caused by overgrowth of *Candida* yeast species in the vagina and is characterized by curd-like vaginal discharge, itching and erythema.(1) Vulvovaginal candidiasis has been associated with considerable direct and indirect economic costs;(2) enhanced susceptibility to HIV infection;(3) and an increased risk of preterm birth.(4,5) Treatment of vulvovaginal candidiasis is warranted when a woman presenting with a complaint of symptoms consistent with vulvovaginal candidiasis also has laboratory confirmation of the presence of *Candida* from a vaginal specimen. Short-course azole-based treatment regimens are considered effective and safe(6) and are accessible and affordable in most settings.

Much of the epidemiologic literature concerning vulvovaginal candidiasis reports on studies in which women were queried on their self-reported history of vulvovaginal candidiasis,(7) but without laboratory-confirmation of infection by *Candida*. Other studies, in which investigators only measure the presence of *Candida* infection of the vagina,(1) are not able to identify women with symptomatic vulvovaginal candidiasis disease; this latter study design is frequently employed for studies conducted in low-income settings. Few studies have diagnosed vulvovaginal candidiasis through laboratory confirmation of infection in symptomatic women, and few studies have measured the incidence of confirmed cases of vulvovaginal candidiasis.

The lack of representative data on the epidemiologic features of laboratory-confirmed vulvovaginal candidiasis has been evident throughout the time in which vulvovaginal candidiasis has evolved from being considered a "nuisance infection" to a clinically relevant condition.(8,9) In India, only two studies have been conducted in which laboratory-confirmed vulvovaginal candidiasis was diagnosed in a community-based sample: Bang et al diagnosed vulvovaginal candidiasis in 35% of 650 adult women living in rural Maharashtra state, and Prasad et al diagnosed vulvovaginal candidiasis in 10% of 451 married, 16-22 year old women in rural Tamil Nadu state.(10,11) However, neither study assessed the incidence of or identified risk factors for vulvovaginal candidiasis.

As reduction of HIV transmission and of adverse birth outcomes remain public policy priorities in India,(12) and studies have shown gynecological morbidity is extremely common,(13–16) additional investigation of the epidemiologic features of vulvovaginal candidiasis is warranted.

Materials and Methods

We examined the incidence, prevalence, and risk factors for vulvovaginal candidiasis among a cohort of women recruited for a study of the presence of bacterial vaginosis and incident Herpes simplex virus - type 2 infection.(17) The recruitment and baseline descriptive features of the cohort of 898 sexually active, non-pregnant women between 16 and 30 years of age from communities around Mysore, India have been previously described.(18,19) Briefly, between 2005 and 2006, women in the cohort completed three study visits (at baseline and at three and six months), comprised of a structured interview, a clinical examination, and collection of cervico-vaginal specimens for laboratory testing. Trained interviewers used a structured interview to collect sociodemographic and behavioral information, as well as reports of symptoms associated with gynecologic morbidity.

Laboratory and clinical methods: Study physicians performed a pelvic examination of each participant and recorded signs of vaginal abnormalities. During the examination, swabs of the posterior fornix of the vagina and blood specimens were collected. Women diagnosed with vulvovaginal candidiasis were treated with a single 150 mg dose of oral fluconazole.

Trichomonas vaginalis infection was treated with a single two-gram dose of oral metronidazole, and participants were given the option of receiving treatment to give to their sex partner. Women were clinically diagnosed as having bacterial vaginosis using criteria developed by Amsel et al(20) those with bacterial vaginosis were treated with 400 mg oral metronidazole administered twice daily for one week.

Women were diagnosed as having *Trichomonas vaginalis* infection if found to be positive on either wet mount microscopy or culture (InPouch, BioMed Diagnostics, White City, OR, USA). A Gram stain was used for laboratory diagnosis of bacterial vaginosis using the criteria developed by Nugent et al, whereby bacterial vaginosis was diagnosed with a score of 7-10, while a score of 4-6 was considered intermediate vaginal flora, and a score of 0-3 was considered negative for bacterial vaginosis.(21) As part of the Nugent-criteria scoring, the average number of Lactobacillus-like cells (morphotypes) detected over several visual fields in light microscopy was enumerated, and given a score corresponding to averages of 0, <1, 1-4, 5-29 and ≥ 30 .

Candida infection was assessed from culture (InTray Colorex Yeast, BioMed Diagnostics, White City, OR, USA). Participants infected with *Candida* were diagnosed as having vulvovaginal candidiasis if they reported vaginal itching or discharge and had vaginal erythema or discharge observed on clinical examination. Herpes simplex virus - type 2 infection was assessed by an index > 1.09 from type-specific ELISA testing of serum (Focus Technologies, Cypress, CA, USA). (Table 4)

Statistical methods: Our outcome measure was laboratory-confirmed diagnosis of vulvovaginal candidiasis. First, we describe prevalence and incidence of vulvovaginal candidiasis using frequencies and percentages. We calculated the visit-specific prevalence of vulvovaginal candidiasis and assessed whether this prevalence changed over the course of the study. Next, we tabulated the number of women's clinic visits with various vaginal symptoms reported or vaginal signs observed. Among observations in which these vaginal signs or symptoms were recorded, we calculated the proportion in which a diagnosis of vulvovaginal candidiasis was made. Finally, we examined the relationship between the prevalence of vulvovaginal candidiasis and sociodemographic characteristics and possible risk factors. We used a separate univariable regression model for each characteristic and risk factor. While counts (e.g. the number of diagnoses of vulvovaginal candidiasis) are reported as observed, percentages and prevalences were estimated using generalized estimating equations (GEE) regression models. GEE allows for parameter estimation when observations are correlated, in this case, due to multiple observations per participant over the course of the study. The GEE models were specified with binary family, identity link, and exchangeable correlation. The GEE models were bootstrapped with 500 repetitions to estimate robust standard errors.

Statistical analysis was conducted using Stata 11.2 (StataCorp, College Station, TX, USA). The study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley, and the Asha Kiran Institutional Review Board of Mysore, India; all participating women provided written informed consent.

Results

The median age of the women at the baseline visit was 26 years (Interquartile range [IQR] 24-29 years), and participants had been with their current sex partner for a median of nine years (IQR 6-12 years). Over one-quarter of the women had no formal education (27%). Nearly one in three women identified as Muslim (29%). Few women were nulliparous (15%), and very few women reported using oral contraception, condoms, or an intrauterine device at any point during the study. Furthermore, less than 5% reported having oral sex or having had more than one lifetime sex partner. No woman reported douching or smoking tobacco. Throughout the duration of the study, the mean prevalences of reproductive tract infections were as follows: *Trichomonas vaginalis* (6%), clinical diagnosis (Amsel criteria) of bacterial vaginosis (12%), laboratory diagnosis (Nugent criteria) of bacterial vaginosis (16%), and infection with Herpes simplex virus - type 2 (13%). At least 30 Lactobacillus morphotypes were detected on the majority (66%) of Gram stains of vaginal swabs, with smaller proportions of swabs with 5-29, 1-4, <1 and 0 morphotypes detected (11, 5, 6 and 12%, respectively).

Vaginal signs and symptoms, and diagnosis of vulvovaginal candidiasis: Including all three study visits, we found that substantial proportions of the women reported vaginal itching (29%) or vaginal discharge (31%), or had vaginal erythema (9%) or vaginal discharge (35%) on examination. The positive predictive values of these signs and symptoms for predicting vulvovaginal candidiasis were low: a minority of women with these symptoms or signs was subsequently diagnosed with vulvovaginal candidiasis (18, 15, 25, and 18%, respectively). Combinations of the vaginal signs and symptoms were increasingly rare, though the probability of a correct diagnosis of vulvovaginal candidiasis increased to 41% when both signs and both symptoms were present. (Table 5)

Diagnosis, prevalence and incidence of vulvovaginal candidiasis: Of the 885 observations in which *Candida* was detected in vaginal specimens, 180 (20%) satisfied the case definition for diagnosis of vulvovaginal candidiasis, while the remaining 705 (80%) were considered asymptomatic infection.

The prevalence of vulvovaginal candidiasis declined over the three study visits: from 77/893 (9%) at baseline to 65/840 at three months (8%) and 38/795 (5%) at six months (p -trend < 0.001). Of the 1487 baseline or three month study visits in which a woman was not diagnosed with vulvovaginal candidiasis, 72 (5%) were positive for vulvovaginal candidiasis at the next study visit. Repeat diagnoses of vulvovaginal candidiasis were common: Of the 137 baseline or three-month study visits in which a woman was diagnosed with vulvovaginal candidiasis, 30 (28%) were again diagnosed with vulvovaginal candidiasis at the next study visit.

Cross-sectional analysis of vulvovaginal candidiasis: We did not find strong evidence for associations between sociodemographic characteristics and the diagnosis of vulvovaginal candidiasis. The prevalence of vulvovaginal candidiasis among women who first had sex before 15 years of age (Prevalence 4.4%, 95% CI 2.6, 6.3) appeared lower than for women who first had sex between 15-18 years (Prevalence 7.5%, 95% CI 6.1, 9.0) or over 19 years of age (Prevalence 8.2%, 95% CI 5.5, 11.0). There was a large difference in prevalence of vulvovaginal candidiasis among those with bacterial vaginosis diagnosed by clinical (Amsel) criteria

(Prevalence 12.0%, 95% CI 8.2, 15.8), compared to the 6.5% prevalence of vulvovaginal candidiasis among women who were not clinically diagnosed with bacterial vaginosis (95% CI 5.3, 7.6). We did not find evidence of differences in the prevalence of vulvovaginal candidiasis by other laboratory diagnoses or behavioral characteristics. (Table 6)

Discussion

Among this cohort of reproductive-age women in India, we found evidence that a presumptive diagnosis of vulvovaginal candidiasis based only on presence of signs or symptoms, in absence of laboratory confirmation, would be mostly incorrect. Consistent with previous research, we could not identify behavioral risk factors for vulvovaginal candidiasis,(22) which provides impetus for additional investigation into intrinsic factors such as the composition of vaginal flora, the presence or absence of genetic factors, and the features of the host and local immune response.

Vaginal discharge, itching and erythema, while quite common, were insufficient to diagnose vulvovaginal candidiasis in the absence of laboratory confirmation. Had syndromic diagnosis been used to diagnose vulvovaginal candidiasis in this cohort, the positive predictive values would have been very low (15-41%). Our results are consistent with other studies detailing the overtreatment that results from the use of syndromic diagnosis based on vaginal discharge to diagnose vaginal conditions.(23–26) Previous findings also demonstrate that a minority of women with vaginal discharge have vulvovaginal candidiasis.(23,27–29) Thus, the diagnosis of vulvovaginal candidiasis based solely on signs or symptoms leads to over-estimation of the prevalence of vulvovaginal candidiasis its over-treatment, while leaving the actual cause of the vaginal symptoms untreated. This finding of misdiagnosis based on symptoms is also relevant for women who self-diagnose vulvovaginal candidiasis.

The prevalence of laboratory-confirmed vulvovaginal candidiasis we observed is consistent with the results of two other community-based studies in India.(11,30) Given that reproductive tract conditions account for nearly half of the days lost due to illness among women in this region of India,(31) it is critical to understand the incidence and prevalence of individual conditions; to our knowledge, this is the first study from India to describe the incidence of and possible risk factors for vulvovaginal candidiasis.

The study visit-specific point prevalence of vulvovaginal candidiasis in this cohort ranged between 5 and 9%. Only 20% of those infected with *Candida* were diagnosed as having vulvovaginal candidiasis, much lower than the 53% found in a community-based study in Tamil Nadu, India.(30) We were not able to determine whether the 28% of women with a diagnosis of vulvovaginal candidiasis on two consecutive visits were cases in which, despite treatment, vulvovaginal candidiasis had cleared and then recurred. More likely, the repeat diagnoses at consecutive visits represent instances in which the vulvovaginal candidiasis was caused by *Candida* species not susceptible to fluconazole treatment. Previous research in India has found a high proportion of women are infected by non-*albicans* *Candida* species,(27,32) which are more resistant to treatment with azoles.(33,34)

Of the sociodemographic and behavioral characteristics we examined, only age at initiation of sexual activity appeared to be associated with the prevalence of vulvovaginal candidiasis, such that those with later initiation of sexual activity had a higher prevalence of vulvovaginal candidiasis. As the number of years women had been with their sex partners was not associated with vulvovaginal candidiasis, these two sociodemographic results appear discrepant and warrant additional investigation.

We found a positive association between having clinically diagnosed bacterial vaginosis and vulvovaginal candidiasis. As both diagnoses include vaginal discharge as a component of their respective diagnostic criteria, it is very likely there is misclassification between vulvovaginal candidiasis and clinically-defined bacterial vaginosis. For example, women infected with *Candida* may have discharge caused by bacterial vaginosis and could thus be misdiagnosed with vulvovaginal candidiasis.(35) Our findings emphasize the problems inherent in making diagnoses of vaginal conditions based on clinical examination alone.(33,36,37)

We found some evidence that the prevalence of vulvovaginal candidiasis varied with the presence of Lactobacillus morphotypes. The evidence for a relationship between the prevalence of vulvovaginal candidiasis and the presence of Lactobacillus in the vagina is conflicting, including studies in which the H₂O₂-production status of Lactobacillus was considered.(22,38–43) Recently, a prospective cohort study of female sex workers in Kenya found the presence of Lactobacillus, regardless of H₂O₂-production status, was positively associated with prevalent vulvovaginal candidiasis (adjusted odds ratio [aOR] 2.3, 95% CI 0.8, 6.4), a relationship that was strengthened after restricting the analysis to women without a diagnosis of bacterial vaginosis (aOR 3.8, 95% CI 1.3, 10.8).(38)

The loss of vaginal Lactobacilli is the hypothesized mediator for the relationship between the receipt of antibiotics and the risk of vulvovaginal candidiasis.(1,6,44,45) The mediation hypothesis also underpins the long-standing interest in use of probiotic interventions to reduce the risk of developing vulvovaginal candidiasis;(1,46,47) the results here do not provide strong support for this hypothesis.

Strengths of this study include a large effective sample size derived from the use of participants' repeated observations, which allows for measurements of prevalence and incidence. Additionally, other studies of vulvovaginal candidiasis in India used samples of symptomatic women recruited from clinics or used syndromic diagnosis, and as a result were not able to estimate the community-level prevalence of vulvovaginal candidiasis. Our study is one of the few to examine the prevalence of vulvovaginal candidiasis across a range of number of Lactobacillus morphotypes detected in the vagina; a dose-response effect, if present, provides better evidence of a causal relationship.

There are also important limitations of our study to consider. First, the women in this cohort were recruited by non-random sampling; unmeasured sampling bias can limit the generalizability of these results. Second, because of the cross-sectional nature of our analysis, we cannot make causal interpretations for the variations in the prevalence of vulvovaginal candidiasis observed here. Third, we did not speciate the *Candida* organisms detected. The associations between sociodemographic characteristics and potential risk factors, and the prevalence of vulvovaginal

candidiasis may differ by the *Candida* species which infect women, which are known to vary considerably by geographical location.(1) Fourth, we could not verify whether participants were self-medicating between visits with antibiotics or antifungals, which would influence the incidence and prevalence measurements of vulvovaginal candidiasis. Finally, given the limited duration of the study, we could not identify a subset of women with recurrent vulvovaginal candidiasis - an important condition with epidemiologic features distinct from acute vulvovaginal candidiasis.(48)

We found that syndromic diagnosis will result in substantial overdiagnosis and overtreatment of vulvovaginal candidiasis-negative women. For correct diagnosis of vulvovaginal candidiasis, laboratory confirmation of vaginal infection with *Candida* is necessary, as is a means of assessing whether the discharge has been caused by bacterial vaginosis. Absent accurate means of diagnosing vulvovaginal candidiasis, women remain at risk for vulvovaginal candidiasis-associated negative birth outcomes and acquisition of sexually transmitted infections. Follow-up studies are needed of women infected with *Candida* yeast species to determine the risk factors for the yeast's overgrowth, as it appears that the examination of behavioral risk factors does not appear to be a fruitful avenue for further inquiry.

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Table 4: Diagnostic criteria and treatment given for laboratory- and clinically- diagnosed gynecological conditions, Mysore, India 2005-2006

Diagnosis	Diagnostic criteria	Laboratory device	Treatment
<i>Candida</i> infection	Positive culture	InTray Colorex Yeast, BioMed Diagnostics, White City, OR, USA	None
Vulvovaginal candidiasis	Positive culture for <i>Candida</i> infection and one clinical sign (vaginal erythema or discharge) and one reported symptom (vaginal pruritis or discharge)		150 mg oral fluconazole, single dose
Trichomoniasis	Positive culture or positive saline wet mount microscopy	InPouch, BioMed Diagnostics, White City, OR, USA	2 g oral metronidazole, single dose
Bacterial vaginosis, laboratory	Score of 7-10 on Nugent criteria from Gram stain		None
Intermediate flora, laboratory	Score of 4-6 on Nugent criteria from Gram stain		None
Abnormal flora, laboratory	Score of 4-10 on Nugent criteria from Gram stain (21)		None
Bacterial vaginosis, clinical	Positive on at least three of four Amsel criteria (elevated pH, positive whiff test, clue cells observed, discharge observed) (20)		400 mg oral metronidazole, bid for 7 days
Herpes simplex virus type 2	Index value > 1.1 on ELISA test of serum	Focus Technologies, Cypress, CA, USA	Acyclovir 400 mg tid for 7-10 days

Table 5: Prevalence of observed clinical signs, reported symptoms, and diagnosis (positive predictive value) of vulvovaginal candidiasis, Mysore, India 2005-2006

Vaginal sign observed or symptom reported	Prevalence (n=2528 clinical visits)	% prevalence (95% CI)**	Diagnosed with vulvovaginal candidiasis	% diagnosed with vulvovaginal candidiasis (95% CI)**
Pruritis reported	735	29.0 (26.8, 31.2)	132	17.8 (14.8, 20.8)
Discharge reported	782	31.0 (29.0, 32.9)	122	15.3 (12.5, 18.1)
Erythema observed	239	9.5 (8.2, 10.8)	61	24.8 (18.7, 30.8)
Discharge observed	894	35.4 (33.4, 37.5)	162	17.6 (14.9, 20.3)
None	887	35.1 (33.0, 37.2)	-	-
Any one	916	36.2 (34.3, 38.1)	-	-
Any two	492	19.5 (17.8, 21.1)	84	16.7 (13.3, 20.1)
Any three	182	7.2 (6.1, 8.3)	75	40.1 (33.7, 47.9)
All four	51	2.0 (1.4, 2.6)	21	40.9 (27.6, 54.2)

** Percentages and 95% confidence intervals calculated using generalized estimating equations, with binary family, identity link, and exchangeable correlation.

Table 6: Prevalence vulvovaginal candidiasis by sociodemographic, behavioral, partner and laboratory measures, Mysore, India 2005-2006

Prevalent vulvovaginal candidiasis (Overall prevalence= 180/2528; 7.1%)		
	Prevalence (%)	Prevalence 95% CI
Age, years		
16-20	9.2	(4.1, 14.3)
21-25	7.4	(5.4, 9.5)
26-30	6.6	(5.2, 8.1)
Years of education		
0	6.6	(4.3, 8.9)
1-7	7.7	(5.7, 9.7)
8-17	7.0	(5.2, 8.8)
Years with sex partner		
0-6	7.5	(5.3, 9.6)
7-19	6.9	(5.6, 8.2)
Socioeconomic status***		
Low	6.3	(4.8, 7.7)
High	7.9	(6.1, 9.8)
Parity		
No	6.0	(3.5, 8.5)
Yes	7.3	(6.1, 8.5)
Religion*		
Non-Muslim	6.4	(5.1, 7.7)
Muslim	8.9	(6.5, 11.3)
Age of initiation of sexual activity, years**		
<14	4.4	(2.6, 6.3)
15-18	7.5	(6.1, 9.0)
19+	8.2	(5.5, 11.0)
Number of vaginal sex acts in prior three months		
0-12	8.2	(6.0, 10.5)
13-120	6.6	(5.4, 7.8)

Table 6: Prevalence vulvovaginal candidiasis by sociodemographic, behavioral, partner and laboratory measures, Mysore, India 2005-2006

Prevalent vulvovaginal candidiasis (Overall prevalence= 180/2528; 7.1%)		
	Prevalence (%)	Prevalence 95% CI
Ever had anal sex		
No	7.1	(5.9, 8.2)
Yes	7.6	(3.6, 11.5)
Husband has other sex partners		
No	7.1	(6.0, 8.2)
Yes	7.8	(3.3, 12.4)
Tubal ligation		
No	7.0	(5.3, 8.8)
Yes	7.2	(5.7, 8.6)
Herpes simplex virus - type 2 infection		
Negative	7.1	(5.9, 8.3)
Positive	6.9	(4.0, 9.8)
<i>Trichomonas vaginalis</i> infection		
Negative	7.1	(5.8, 8.3)
Positive	8.0	(3.4, 12.6)
Vaginal pH		
<4.5	6.0	(4.3, 7.7)
≥4.5	7.7	(6.4, 9.0)
Clinical (Amsel) criteria diagnosis for bacterial vaginosis**		
Negative	6.5	(5.3, 7.6)
Positive	12.0	(8.2, 15.8)
Laboratory (Nugent) criteria diagnosis for bacterial vaginosis		
Negative	6.9	(5.6, 8.3)
Intermediate	9.8	(6.8, 12.8)
Positive	5.7	(3.1, 8.2)

Table 6: Prevalence vulvovaginal candidiasis by sociodemographic, behavioral, partner and laboratory measures, Mysore, India 2005-2006

Prevalent vulvovaginal candidiasis (Overall prevalence= 180/2528; 7.1%)		
	Prevalence (%)	Prevalence 95% CI
Lactobacillus morphotypes detected*		
≥30	7.5	(6.0, 9.0)
5-29	9.6	(5.9, 13.3)
1-4	3.7	(0.3, 7.0)
<1	4.5	(0.8, 8.2)
0	5.5	(2.6, 8.4)

Prevalences, 95% confidence intervals and p-values calculated using generalized estimating equations with binary family, identity link and exchangeable correlation.

* $0.05 \leq p < 0.10$

** $p < 0.05$

*** An socioeconomic index was calculated using the first factor from a principle components analysis of household consumer goods, toilet type, financial instruments, and stove type, and then recoded into a binary score of Low and High socioeconomic status.(49)

Syndromic diagnosis of vaginal infections: Comparison of models from logic regression with a WHO diagnostic algorithm

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Abstract

Background: Syndromic diagnostic algorithms, such as those developed by the World Health Organization (WHO), are commonly used to diagnose reproductive tract infection in women presenting with symptoms in low-income settings. Logic regression is a statistical method that can be used to construct a syndromic diagnosis algorithm to predict vaginal infections, using combinations of measures collected in a clinical interview, pelvic examination, and simple laboratory testing.

Methods: We used logic regression to develop predictive models for syndromic diagnoses of vaginal infection among symptomatic, reproductive-age women in south India. We assessed the sensitivities, specificities, positive predictive values and negative predictive values of the logic models and a WHO algorithm against laboratory-confirmed diagnoses of two conditions: bacterial vaginosis or trichomoniasis (BV/TV), and vulvovaginal candidiasis (VVC).

Results: The WHO algorithm had moderately high sensitivities for diagnosing vaginal infections (73% for BV/TV and 68% for VVC) while the logic models were comparable for BV/TV (68% sensitivity) and superior for VVC (99% sensitivity). The positive predictive value for BV/TV was higher for the logic model than for the WHO algorithm (58% to 32%), while the opposite was true for the positive predictive value for VVC (42% to 52%). A majority (72%) of women with bacterial vaginosis or trichomoniasis do not report symptoms that would motivate them to seek care.

Conclusion: The logic regression package can be implemented using free software, and offers more flexibility to create a syndromic diagnosis algorithm than is possible with the other established algorithms. The performance of the logic models was frequently comparable or superior to that of the WHO algorithm.

Background

Syndromic diagnostic algorithms are commonly used to diagnose reproductive tract infection in women presenting with symptoms in low-income settings. The clinics using syndromic diagnosis algorithms are characterized by their lack of state-of-the-art diagnostic capacity, and the women being seen at the clinics are ideally diagnosed and treated in a single visit. The World Health Organization (WHO) has developed and disseminated diagnostic algorithms which are adaptable to local conditions, allowing for the possibility of incorporating information collected from a bimanual pelvic examination, light microscopy of vaginal specimens, a clinical interview regarding behavioral risk factors, and the expected prevalences of reproductive tract infections.(1)

Validation studies in low-income settings have shown the specificity of established syndromic diagnosis algorithms for bacterial vaginosis, trichomoniasis and vulvovaginal candidiasis – collectively known as vaginal infections - to be around or below 50%.(2–7) The correspondingly low positive predictive values of syndromic diagnoses indicate that the use of these algorithms leads to substantial overdiagnosis and overtreatment of women, and an increase in the average cost per true case treated.(5) Attempts have been made to improve on the established syndromic diagnosis algorithms, with modest improvement, at best, in diagnostic accuracy.(2,5)

Logic regression is a nonparametric statistical method which has been used to identify combinations of binary measures which may be associated with an outcome measure.(8) The combination of measures can be expressed as a Boolean logic statement (e.g. (A AND B) OR NOT C)). In contrast to other machine-learning processes for prediction, logic regression produces highly interpretable output, appropriate for implementation in a clinical setting. Logic regression was initially developed and applied to explore high-order interactions of single nucleotide polymorphisms with disease outcomes. More recently, logic regression has been employed to use biospecimen data to predict a range of clinical outcomes(9) and to use sociodemographic and behavioral characteristics to pre-screen individuals at high-risk for colorectal cancer.(10)

Here, logic regression will be used to develop predictive models for syndromic diagnoses of laboratory-confirmed bacterial vaginosis or trichomoniasis, and of vulvovaginal candidiasis, among reproductive-age women in south India.

Methods

The Prerana dataset was collected as part of a six-month prospective cohort study of 898 women living in and around Mysore, India between 2005 and 2006. The methods used to recruit the cohort have been described elsewhere.(11) Briefly, women were recruited from obstetrics and gynecology outpatient clinics and women's self-help groups in the peri-urban and rural areas around Mysore. Eligibility criteria were: female sex, age between 15 and 30 years, not pregnant at time of enrollment, sexually active, planning on residing in the area for at least six months, and willing to undergo study procedures. Women provided written informed consent for the study at the time of enrollment. The study protocol was approved by the Committee for Protection of Human Subjects at the University of California, Berkeley and the Asha Kiran Hospital Institutional Review Board.

Interview and clinical examination: Study visits were completed at baseline, and follow-up visits three and six months later. As data collection procedures were changed at the three-month visit,

only data from the three- and six-month visits will be analyzed here. The study visits involved an interviewer-administered questionnaire in Urdu or Kannada; a pelvic examination conducted by a female study physician; and collection of vaginal specimens for laboratory testing. The interviewer-administered questionnaire collected information concerning sociodemographic characteristics; sexual and reproductive health history; history and current complaints of abnormal vaginal symptoms; and sexual partner characteristics. During the pelvic examination, the study physician noted and recorded on study forms the absence or presence of abnormal vaginal findings and collected swab samples from the posterior fornix of the vagina.

Laboratory tests and diagnoses: Laboratory testing was conducted at the laboratories of the Holdsworth Memorial Hospital and Vikram Hospital in Mysore. Vaginal swab samples were used for pH testing, Gram staining, and saline wet mount microscopy to detect motile trichomonads. The presence of amines was evaluated by dropping KOH on a smeared vaginal swab sample (whiff test). The vaginal swab samples were also used to culture *Trichomonas vaginalis* (InPouch, BioMed Diagnostics, White City, OR, USA) and *Candida* (InTray Colorex Yeast, BioMed Diagnostics).

A laboratory diagnosis of bacterial vaginosis was made using the Gram stain scoring criteria identified by Nugent et al: a total score of 7-10 was considered positive for bacterial vaginosis.(12) A diagnosis of trichomoniasis was made if motile trichomonads were detected on microscopy from a vaginal swab specimen or on culture within five days. Women were considered to be colonized by *Candida* if positive on culture. A vulvovaginal candidiasis diagnosis was made if, in addition to *Candida* colonization, women reported at least one of two vaginal symptoms (itching or discharge) in the interview and the study clinician observed at least one of two vaginal signs (erythema or discharge) during the pelvic examination.

Women were treated per CDC guidelines if clinically diagnosed with bacterial vaginosis or if they had a laboratory-confirmed diagnosis of *Trichomonas vaginalis* infection or vulvovaginal candidiasis.(13) Treatment was offered to the women for their sex partners upon diagnosis of trichomoniasis.

Statistical methods: Two conditions are considered as outcomes for evaluation of syndromic diagnoses: 1) bacterial vaginosis or trichomoniasis (BV/TV), and 2) vulvovaginal candidiasis (VVC). Both outcomes were measured at the three- and six-month study visits. As the presence of bacterial vaginosis and infection with *Trichomonas vaginalis* have similar clinical presentation, are commonly present together, and can be treated with the same antibiotic regimen, they are considered as a single condition here. Similarly, the WHO algorithm we are evaluating here does not require the presence of bacterial vaginosis and infection by *Trichomonas vaginalis* to be distinguished from each other, while additional information is required to diagnose vulvovaginal candidiasis.(1)

First, we will describe the prevalence of BV/TV and of VVC among study visits in which a participating woman reported at one or more symptom associated with vaginal infection (i.e. vaginal itching, discharge or burning), and then among study visits in which a participating woman did not report any of the symptoms. These symptoms predominate among women who opt to visit a health clinic for treatment, and are thus used as the entry point on the WHO algorithm.

Second, the WHO algorithm for syndromic diagnoses of vaginal infections will be evaluated against laboratory-confirmed diagnoses of bacterial vaginosis or trichomoniasis, and of vulvovaginal candidiasis. The WHO algorithm used here allows for use of measures collected from a clinical interview and a non-invasive pelvic examination; it is modified to make only diagnoses of vaginal infections, rather than both vaginal and cervical infections (i.e. *Neisseria gonorrhoeae* and *Chlamydia trachomatis*)(Figure 3). Other WHO algorithms involve use of a speculum for examination of the cervix, bimanual examination, and of microscopic examination of vaginal specimens, which were not thought to be appropriate or feasible in this setting. The WHO algorithm we selected for evaluation will be applied to the subset of observations in which women reported one or more symptom associated with vaginal infection (i.e. vaginal itching, discharge or burning). The syndromic diagnoses will be compared to the laboratory-confirmed diagnoses of BV/TV and of VVC to calculate the respective sensitivities, specificities, positive predictive values, and negative predictive values of syndromic diagnosis.

Third, the same subset of observations in which women reported one or more symptom associated with vaginal infections will be used to create logic models for syndromic diagnosis of BV/TV and of VVC. Candidate variables for logic models are all binary measurements, and include women's reports of vaginal symptoms (i.e. report of current itching, burning, discharge, and discharge present in the past three months); vaginal features observable as part of the pelvic examination (i.e. presence or absence of vaginal erythema, abnormal discharge, and curdy discharge); the findings from laboratory testing of vaginal specimens (i.e. $\text{pH} \geq 4.5$, positive whiff test); and a sexual partner characteristic (i.e. whether he may have additional sex partners)(Table 7). The selection of candidate variables was guided by their inclusion on the WHO algorithm, along with other locally-appropriate measures that are relatively simple to collect when taking a clinical history or performing a pelvic examination. The laboratory tests selected for inclusion involve non-invasive vaginal specimen collection by the physician or by the woman herself,(14) and are simple, low-cost, and readily available in low-income settings.

As part of the logic regression procedures, a logic model is nested into a logistic regression equation, whereby the logic model is a binary predictor (evaluated as True=1 or False=0) and the log odds of the vaginal condition is the outcome (Figure 4). A logic model's fit is assessed by the deviance calculated by logistic regression's maximum likelihood procedure; lower deviance is evidence of a better logic model fit. As the initial 'best' model identified by logic modeling procedures on the full dataset is likely overfitted, cross-validation and permutation testing will be used to identify a logic model with comparable performance to the best model, but with fewer predictors required. A more parsimonious model also enhances its acceptability for clinicians' use to diagnose women. Further details concerning logic regression's fitting procedures and performance in comparison to other machine-learning processes are available.(9,15)

The logistic regression equation associated with the chosen logic model can be solved to calculate the positive and negative predictive values for diagnosis (Figure 4). These predictive values, along with the probability of the logic model being true in the sample, provide the inputs for calculation of the sensitivity and specificity of syndromic diagnosis.

The analysis was completed using R 2.13.2 (R Foundation for Statistical Computing, Vienna, Austria), the LogicReg package 1.4.14(16) and Stata 11.2 (StataCorp, College Station, USA).

Results

The demographic characteristics of the 898 women in the cohort have been described in detail, as has the baseline prevalence of signs and symptoms associated with reproductive tract infections.(17,18) Over the study visits at three and six months, from which the analysis sample is drawn, 853 women contributed a total of 1653 study visits. Of the 1653 study visits, 335 study visits (20%) involved report of at least one symptom associated with vaginal infection (of which 61% of visits involved a report of discharge, 38% of itching and 29% of burning). The 335 study visits were collected from 272 women (209 women provided one observation, 63 women provided two observations). For the 335 study visits for which a laboratory result was available, 76/322 (24%, with 13 missing results) had a confirmed diagnosis of BV/TV, and 72/331 (22%, with four missing results) had a confirmed diagnosis of VVC, including 14 cases in which both conditions were diagnosed in the same woman at the same visit. Of the 1318 study visits at which no symptom associated with vaginal infection was reported and a laboratory result was available, 207/1230 (17%, with 88 missing results) had a confirmed diagnosis of BV/TV, and 31/1304 (2%, with 14 missing results) had a confirmed diagnosis of VVC, including three cases in which both conditions were diagnosed in the same woman at the same visit. (Figure 2) Thus, 76/283 (27%) cases of BV/TV, and 72/103 (70%) cases of VVC presented with at least one symptom associated with vaginal infection.

Using the WHO algorithm (Figure 2) to make a syndromic diagnosis, the sensitivity, specificity, positive predictive value and negative predictive value for BV/TV were 73% (56/76), 52% (128/246), 32% (56/174), and 86% (128/148), respectively, and for VVC were 68% (49/72), 82% (213/259), 52% (49/95) and 90% (213/236), respectively. (Table 8)

Using logic regression to create a model for the syndromic diagnosis of BV/TV, a model with three leaves was selected due to its having the lowest deviance in cross-validation and the diminishing marginal improvement of larger models in the permutation test. For BV/TV, the logistic regression equation is described in

and

$$\frac{NPV * p_{Logic\ model=False}}{NPV * (1 - p_{Logic\ model=True}) + (1 - PPV)(p_{Logic\ model=True})} = Specificity$$

Figure 5, and the logic model is shown in Figure 7. For the syndromic diagnosis of VVC, a model with four leaves was selected due its cross-validation deviance being approximately equivalent to the neighboring models and the diminishing marginal improvement of larger models on the permutation test. For VVC, the logistic regression equation is described in Figure 6, and the logic model is shown in Figure 8.

Using the logic models, the sensitivity, specificity, positive predictive value and negative predictive value for syndromic diagnosis of BV/TV were 68% (52/76), 85% (208/246), 58% (52/90), and 90% (208/232), respectively, and for VVC were 99% (71/72), 62% (160/259), 42% (71/170) and 99% (160/161), respectively. (Table 8)

Discussion

This is the first evaluation of a syndromic diagnosis algorithm of vaginal infection in this region of south India. We assessed the performance of the WHO algorithm for diagnoses of bacterial vaginosis or trichomoniasis, and of vulvovaginal candidiasis, among a cohort of symptomatic reproductive-age women. Building from the measures used for the WHO algorithm, we included additional locally-appropriate measures to develop diagnostic models with logic regression. The logic model for diagnosis of bacterial vaginosis or trichomoniasis met or exceeded the performance characteristics of the WHO algorithm. However, our results also indicate that substantial proportions of women with bacterial vaginosis or trichomoniasis do not report symptoms that would motivate them to seek care.

In south India, previous research has established high levels of reproductive tract symptoms – particularly of vaginal discharge - in spite of the low prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection.(19–27) Vaginal infections caused by bacterial vaginosis, *Trichomonas vaginalis* and *Candida* species are thought to increase risk of infection by HIV and adverse birth outcomes, and to be responsible for substantial psychological distress and economic costs.(28–30) To reduce the prevalence of vaginal infections, women must a) be aware of the presence of vaginal abnormalities, b) be encouraged to seek care upon awareness of any abnormality, and c) have access to clinical providers with the tools necessary to provide a rapid and accurate diagnosis. We found that only 27% of cases of bacterial vaginosis or trichomoniasis were associated with reports of symptoms. Thus, for purposes of population-level control of these vaginal conditions, the utility of any syndromic diagnosis algorithm is limited. Yet, absent the availability of inexpensive point-of-care diagnostic tests, syndromic algorithms remain the primary means of providing diagnosis and treatment to women with vaginal infections.

The WHO algorithm for diagnosis of bacterial vaginosis or trichomoniasis among women reporting one or more symptom associated with vaginal infection (i.e. report of vaginal discharge, itching or burning) required an additional two measures (i.e. observation of vaginal discharge or erythema) to be collected. While the sensitivity of syndromic diagnosis of bacterial vaginosis or trichomoniasis was moderately high (73%), the low specificity (52%) resulted in a correspondingly low positive predictive value (32%), as false positive diagnoses would have outnumbered true positives. The WHO algorithm for syndromic diagnosis of vulvovaginal candidiasis required one additional measure to be collected (observation of curd-like discharge), beyond those measures required for syndromic diagnosis of bacterial vaginosis or trichomoniasis. For syndromic diagnosis of vulvovaginal candidiasis, the specificity (82%) was largely responsible for a modest positive predictive value (52%) as false positive and true positive diagnoses were made in nearly equal numbers.

The logic regression procedure considered ten measures: all six measures used in the WHO algorithm, one additional symptom (i.e. report of vaginal discharge in the past three months), one partner characteristic (i.e. whether he may have additional sex partners), and two simple laboratory tests (i.e. whiff and pH). For diagnosis of bacterial vaginosis or trichomoniasis among women reporting one or more symptom associated with vaginal infection, we selected a logic model requiring collection of three measures (i.e. whiff test, pH test, and observation of erythema). The logic model for diagnosis of bacterial vaginosis or trichomoniasis had similar sensitivity (68%) as the WHO algorithm, but higher specificity (85%), resulting in a higher

positive predictive value (58%) than the WHO algorithm. For diagnosis of vulvovaginal candidiasis, we selected a model requiring four measures, only one of which (i.e. observation of discharge) was not in the initial set of presenting symptoms, or included in the logic model for diagnosis of bacterial vaginosis or trichomoniasis. The logic model for diagnosis of vulvovaginal candidiasis had higher sensitivity (99%), but its lower specificity (62%), resulted in a lower positive predictive value (42%) than the WHO algorithm.

Our results provide evidence that only a limited amount of easily collectible clinical information is needed to meet or exceed the diagnostic performance of the WHO algorithm. The utility of pH testing and the whiff test for diagnosis of bacterial vaginosis and trichomoniasis has long been evident, and these two comprise two of the four criteria for the clinical diagnosis of bacterial vaginosis identified by Amsel et al.(31) Vaginal erythema is also known as a potential sign of trichomoniasis, though not of bacterial vaginosis.(32)

The presence of vaginal erythema was also part of the logic model for vulvovaginal candidiasis, along with a clinical observation of discharge and reported vaginal itching. Our definition of a confirmed case of vulvovaginal candidiasis involved these three measures, so it is unsurprising logic regression selected the three for the model shown in Figure 8. That these three measures were selected gives an encouraging indication of logic regression's ability to detect the existence, if any, of high-order relationships between the included predictors, as they related to an outcome measure.

We have fitted logic regression models and sought to minimize overall deviance, and reported the positive predictive values associated with the selected models. Additional modifications to the logic regression fitting procedures that may improve the positive predictive value are possible. The improvement can be accomplished by 1) inclusion of additional logic models in the logistic regression equation, 2) up-weighting cases relative to non-cases, and 3) inclusion of additional variables, for example by dummy-coding continuous measurements. Though we opted to use the deviance from a logistic regression equation as the measure of fit quality here, logic regression can be run with a different measure of fit quality, such as misclassification, the cost per true case detected, or the positive likelihood ratio (LR+). The process by which logic regression derives a model for diagnostic prediction can be implemented for other disease conditions and in other populations, using free software, making it an appealing option for low-income settings.

Though we used logic regression's cross-validation procedure to select our final logic models, these models require additional validation in a similar population of women reporting symptoms associated with vaginal infections. Our results may not be generalizable to populations of women with differing prevalences of other sexually transmitted infections – particularly those which cause abnormal discharge - or to women who are more or less likely to be aware of and report vaginal symptoms. Syndromic diagnosis requires accurate reporting of symptoms to clinicians; in particular, the validity of reports of vaginal discharge in south Asia has been called into question.(33,34) Therefore, if a logic regression-derived model is to be used to develop a syndromic diagnosis algorithm in a new population, it must be validated against a gold-standard diagnosis. The validation exercise can also be set up to collect enough data to develop new, locally-appropriate, syndromic diagnosis algorithms using logic regression, if necessary.

We found that, using data collected from a cohort of reproductive-age women in south India, syndromic diagnosis of bacterial vaginosis or trichomoniasis, and of vulvovaginal candidiasis, is possible using a model created by logic regression. The performance of the logic models was comparable to that of the WHO algorithm. Logic regression offers considerable flexibility to create a syndromic diagnosis algorithm using measures that are appropriate in the local setting, can be specified to maximize any performance characteristic of a diagnostic algorithm (eg sensitivity, positive predictive value, etc), and to identify a minimum set of measures necessary to collect for diagnosis. While we used logic regression to create an algorithm for syndromic diagnosis of vaginal infections, the methods described here can be extended to other health conditions for which there may be a combination of predictors collected from a clinical history, examination or laboratory testing. Finally, the models created by logistic regression are highly interpretable, either as a decision tree or as a Boolean logic statement, making them appropriate for use in both low- and high-income settings.

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Table 7: Measures selected for evaluation of the WHO algorithm for syndromic diagnosis of vaginal infections among symptomatic reproductive-age women, and for inclusion in logic regression modeling, Mysore, India, 2005-2006

Measures on the WHO algorithm

- Vaginal discharge reported
- Vulval itching reported
- Burning reported
- Abnormal discharge observed
- Curd-like discharge observed
- Vulval erythema observed

Additional symptoms and behaviors included for logic regression

- Discharge in the past three months reported
- Sex partner may have other partners reported

Additional signs and laboratory tests included for logic regression

- Vaginal pH ≥ 4.5
 - Whiff (KOH) test for presence of vaginal amines
-

Figure 2: Reports of vaginal-infection associated symptoms and laboratory-confirmed diagnoses of bacterial vaginosis or trichomoniasis (BV or TV), or vulvovaginal candidiasis (VVC) among reproductive-age women: Mysore, India 2005-2006

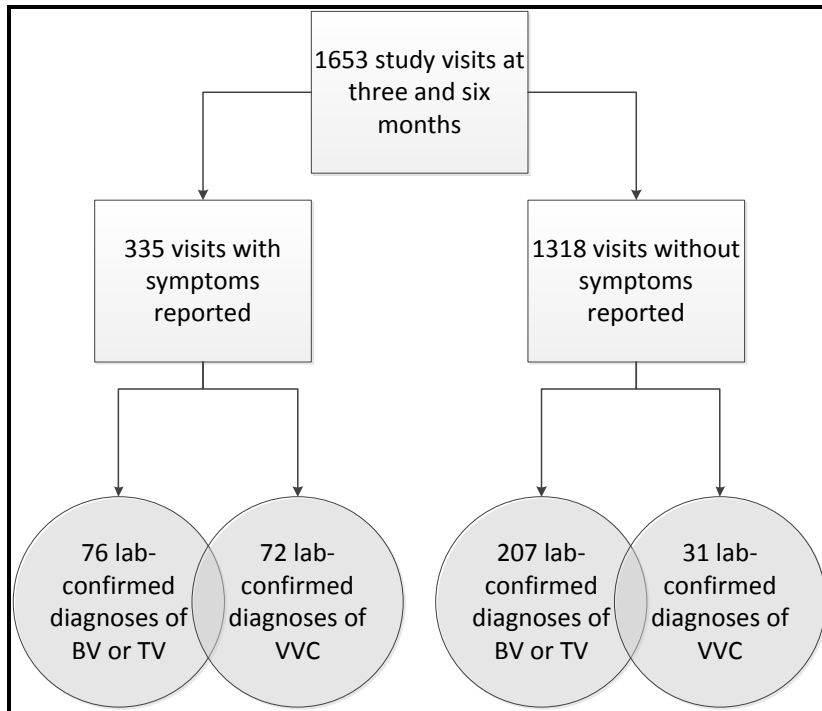


Figure 3: Modified WHO algorithm for syndromic diagnosis of bacterial vaginosis, trichomoniasis, or vulvovaginal candidiasis.

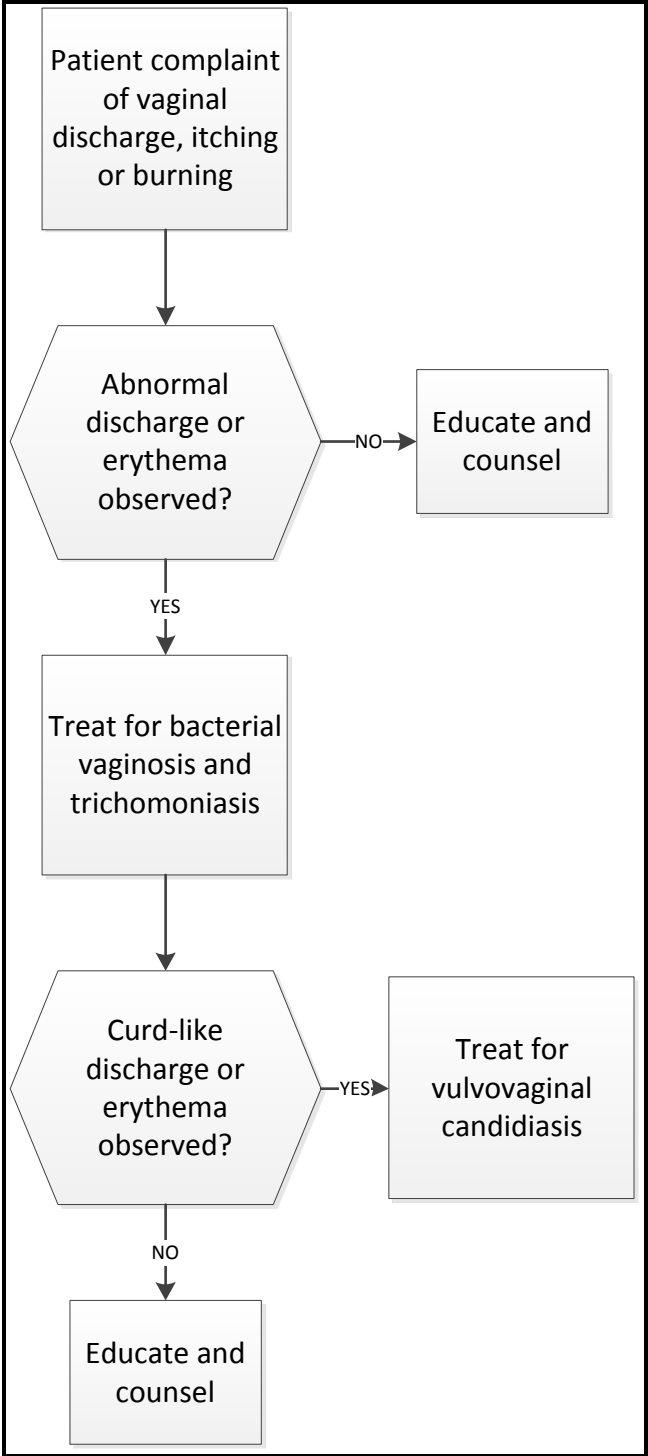


Figure 4: Logic model nested in a logistic regression equation, for diagnosis of infection (by bacterial vaginosis or *Trichomonas vaginalis*, or by vulvovaginal candidiasis)

$$\log\left(\frac{p_{infection=positive}}{1 - p_{infection=positive}}\right) = a + b * (Logic\ model = True)$$

Where

$$\frac{\exp^{(a+b*1)}}{1 + \exp^{(a+b*1)}} = \text{Positive predictive value (PPV) for diagnosis of infection}$$

and

$$1 - \frac{\exp^{(a+b*0)}}{1 + \exp^{(a+b*0)}} = \text{Negative predictive value (NPV) for diagnosis of infection}$$

therefore

$$\frac{PPV * p_{Logic\ model=True}}{PPV * p_{Logic\ model=True} + (1 - NPV)(1 - p_{Logic\ model=True})} = \text{Sensitivity}$$

and

$$\frac{NPV * p_{Logic\ model=False}}{NPV * (1 - p_{Logic\ model=True}) + (1 - PPV)(p_{Logic\ model=True})} = \text{Specificity}$$

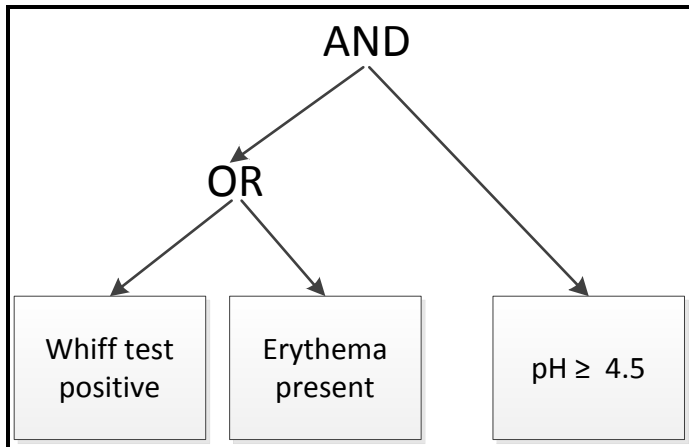
Figure 5: Logistic regression equation for syndromic diagnosis of bacterial vaginosis or trichomoniasis (BVTV)

$$\log\left(\frac{p_{BVTV=positive}}{1 - p_{BVTV=positive}}\right) = -2.16 + 2.47 * ((whiff\ positive\ OR\ erythema\ present) AND\ pH \geq 4.5) = True$$

Figure 6: Logistic regression equation for syndromic diagnosis of vulvovaginal candidiasis (VVC)

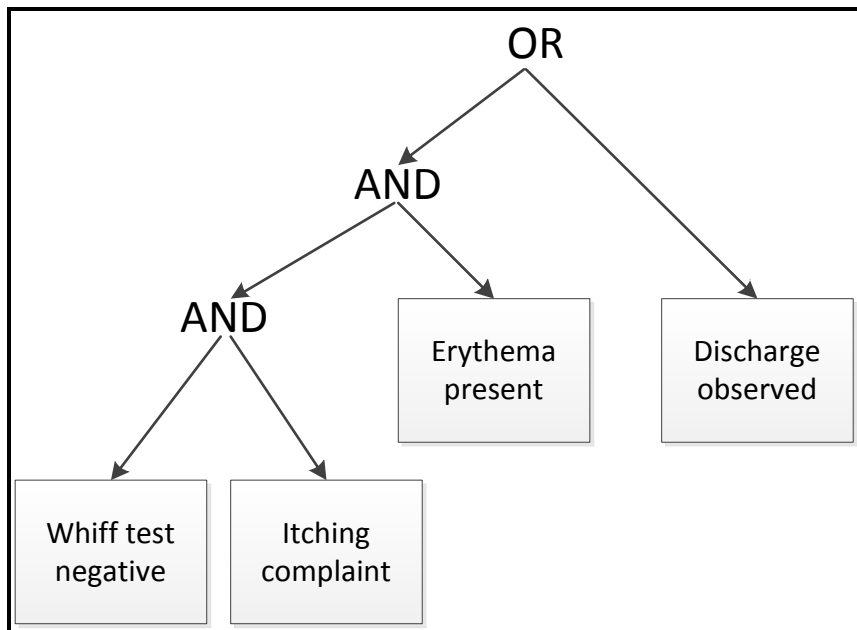
$$\log\left(\frac{p_{VVC=positive}}{1 - p_{VVC=positive}}\right) = -5.08 + 4.74 * \left(((whiff\ test\ negative\ AND\ itching\ complaint\ AND\ erythema\ present) OR\ discharge\ observed) = True \right)$$

Figure 7: Logic model for syndromic diagnosis of bacterial vaginosis or trichomoniasis



Among women reporting symptom of vaginal discharge, itching or burning, a syndromic diagnosis of bacterial vaginosis or trichomoniasis is made if the logic model is evaluated as TRUE using Boolean operation rules, and is otherwise FALSE.

Figure 8: Logic model for syndromic diagnosis of vulvovaginal candidiasis



Among women reporting symptom of vaginal discharge, itching or burning, a syndromic diagnosis of vulvovaginal candidiasis is made if the logic model is evaluated as TRUE using Boolean operation rules, and is otherwise FALSE.

Table 8: Syndromic diagnosis of vaginal infections among symptomatic reproductive-age women using a WHO algorithm and logic models: Mysore, India 2005-2006

Vaginal infection(s) and diagnostic algorithm	Se	Sp	PPV	NPV
Bacterial vaginosis or trichomoniasis (Prevalence = 24%, 76/322)				
WHO algorithm	73%	52%	32%	86%
Logic model	68%	85%	58%	90%
Vulvovaginal candidiasis (Prevalence = 22%, 72/331)				
WHO algorithm	68%	82%	52%	90%
Logic model	99%	62%	42%	99%

Se = Sensitivity, Sp=Specificity,

PPV=Positive predictive value, NPV=Negative predictive value

Conclusion

Summary of hypotheses

Paper 1: Bacterial vaginosis and risk for *Trichomonas vaginalis* infection

Hypothesis: Women with abnormal vaginal flora detected over a three-month period will have an increased risk of a diagnosis of *Trichomonas vaginalis* infection at the end of three months, relative to women with normal vaginal flora over the three-month period.

We found evidence for a 4- to 9-fold increased risk of TV infection among women who had abnormal vaginal flora within a three month span, with the highest risk among those women found to have abnormal flora at consecutive visits. Our alternate GEE models indicate the relationships are generally maintained when other widely accepted abnormal flora measurement criteria are used, and for incident TV infection.

Paper 2: Epidemiologic features of vulvovaginal candidiasis

Hypothesis: The prevalence of vulvovaginal candidiasis will increase with increasing numbers of vaginal *Lactobacillus* morphotypes detected.

We found some evidence that the prevalence of vulvovaginal candidiasis varied with the number of vaginal *Lactobacillus* morphotypes detected.

Paper 3: Machine learning for syndromic diagnosis of vaginal infections

Hypothesis: The sensitivity, specificity, positive predictive value, and negative predictive value of the logic regression-derived diagnostic algorithms will be higher than those of the WHO algorithm for the laboratory-confirmed diagnoses of bacterial vaginosis, trichomoniasis or vulvovaginal candidiasis.

Among women with symptoms of vaginal infections, the WHO algorithm had moderately high sensitivities for diagnosing vaginal infections (73% for BV/TV and 68% for VVC) while the logic models were comparable for BV/TV (68% sensitivity) and superior for VVC (99% sensitivity). The positive predictive value for BV/TV was higher for the logic model than the WHO algorithm (58% to 32%), while the opposite was true for the positive predictive value for VVC (42% to 52%).

Further steps

The prevalence of vaginal infections in this setting is in the range found by other studies conducted in south India. On a population-level, the total morbidity caused by vaginal infections is likely to be substantial, either directly through its economic and psychological costs, or indirectly via increased risk of acquisition of sexually transmitted infections or of adverse birth outcomes. In contrast to many other reproductive tract conditions, vaginal infections are relatively simple to diagnose and treat. A primary and necessary step to addressing the burden of disease caused by vaginal infections is developing a greater understanding of the process by which women recognize the presence of gynecological abnormalities and opt to present to a health clinic for diagnosis and treatment.

The evidence that bacterial vaginosis, and in particular of the absence of vaginal *Lactobacillus*, plays a role in enhancing a woman's risk of acquiring a reproductive tract infection continues to grow. Here, we found women with bacterial vaginosis had higher risk of infection by *Trichomonas vaginalis*, and women with fewer detectable *Lactobacillus* morphotypes had a higher prevalence of vulvovaginal candidiasis. Unfortunately, we found that syndromic diagnosis is insufficient for population-level control of bacterial vaginosis. And while the Amsel criteria for diagnosis of bacterial vaginosis are appropriate for use in low-income settings or where women must be screened and treated in a single visit, absent further microscopic examination of vaginal specimens, a positive result can be conflated with vulvovaginal candidiasis. The Nugent criteria, unfortunately, require time and laboratory equipment for Gram staining and light microscopy, and most likely require a woman to return to the clinic for her diagnosis.

As the Amsel and Nugent criteria for diagnosis of bacterial vaginosis each have significant limitations for the population-level control of bacterial vaginosis, so do the existing syndromic diagnosis algorithms. Aside from the challenges of increasing awareness and access for infected women, the performance of the algorithms must be improved. At a minimum, additional measures collectable during the clinical history-taking and examination that are predictive of infection must be identified. As it is unknown whether a more complete set of measures will improve the sensitivity and specificity of diagnoses of vaginal infections, the impetus to develop inexpensive and accurate point-of-care diagnostics remains.

Appendix: R code for Logic Regression

```
# Install necessary packages
library(foreign)
library(LogicReg)
rm(list=ls(all=TRUE))

# Make the graphs wait, with proper margins
par(mar=c(3,3,3,3))
par(ask=TRUE)

# The full dataset from Stata
prerana <- read.dta(file="C:/users/Sujit/dropbox/mysore/prerana.dta", convert.factors=FALSE)

# Keep only the necessary vars
dat0 <- prerana[,c("id", "visit", "vds", "bvtv", "candidasx", "vag_dis_his", "itching_vag", "burn_mictu", "hus_opart", "vagdis3mo", "ph_2cat", "whiff_new", "discharge_sign", "vagi_erythema", "curdy")]
#1-3 Other vars
#4-5 Outcomes
#6-10 Sx/behavior
#11-15 Signs/feat

# Keep only symptomatic women
dat1 <- subset(dat0, vds==1,.)

#### LR specifications
y <- 5 # Which outcome: 4=BVTV 5=VVC
trees <- 1 # How many trees
wt <- 1 # Up-weight cases
rep <- 500 # How many permutations
iter <- 50000 # Simulated annealing parameter

# Set the annealing parameters
myanneal <- logreg.anneal.control(start=-1, end=-4, iter=iter, update=0)
# Exclude missing outcome, incorporate weight, drop visit 1
dat1$weight <- (dat1[,y]==0) + wt*(dat1[,y]==1)
data.frame(dat2)
dat2 <- subset(dat1, dat1[,y]==0 | dat1[,y]==1,.)
dat2 <- subset(dat2, visit!=1)
table(dat2$visit)
table(dat2[,y], useNA="always")
# Find best scoring model (select=1) with tree and weight set
set.seed(1980)
fit1 <- logreg(resp=dat2[,y], bin=dat2[,6:15], type=3, select=1, wgt=dat2$weight,
ntrees=trees, anneal.control=myanneal)
print(fit1)
# Null model permutation test (select=4)
fit4 <- logreg(select=4, anneal.control=myanneal, oldfit=fit1, nrep=rep)
```

```

    plot(fit4, pscript=F)
# Test (select=2) to assess model size
  fit2 <- logreg(oldfit=fit1,select=2,ntrees=c(1,trees),nleaves=c(1,10), anneal.control=
myanneal)
  #plot(fit2,pscript=F)
  fit2
# 10-fold cross-validation (select=3) on the trees in fit2
  fit3 <- logreg(select=3,oldfit=fit2)
  plot(fit3,pscript=F)
  n1<-seq(10,dim(fit3$cvscores)[1],10);n2<-c(1,2,6,8)
  print(round(fit3$cvscores[n1,n2],3))
# Permutation test (select=5) for model selection
  fit5 <- logreg(select=5, oldfit=fit2, nrep=rep)
  plot(fit5,pscript=F)
# print all scores
  print(fit2$allscores)
# plot the trees for the best model
  par(mfrow=c(1,1),mar=c(rep(1,4)))
  plot(fit2$alltrees[[5]],coef.rd=4)

# Select the final model. The [[X]] refers the the model in fit2$allscores
  print(fit2$allscores)
  fit2$alltrees[[3]]$coef
# Predict the probabilities of BVTV outcome, using logistic
  dat2[,17] <- predict(fit2, msz=3)
  dat2[,17] <- predict(fit2, msz=4)

```