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An evaluation of the natural history of bacterial vaginosis using transition models

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Abstract

Background—The natural history of bacterial vaginosis (BV) is complex given the variability across and within women over time. This paper considers three different transition models for analyzing longitudinal BV data.

Methods—Data from the Longitudinal Study of Vaginal Flora (LSVF) was used to evaluate three transition modeling strategies: 1) A Markov regression, 2) A Markov regression with random effects, and 3) a mover-stayer model. The effect of covariates on the transition process of BV, defined as a Nugent score of 7-10, was estimated using a logistic regression parameterization. Models were compared using various model assessment techniques. We analyzed a subset of women completing all 5 visits ($n = 1,731$) as well as the complete data ($n = 3,626$), where one or more visit measurements were missing.

Results—The Markov regression model had a poor fit to the data. A random-effects or mover-stayer model accounted for additional unexplained heterogeneity and had a better fit to the data. Across all models, douching was significantly associated with BV fluctuation. In the mover-stayer model, both douching and number of sexual partners were associated with persisting with ($\lambda_{11}=0.90$, $p<0.001$; $\lambda_{12}= -0.41$, $p<0.03$, respectively) or without ($\lambda_{01}= -0.73$, $p<0.001$; $\lambda_{02}= -0.33$, $p=0.023$, respectively) BV across all visits. Using a random effects model, we demonstrated that an individual propensity to initiate BV was positively associated with their propensity to resolve BV.

Conclusions—Transition models that account for additional heterogeneity provide an attractive approach for describing the effect of covariates on the natural history of BV.

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INTRODUCTION

Bacterial Vaginosis (BV) is described as the depletion of lactobacilli with an overgrowth of mixed anaerobic organisms and *Gardnerella vaginalis*.¹ BV is the most common form of vaginitis among women of childbearing age and has been associated with adverse reproductive and obstetric health consequences, such as pelvic inflammatory disease, preterm birth, low birth weight, and sexually transmitted infections and HIV acquisition.^{2, 3} These risks along with an unknown etiology make it important to understand the natural history of BV and explanatory variables that influence vaginal flora status.

Studying the natural history of BV is complicated by substantial temporal fluctuation in vaginal flora within women. Longitudinal studies have shown marked variability in vaginal flora over time in some women, while other women demonstrate persistently high or low Nugent scores over follow-up.⁴⁻⁶ Of interest, is understanding how BV status fluctuates within and between women over time. One approach for analyzing such longitudinal data is transition modeling⁷, where transitions between BV states may depend on covariates, such as douching, and may vary across individuals. For example, douching may increase the likelihood of BV onset but have no effect on BV persistence.^{8, 9} Various classes of transition models incorporate heterogeneity in transitions over time in different ways. This paper proposes three different transition models, all of which incorporate a basic Markov model assumption (i.e., the occurrence of BV depends on the history of BV), for exploring the natural history of BV and the effect of covariates on this natural history for women enrolled in the Longitudinal Study of Vaginal Flora (LSVF). The basic Markov model described is a random process where the conditional distribution of the future given the past depends only on the present. We extend the basic model in two directions to account for unexplained heterogeneity across women.

METHODS

Our motivating data came from the Longitudinal Study of Vaginal Flora (LSVF) described previously.¹⁰ Briefly, non-pregnant women ages 15 to 44 were recruited from 1999 to 2002 from 12 clinics in Birmingham, Alabama. Women were excluded if they had significant medical or gynecologic conditions, planned to move from the area within a year, or had conditions precluding informed consent. Participants were seen at baseline and 4 subsequent visits spaced approximately 3 months apart. Face-to-face interviews were conducted at each visit to ascertain information on demographic characteristics, sexual behaviors, vaginal symptoms, and hygiene habits. A vaginal smear was collected and evaluated at each visit using Nugent Gram stain criteria.¹¹ BV was defined as a Nugent score of 7 to 10 and women with a score of 0 to 6 were considered BV-negative. The study was approved by Institutional Review Boards of the Jefferson County Department Health, the University of Alabama at Birmingham, and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development.

Initially, we used only complete observations, i.e. women who had a Nugent score at all five visits in this analysis, giving a sample size of 1,731 women. Later, we fit models which allowed for missing response data. Descriptive characteristics were compared between the full sample (n=3,626) and women with complete (n=1,731) and incomplete (n=1,895) observations. For each woman, we summed each reported characteristic and divided by the total number of completed observations to get an overall average across all observed visits. A Wilcoxon rank-sum test was used to compare differences between complete and incomplete samples. The fact that the women were followed on a schedule of almost equally spaced intervals allowed us to fit discrete time Markov chain models. A logistic regression parameterization was employed to model the odds of BV accounting for prior BV status,

douching since the prior visit (Yes/No), and number of sexual partners since the prior visit (0,1,2, or 3 or more).

Modeling Approaches

Simple Markov regression model—We begin by presenting a simple Markov regression model. The Markov regression model presented is a logistic regression model, which incorporates a prior (i.e., lagged) response term as an additional covariate.¹² This model assumes that the correlation within women is explained by their prior BV status. Let Y_{it} be the binary response for the i th subject at the t th visit. Then a first order Markov model using logistic regression would have the form:

$$\text{logit } P(Y_{it}=1|Y_{it-1})=\beta_0+\alpha Y_{it-1}, \quad i=1,\dots,n \quad t=1,2,\dots \quad (1)$$

This allows us to define the following transition probability,:

$$P(Y_{it}=1|Y_{it-1})=\frac{e^{\beta_0+\alpha Y_{it-1}}}{1+e^{\beta_0+\alpha Y_{it-1}}}$$

which is the conditional probability of being positive for BV at the current visit given the BV status at the previous time point, Y_{it-1} . Covariates can easily be added to Model (1).¹³ A first order model with one covariate (x_t) would have the form:

$$\text{logit } P(Y_{it}=1|Y_{it-1}, x_t)=\beta_0+\alpha Y_{it-1}+\lambda x_t, \quad i=1,\dots,n \quad t=1,2,\dots$$

The above expression allows us to estimate the effect of changes in a covariate x_t on the probability of making a transition over two successive time points. Lamda (λ) represents the natural log of the odds ratio of BV for a 1 unit change in x_t controlling for BV at the prior visit. Additionally, Markov regression models can easily be extended to allow for k th-order Markov dependence (i.e., depends on k prior response measurements) by including additional lagged terms, and interpreted as the probability that an individual is positive given the diagnosis at k previous time points. The models can also be extended to allow for additional covariates. The above models, which can be viewed as a generalized linear model, can be estimated using most statistical software packages through maximum likelihood estimation.¹⁴

Random-effects Markov model—A natural extension of the Markov regression model is to include a random effect for individuals to allow for additional between-person variation in the model, as discussed by Cook.¹⁵ Model (2) applies two random effects terms: an intercept term (b_{i1}) and one for the previous visit (b_{i2}). This model assumes that the random effects come from a Gaussian distribution with mean zero and variance Σ .¹⁵ Adding a Gaussian random effect for each individual extends the simple Markov regression model above to:

$$\text{logit } P(Y_{it}=1|Y_{it-1})=\beta_0+\alpha Y_{it-1}+\lambda x_t+b_{i1}+b_{i2}Y_{it-1}, \quad b_i \sim MVN(0, \Sigma) \quad i=1,\dots,n \quad t=1,2,\dots \quad (2)$$

The random-effects terms allow one to incorporate additional heterogeneity in transition patterns across individuals, which is not explained by a simple Markov regression model.

The interpretation of λ for a simple Markov model is different from that of a random-effects Markov model in that the latter interpretation corresponds to how a changing covariate affects a typical subject's transition probabilities, while the former corresponds to an effect of the covariate on the average transition rate in the population.¹⁶ We estimated the parameters from this model using the program R, which utilizes adaptive Gaussian Hermite quadrature.¹⁴

Mover-Stayer Model—As described previously, women may persist in a BV or non-BV state over time. Similarly, a large number of women in our dataset were persistently negative or positive for BV at all five of their visits. This led us to consider the mover-stayer model¹⁷, which allows for a fraction of the population to remain in its original state, while the remaining fraction transition between infection states based on a first order Markov model. In our example, there are three groups of people: women who remain BV-negative throughout the entire study, women who persisted with BV throughout the entire study, and women who transition between states in a process similar to a simple Markov regression model. Additional heterogeneity is accounted for in this model by estimating the effect of covariates on the likelihood of transitioning between visits among movers and the likelihood of being in a particular group of movers or stayers using a polychotomous logistic regression parameterization, which is shown in a footnote to Table 4. We used the Nelder-Mead method to optimize the likelihood in R.¹⁴

Model Comparisons—First, second, and third order Markov regression models were assessed, which include lagged response terms for the prior, two visits prior, and three visits prior, respectively. Although higher order lagged terms in the simple Markov regression model were statistically significant, these models do not effectively use all of the longitudinal data. Explicitly, a Markov regression model with third-order lagged terms only uses the last two longitudinal measurements as responses in the analysis. The random-effects and mover-stayer models are effective ways to incorporate this dependence structure without substantially reducing the amount of data used to model these relationships.

Random-effects and mover-stayer models assumed only a first-order Markov assumption given that these models allow for a richer set of dependence structures without needing additional lagged terms in the models. Across all three models, interactions between covariates and covariates with lagged response terms were assessed. For simplicity, models were fit without the additional covariates of douching and sex partners and compared to assess model fit using three different approaches. The first approach simulated data under each model to determine the expected number of individuals who would be positive zero, one, two, three, four, and five times. The second approach simulated data to calculate the autocorrelation between visits, which is the correlation between pairwise measurements separated by 1 to 4 visits, and these expected autocorrelations were compared with empirically estimated autocorrelations from the data. Finally, we compared the log likelihood (LL) and the Akaike's Information Criteria (AIC) values. In addition, individual transition probabilities were estimated from a random effects model to assess the relationship between BV onset (BV-negative to BV) and resolution (BV to BV-negative) in this population of women. As a sensitivity analysis, a first-order Markov random effects model controlling for douching and sex partners was fit to the entire dataset.

RESULTS

There were 3,626 women involved in the study. Table 1 presents descriptive statistics for the entire sample compared to those with complete and incomplete observations. Those with missing observations had a higher median number of sex partners and greater frequency of vaginal discharge, odor, itching, and irritation and lower frequency of dysuria and

abdominal pain compared to those with complete observations. Both sets of women reported a similar mean Nugent Gram stain score, BV prevalence, and douching prevalence.

A first, second, and third order Markov regression model using douching status and the number of sexual partners as covariates was assessed (Table 2). The lagged BV status terms and douching were significant while the number of sexual partners was not for all first, second, and third order models. Interaction terms between douching and number of sex partners and between covariates and prior BV status were not significant (data not shown).

The first order Markov regression model was extended to account for additional heterogeneity using a random intercept and random slope for the lagged term (Table 3). Similar to the simple Markov regression model, only the previous visit and douching were significant. Figure 1 shows a strong positive correlation between transition probabilities estimated from a Markov regression model with random effects in that women who had a low probability of BV resolution ($P(Y_t=0|Y_{t-1}=1)$) also had a low probability of BV onset ($P(Y_t=1|Y_{t-1}=0)$).

Estimates from the mover-stayer model are described separately for those who transition (movers) and those who persist with BV or remain free of BV over follow-up (stayers) in Table 4. Among movers, only the lagged response term and douching remained significant, which is consistent with the other models. Among stayers, both douching and number of sex partners were significant. The coefficients for douching among those who remained BV-negative ($\lambda_{01} = -0.731$) and BV-positive ($\lambda_{11} = 0.904$) across all visits implies that douching decreases the probability of a woman remaining in the BV-negative group and increases the probability of remaining in the persistent BV group, respectively. Whereas, multiple partners decreased the probability of remaining in the persistent BV ($\lambda_{12} = -0.412$) and BV-negative ($\lambda_{02} = -0.325$) groups, both relative to being in the transitioning (movers) group.

The expected versus observed counts by frequency of BV visits were compared for different modeling approaches in Table 5. The greatest differences in expected versus observed counts were found in the simple Markov regression model; whereas, accounting for additional heterogeneity through the random-effects or mover-stayer model provided a better fit. Figure 2 presents the autocorrelation plots based on all 3 modeling approaches and empirical data. This plot shows that the mover-stayer model is closest to the empirical data. Finally, the log likelihood and AIC values were compared. The simple Markov regression model had the smallest log likelihood (LL = -5065.3) and largest AIC (10,134) indicating a poorer fit compared to the random effects model (LL = -4671.7, AIC = 9354) and the mover-stayer model (LL = -4924.8, AIC = 9859.6). A sensitivity analysis revealed that estimates from a first-order Markov with covariates and a random intercept and slope fit to the entire dataset were similar to estimates from the sample of only complete observations shown in Table 3. For the entire dataset, the corresponding β_0 (SE) was -1.71 (0.098), β_1 (SE) was 2.24 (0.067), λ_1 (SE) was 0.35 (0.059), λ_2 (SE) was 0.18 (0.081), b_{11} (SE) was 1.20 (1.097), and b_{12} (SE) was 3.32 (1.823). The only difference in inference between the complete and entire datasets was that the coefficient for number of sexual partners was statistically significant in the entire sample ($p = 0.025$); however, the magnitude of the coefficients were similar in both analyses.

DISCUSSION

Bacterial vaginosis is a complex condition of unknown etiology. In this paper, we presented three transition models that might be used to analyze longitudinal patterns of BV. A simple Markov regression model is easy to fit with standard software, but does not take into

account additional heterogeneity not explained by prior BV status as shown in the autocorrelation comparison (Figure 2). While both the random effects and mover-stayer model account for additional heterogeneity in the data (Figure 2) and are a better fit to the data based on expected counts, log likelihood, and AIC values, the choice of models also depends on the scientific question of interest. The random-effects model is useful for describing the effect of covariates on transitions for a given individual (i.e., subject-specific changes). The mover-stayer model is useful for understanding covariate effects on transition patterns over time as well as difference across groups of women that either transition or remain in their initial state throughout the study period. Evaluating the performance of particular modeling strategies for this type of data are necessary to better understand the natural history of BV and make inferences regarding the effects of particular covariates.

Prior longitudinal studies of BV have focused on generalized estimating equations, conditional logistic regression, marginal structural modeling,¹⁸⁻²¹ or modeling time-to-BV acquisition or resolution separately to assess covariate effects²². These methods have advantages, including ease of interpretation and use with standard software programs. However, they may not appropriately account for the temporal variability in vaginal flora within or across women, which could have important implications for understanding the natural history of BV. Transition models offer an additional approach for incorporating prior history of BV when assessing the effect of personal characteristics, such as sexual behaviors, on current BV. For example, the mover-stayer model showed that the number of sexual partners did not significantly change the probability of transitioning between visits; however, it was significant in determining whether a woman would persist with or without BV over follow-up (Table 4). Thus, one may conclude that having multiple partners or changing partner status affects whether women persist within a given vaginal flora state, but does not influence their likelihood of transitioning over 3-month intervals. In addition, transition probabilities estimated from a random-effects model showed that BV resolution and onset were highly, positively correlated (Figure 1). This would suggest that there are women who tend to transition frequently and those that do not. While we applied simple models to look at these transition probabilities, one can extend this to account for additional covariates to determine their effect on BV resolution and onset. These patterns are in keeping with other studies evaluating the natural history of BV, which suggested that there may be different groups of women that transition frequently or persist in a given vaginal flora state over time.⁴⁻⁶ Here we provide a formal modeling framework in which to apply these hypotheses in future studies to account for different groups and individual transition patterns across a large sample of women. For example, one can assess the effect of other covariates on whether women are long-term persisters or transitioners and how these covariates affect the probability of transitioning over time. In addition, there may be inherent variability across women, such as vaginal flora composition, that influence these patterns. A model which extends the random effects model to allow for groups of women who remain in their initial BV state (i.e., combining the random effects and mover-stayer models) may better describe the natural history of BV.

This is the first paper we know of to compare different approaches for modeling BV using transition models, and each approach has its own strengths and weaknesses. All models are subject to some loss of data due to the autoregressive correlation structure. The benefit of a simple Markov model is that it is relatively easy to fit with standard statistical software; however, it may not account for additional heterogeneity across women. We showed that extensions of this model may better fit the data providing a more complete description of the natural history of BV and covariate effects while accounting for additional unexplained heterogeneity. An advantage of the random-effects model is that it can be fit using most standard statistical software and is easily extended to account for missing data; however inferences are limited to a subject-specific interpretation. Few epidemiologic studies have

considered the mover-stayer model to describe biologic phenomena. This paper shows that this modeling strategy describes longitudinal patterns of BV well; however, standard software to implement this type of analysis is limited. In addition, an extended model incorporating both mover-stayer and random effects may provide the best fit to the data, but is computationally complex and left to future work. Finally, for comparing all three models, we were limited to looking at a subset of women with complete observations across all visits. While descriptive characteristics differed across samples (Table 1), we saw very few differences in magnitude of estimates when applying a random-effects model to the entire sample or to the subset of women who completed all visits.

There are several possibilities for future work and applications of these methods. First, the mover-stayer model may be extended to account for missing data by integration or imputation to handle missing values. Additional work might also consider using an ordinal instead of a dichotomous outcome. The Nugent score is often examined as a trichotomous outcome of normal, intermediate, or BV rather than a dichotomous classification as was used in this paper.²¹ Exploiting the full ordinal outcome may elicit a finer distinction between groups of women. This could be done by employing a polychotomous logistic regression framework to the proposed models. Finally, these models may also be applied to studying the natural history of sexually transmitted infections, such as human papillomavirus, in which interest is in describing factors related to onset, persistence, resolution, and recurrence.

In summary, each model provides a different way to characterize the natural history of BV. The Markov regression model dictates that the probability of BV on a given visit depends solely on the past observations of the women, while the random effects and mover-stayer model incorporate additional variation by allowing transition probabilities to vary across women. The mover-stayer model is particularly appealing since it allows us to examine which factors dictate a women's propensity to persist with BV, persist without BV, or to transition between BV states. A better understanding of how covariates affect a woman's chances of being in each of these groups could aid in the prevention and treatment of this common gynecologic condition.

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Summary

Transition models that account for additional heterogeneity provide an attractive approach for evaluating the effect of covariates on the natural history of BV.

Figure 1. Estimated transition probabilities from a Markov regression model with a random intercept and slope.

Figure 2. Autocorrelation between visits for the empirical data and a first-order Markov model, a first-order Markov model with random effects, and a mover-stayer model

This figure compares the observed versus the predicted correlation structure for each type of model. The autocorrelation represents the correlation between all pairwise response measurements separated by 1 to 4 visits (lag).

Table 1

Descriptive characteristics of women in the Longitudinal Study of Vaginal Flora (LSVF) stratified by complete and incomplete observations

Covariate	Entire Sample	Complete Observations	Incomplete Observations	p-value ¹
Total (n)	3,626	1,731	1,895	
Nugent Score	5.05 (3.18)	5.01 (3.22)	5.09 (3.11)	0.27
BV	0.41 (0.38)	0.40 (0.35)	0.41 (0.41)	0.61
Douching	0.44 (0.43)	0.42 (0.42)	0.46 (0.45)	0.013
Number of sex partners, median (IQR)	1 (1-3)	1(1-1)	2(1-4)	<0.001
Vaginal Wetness	0.26 (0.32)	0.23 (0.25)	0.29 (0.37)	0.27
Vaginal Discharge	0.29 (0.33)	0.25 (0.26)	0.34 (0.38)	<0.001
Vaginal Odor	0.13 (0.25)	0.11 (0.19)	0.15 (0.29)	0.016
Vaginal Itching	0.09 (0.20)	0.08 (0.15)	0.10 (0.24)	0.003
Vaginal Irritation	0.07 (0.18)	0.06 (0.13)	0.08 (0.21)	<0.001
Abdominal Pain	0.07 (0.18)	0.06 (0.14)	0.08 (0.22)	<0.001
Dysuria	0.04 (0.13)	0.03 (0.09)	0.04 (0.16)	0.002

Note: Tables present means unless specified otherwise; IQR: Interquartile range

¹ P-values are based on Wilcoxon rank-sum test of differences between women with complete observations verse those without

Parameter estimates for a first-, second-, and third-order Markov regression model controlling for douching and number of sexual partners, n = 1,731

Table 2

Parameter ¹	First-order Model Estimate (SE)	p-value	Second-order Model Estimate (SE)	p-value	Third-order Model Estimate (SE)	p-value
β_0 (intercept)	-1.59 (0.10)	<0.001	-1.67 (0.12)	<0.001	-1.65 (0.15)	<0.001
α_1 (first-order lag term) ²	1.94 (0.061)	<0.001	1.65 (0.08)	<0.001	1.64 (0.10)	<0.001
α_2 (second-order lag term) ²	-	-	1.01 (0.08)	<0.001	0.76 (0.10)	<0.001
α_3 (third-order lag term) ²	-	-	-	-	0.65 (0.10)	<0.001
λ_1 (douching)	0.39 (0.061)	<0.001	0.33 (0.07)	<0.001	0.30 (0.09)	0.001
λ_2 (number of sexual partners)	0.13 (0.084)	0.133	0.01 (0.10)	0.896	-0.15 (0.13)	0.25

¹Odds ratios and 95% confidence intervals can be obtained for all non-intercept parameter estimates as follows: e(Parameter estimate)±1.96(SE)

²First, second, and third-order lagged terms correspond to the effect of the previous, two visits prior, and three visits prior, respectively.

Table 3

Parameter estimates for a first-order Markov regression model with a random intercept and random slope for the lagged response variable controlling for douching and number of sexual partners, n = 1,731

Parameter ¹	Estimate (SE)	p-value
β_0 (intercept)	-1.75 (0.12)	<0.001
α_1 (first-order lag term) ²	2.36 (0.08)	<0.001
λ_1 (douching)	0.42 (0.07)	<0.001
λ_2 (number of sexual partners)	0.14 (0.09)	0.15
b_{i1} (random intercept)	1.22 (1.11)	-
b_{i2} (random slope)	3.71 (1.92)	-

Note: the correlation between b_{i1} and b_{i2} is -1.0

¹ Odds ratios and 95% confidence intervals can be obtained for all non-intercept parameter estimates as follows: $e^{(\text{Parameter estimate}) \pm 1.96(SE)}$

² First-order lagged term corresponds to the effect of the previous visit

Table 4

Parameter estimates for a mover-stayer model controlling for douching and number of sexual partners, n = 1,731

Parameter ¹	Estimate (SE)	p-value
Mover (transition between visits)²		
β_0 (intercept)	-0.79 (0.13)	<0.001
α_1 (first-order lag term) ³	0.89 (0.10)	<0.001
λ_1 (douching)	0.32 (0.09)	<0.001
λ_2 (number of sexual partners)	0.06 (0.10)	0.55
Stayer (BV-negative at all visits)⁴		
γ_{00} (intercept)	-0.01 (0.42)	0.98
λ_{01} (douching)	-0.73 (0.18)	<0.001
λ_{02} (number of sexual partners)	-0.33 (0.15)	0.023
Stayer (BV-positive at all visits)⁵		
γ_{10} (intercept)	-1.59 (0.53)	0.003
λ_{11} (douching)	0.90 (0.26)	<0.001
λ_{12} (number of sexual partners)	-0.41 (0.19)	0.028

¹ Odds ratios and 95% confidence intervals can be obtained for all non-intercept parameter estimates as follows: $e^{(\text{Parameter estimate}) \pm 1.96(SE)}$

² $P(\text{Mover}) = 1 - P(\text{Stayer, BV-negative at all visits}) - P(\text{Stayer, BV-positive at all visits})$

³ First-order lagged term corresponds to the effect of the previous visit

⁴

$$P(\text{Stayer, BV-negative at all visits}) = \frac{e^{\gamma_{00} + \lambda_{01}(\text{douching}) + \lambda_{02}(\# \text{ of sex partners})}}{1 + e^{\gamma_{00} + \lambda_{01}(\text{douching}) + \lambda_{02}(\# \text{ of sex partners})} + e^{\gamma_{10} + \lambda_{11}(\text{douching}) + \lambda_{12}(\# \text{ of sex partners})}}$$

⁵

$$P(\text{Stayer, BV-positive at all visits}) = \frac{e^{\gamma_{10} + \lambda_{11}(\text{douching}) + \lambda_{12}(\# \text{ of sex partners})}}{1 + e^{\gamma_{00} + \lambda_{01}(\text{douching}) + \lambda_{02}(\# \text{ of sex partners})} + e^{\gamma_{10} + \lambda_{11}(\text{douching}) + \lambda_{12}(\# \text{ of sex partners})}}$$

Table 5

Observed versus expected counts by number of visits with BV for each transition model

Number of visits with BV	Observed counts (proportion)	Expected Counts (proportion)		
		Simple first-order Markov Model	First-order Markov with Random Effects Model	Mover-Stayer Model
0	523 (0.302)	388 (0.224)	479 (0.276)	521 (0.301)
1	275 (0.159)	373 (0.215)	267 (0.153)	256 (0.148)
2	251 (0.145)	330 (0.191)	324 (0.187)	315 (0.182)
3	251 (0.145)	290 (0.168)	273 (0.158)	268 (0.155)
4	217 (0.125)	203 (0.177)	170 (0.098)	156 (0.090)
5	214 (0.124)	174 (0.085)	221 (0.127)	214 (0.124)