Factors Associated With Incidence and Spontaneous Clearance of Molecular-Bacterial Vaginosis: Results From a Longitudinal Frequent-Sampling Observational Study

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Background: We sought to assess time-independent and time-varying factors associated with incidence and spontaneous clearance of molecular-bacterial vaginosis (BV; without treatment).

Methods: Midvaginal samples were self-collected daily by 100 participants recruited at the University of Alabama Birmingham for 10 weeks (4778 samples). Vaginal microbiota was characterized by 16S rRNA gene amplicon sequencing and clustered into community state types (CSTs). A low-*Lactobacillus* CST defined the molecular-BV outcome in this study. Factors associated with molecular-BV incidence and spontaneous clearance were modeled using Andersen-Gill recurrent event Cox models. Community class identified the predominant CST of a participant during follow-up.

Results: Menstruations (adjusted odds ratio [aHR], 2.09 [95% confidence interval, 1.51–2.89] in the prior 24 hours) and CST III (*Lactobacillus iners* dominated) at the previous sample (aHR, 2.25 [1.48–3.40]) were associated with increased molecular-BV incidence. Participants with a majority of *L. iners*–dominated samples longitudinally (community class LI) displayed less stable patterns of vaginal microbiota. In LI participants, reduced molecular-BV spontaneous clearance was observed in African American participants (aHR, 0.44 [0.26–0.75]) compared with White participants, older participants (age, 40–49 years [aHR, 0.38; 0.23–0.61]; age, 30–39 years [aHR, 0.48; 0.28–0.83]) compared with participants aged 18 to 29 years, and after douching (0.45 [0.28–0.73] within prior 72 hours).

Conclusions: Although it is now well documented that vaginal microbiota are dynamic, there are few available data on factors associated with spontaneous clearance of molecular-BV. *Lactobacillus iners*–dominated vaginal microbiota are more likely to be dynamic and associated with different risk factors for incidence and clearance of BV. Among *L. iners*–dominated participants, age, race, and douching were linked to reduced clearance. Most transitions to molecular-BV during menstruations were short-lived.

Bacterial vaginosis (BV) affects nearly 30% of reproductive-aged North American women[1] and is characterized by a vaginal microbiota with low abundance of *Lactobacillus* species and higher abundances of anaerobic bacteria.[2] Bacterial vaginosis is associated with vaginal symptoms[3] and a significant 2- to 4-fold increased risk of sexually transmitted infections,[4–6] including HIV.[7,8] Community state types (CSTs) can be defined based on the clustering of vaginal bacterial community compositions determined by 16S rRNA gene amplicon sequencing.[9] Community state type IV is characterized by low relative abundance of *Lactobacillus* species and a wide array of strict and facultative anaerobes associated with BV and was termed molecular-BV by McKinnon et al.[10] Recently, *Lactobacillus iners*–dominated communities have gained attention as being a risk factor for sexually transmitted infections and other adverse outcomes,[11] hypothesized to be due in part to the inability of *L. iners* to produce D-lactic acid.[12]

Previous longitudinal studies focusing on daily or frequent sampling with Gram stain assessment (termed Nugent-BV[10]) have described several patterns of vaginal microbiota dynamics. In 1999, Schwebke et al.[13] demonstrated that there are stable patterns of normal microbiota, intermediate microbiota or BV, or short transitions in and out of BV, and these findings have been observed in other studies.[14–18] These fluctuation patterns have been documented in BV treatment studies as well, where molecular characterization of the vaginal microbiota demonstrated increases of BV-associated bacteria during menstruations, whereas women

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Contribution to Authorship: J.R. and R.M.B. were involved in the conception, planning, and carrying out of the HMP-UMB study. R.M.B., J.T., and M.D.S. determined the research question and analysis plan. J.T. analyzed the data, with the deep support of M.D.S. and R.M.B. J.R. also contributed to analysis and translation. J.T. wrote the first draft of the manuscript, and all authors read, gave their feedback, and validated the final version of the manuscript.

Details of Ethics Approval: The study protocol of the HMP-UMB study was approved by the institutional review boards of the University of Alabama Birmingham (No. F090430006; August 21, 2009) and the University of Maryland Baltimore (No. HP-00041351; February 18, 2010). All participants provided written informed consent.

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with BV could face recurrent BV quickly after antibiotic treatment.[19,20] Such data expanded and challenged research practice and pointed to a need for vaginal microbiota to be more frequently assessed to gain a better understanding of its natural history and pathogenesis.

Studies using mostly Nugent-BV assessment and frequent sampling designs have shown that fluctuations in the vaginal microbiota are associated with menstruations, spermicide, and lack of condom use, whereas vaginal intercourse and receptive oral sex have been suspected but with inconsistent results.[13–18] Other longitudinal studies with infrequent sampling have demonstrated that sex with a new partner,[21] digital sex,[22] and vaginal douching[23,24] were associated with incident BV, whereas condom use was associated with lower BV risk.[25,26] However, factors associated with spontaneous clearance of BV (without antibiotic treatment) have been seldom studied.

We aimed to assess the association between both time-fixed and daily time-varying factors and incidence and spontaneous clearance of molecular-BV defined by 16S rRNA gene amplicon sequencing[10] in a cohort of 100 participants who collected samples daily for 10 weeks.

METHODS

This is a secondary data analysis using data from an observational cohort study in which factors associated with incidence and spontaneous clearance of molecular-BV are assessed.

Human Microbiome Project—University of Maryland

The Human Microbiome Project—University of Maryland (HMP-UMB) study recruited 135 nonpregnant reproductive-age cisgender women (between 18 and 49 years old) at the University of Alabama at Birmingham between September 2009 and July 2010 to a prospective observational study that has been previously described.[27] In brief, participants self-collected midvaginal swabs daily for 10 weeks. After exclusion of participants with fewer than 5 samples with 16S rRNA gene sequence data over the course of the study, this analysis included 100 participants with 4778 vaginal samples. A clinician performed a pelvic examination and clinical evaluation at baseline, week 5 and week 10, or at an interim visit if a participant returned for a visit due to vulvovaginal symptoms. A questionnaire was administered at all clinical visits, and participants also reported time-varying behaviors and menstrual bleeding on daily diaries, which were submitted weekly along with vaginal samples. During the clinical visits, BV was diagnosed according to Amsel's criteria (at least 3 of the following 4 findings): (i) vaginal pH >4.5, (ii) homogenous white/gray vaginal discharge, (iii) the presence of clue cells, and (iv) a positive whiff test result (fishy odor after the addition of 10% potassium hydroxide). When symptomatic BV was diagnosed, the participant was prescribed antibiotic treatment after standard practice (metronidazole or clindamycin).[28] Antibiotic use for BV (16 events) from the moment it was taken until the end of the study was controlled for in all analyses as a time-varying covariate. In addition to antibiotic treatment for BV, many participants also reported antibiotic use for other indications over the course the study. This additional antibiotic consumption was not taken into account in the present study (antibiotic use in the last 30 days was reported 32 times throughout the study). The study protocol was approved by the institutional review boards of the University of Alabama Birmingham and the University of Maryland Baltimore. All participants provided written informed consent.

Vaginal Microbiota Characterization

DNA was extracted from a Copan ESwab placed in Amies liquid transport, using enzymatic and physical lysis of bacterial cells followed by purification of genomic DNA using a QIAsymphony robotic platform and QIAGEN CellFree 500 kits (QIAGEN, Valencia, CA) according to the manufacturer's instructions.[27] The V3–V4 regions of the 16S rRNA gene were amplified and sequenced on an Illumina HiSeq 2500 instrument.[29] Bioinformatic sequence processing and taxonomic assignments also followed the procedures of Holm et al.[29] and resulted in a bacterial phylotypes relative abundance table. Community state types (Fig. S1, http://links.lww.com/OLQ/A836) were assigned using VALENCIA, a nearest centroid-based classification algorithm (git-hub.com/ravel-lab/valencia).[30] VALENCIA identified 7 CSTs, 4 of which were dominated by the indicated *Lactobacillus* species: CST I, *Lactobacillus crispatus*; CST II, *L. gasseri*; CST III, *L. iners*; and CST V, *L. jensenii*. Community state type IV was characterized by low levels of *Lactobacillus* species and was subdivided into 3 sub-CSTs based on the most abundant organisms detected: CST IV-A, BVAB1 and *Gardnerella vaginalis*; CST IV-B, *G. vaginalis* and *Atopobium vaginae*; and CST IV-C, *Streptococcus* species and *Corynebacterium.*[10] For some analyses, CSTs were also grouped into a binary outcome: *Lactobacillus*-dominated (CST I, II, III, V) versus molecular-BV (CST IV).

Community Classes

The community class variable reflects a profile of longitudinal sampling and was defined by hierarchical clustering of a participant's CST profile using Euclidian distance and Ward linkage, as described previously.[31s] The community classes that we observed were LC (CST I dominated, mostly *L. crispatus*), LG (CST II dominated, mostly *L. gasseri*), LI (CST III dominated, mostly *L. iners*), DA (CST IV-A dominated, diverse), DB (CST IV-B dominated, diverse), and DC (CST IV-C dominated, diverse; Fig. 1).

Statistical Analyses

Comparison of baseline characteristics across community classes was performed using Fisher exact test. Incidence of molecular BV was defined as at least 1 sample categorized as molecular-BV (CST IV) after a non–molecular-BV sample (any other CST). Clearance of molecular BV was defined as at least 1 non–molecular-BV sample after a molecular-BV sample, that is, a spontaneous transition from molecular-BV to *Lactobacillus*-dominated state. The effects of time-independent and time-dependent covariates on the rate of transition from *Lactobacillus*-dominated state to molecular-BV (incident molecular-BV vs. nonincidence) and molecular-BV to a *Lactobacillus*-dominated state (molecular-BV clearance vs. nonclearance) were assessed with Andersen-Gill recurrent event Cox models, using the "survival" package (version 3.2-11) in R (version 4.1.0). The models included assessment for time-varying factors such as douching and sexual exposure variables in different time windows (within prior 24 hours, within prior 48 hours, within prior 72 hours of the outcome) and CST at the previous sample (approximately 1.5 days prior), and time-independent factors such as age, race, and contraception. The effect of condom use was evaluated by comparing condom use versus no condom use during vaginal intercourse. The effect of condomless vaginal intercourse was evaluated by comparing vaginal intercourse without condom use versus no vaginal intercourse. For each exposure assessed, multivariate models were computed using relevant confounders. Transition intensities (rates), daily probabilities of transition, and sojourn length were

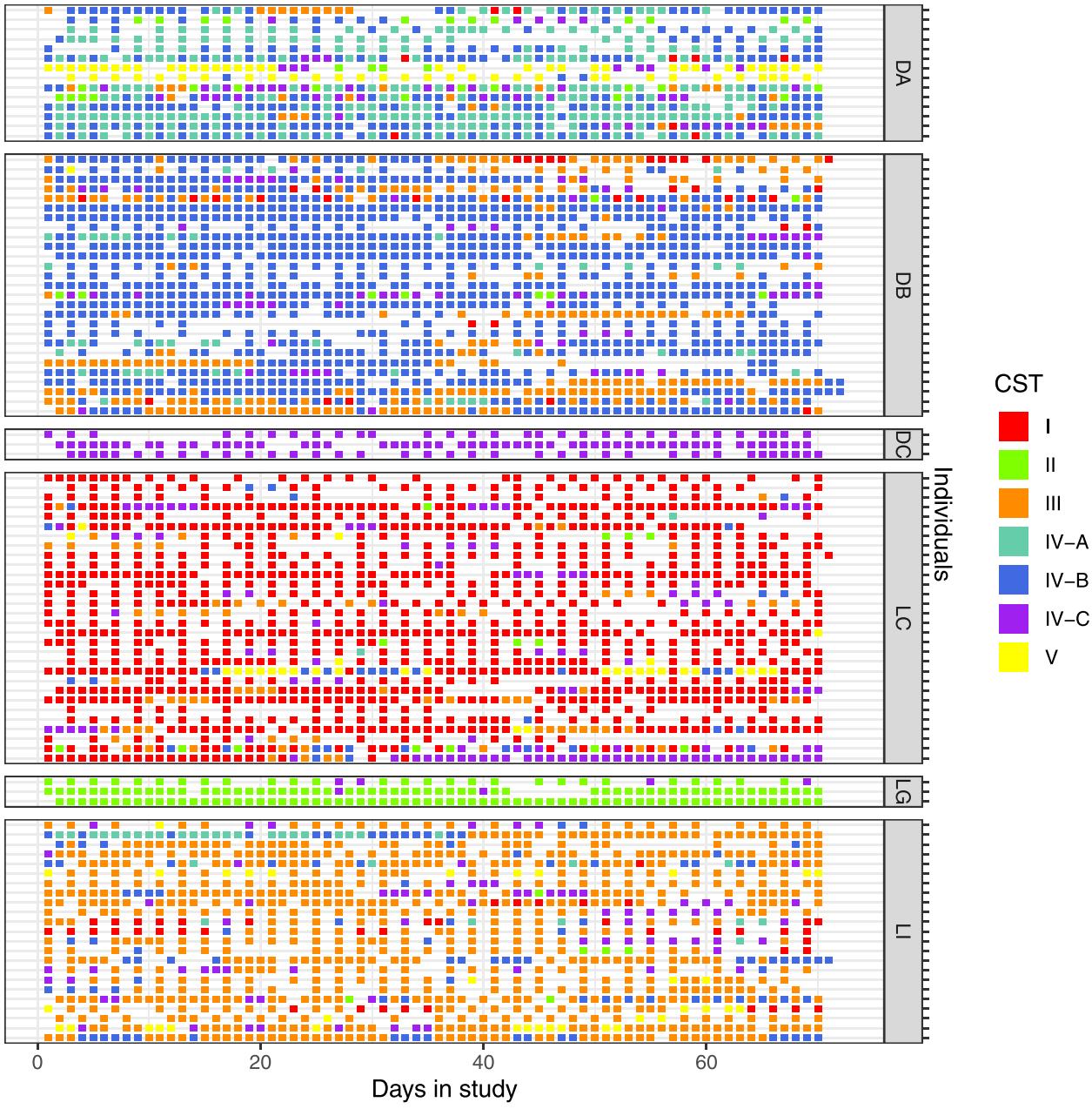

Figure 1. Community state type dynamics per participant over the course of the study, in the HMP-UMB study on 100 participants in Birmingham, AL. Each of the 4778 samples of the study is represented by a rectangle and colored according to its CST. Each row represents 1 participant. Participants were grouped into community class according to their main CST over the course of the study, determined through hierarchical clustering on the proportions of each CST. LC (*L. crispatus*): mostly CST I; LG (*L. gasseri*): mostly CST II; LI (*L. iners*): mostly CST III; DA: mostly CST IV-A; DB: mostly CST IV-B, DC: mostly CST IV-C.

computed from multistate models with the "msm" package (version 1.6.9) in R.

RESULTS

Participants contributed a median of 50 (interquartile range, 35–65) midvaginal samples. Individuals with a low-*Lactobacillus* CST IV as their main CST over the course of the study (community classes DA/DB/DC) were more likely to be African American ($P = 0.011$) and report history of vaginal douching ($P = 0.060$), and were less likely to use hormonal contraception ($P = 0.060$) compared with those in other community classes (Table 1).

The temporal dynamics of CST by community class are presented in Figure 1. Eight participants (8%) remained in the same CST for the length of the study, 3 from the LC community class (*L. crispatus*–dominated CST I), 1 from the LG community class (*L. gasseri*–dominated CST II), 1 from the DB community class (CST IV-B), and 3 from the DC community class (CST

TABLE 1. Baseline Characteristics of Participants in *L. crispatus*–Dominated and *L. gasseri*–Dominated Community Classes (LC/LG), *L. iners*–Dominated Community Class (LI), and Diverse Community Classes (DA/DB/DC) in the HMP-UMB Study in Birmingham, AL (N = 100)

	Total No. Observations		Participants in LC/LG Community Class* (n = 33)		Participants in LI Community Class* (n = 23)		Participants in DA/DB/DC Community Class* (n = 44)		$P^{\dagger}$
Age, y	100								0.163
40+	9	9%	4	12%	2	9%	3	7%	
30–39	37	37%	17	52%	7	30%	13	30%	
18–29	54	54%	12	36%	14	61%	28	64%	
Race	100								0.011
African American	61	61%	13	39%	14	61%	34	77%	
Hispanic or other	6	6%	3	9%	1	4%	2	5%	
White	33	33%	17	52%	8	35%	8	18%	
Contraception	86								0.060
Hormonal	17	20%	7	26%	7	33%	3	8%	
IUD	5	6%	1	4%	0	0%	4	11%	
Nonhormonal	64	74%	19	70%	14	67%	31	82%	
Lifetime number of partners	100								0.531
>7	41	41%	15	45%	7	30%	19	43%	
1–6	59	59%	18	55%	16	70%	25	57%	
High school education	100								0.179
Graduate	43	43%	18	55%	6	26%	19	43%	
More than high school	54	54%	15	45%	16	70%	23	52%	
Less than high school	3	3%	0	0%	1	4%	2	5%	
Marital status	100								0.488
Not married	54	54%	15	45%	12	70%	27	61%	
Separated	20	20%	6	18%	6	26%	8	18%	
Married	26	26%	12	36%	5	22%	9	20%	
Smoking in last 2 mo	100								0.730
Yes	16	16%	5	15%	5	22%	6	75%	
No	84	84%	28	85%	18	78%	38	86%	
Alcohol consumption in last 2 mo	100								0.402
Yes	77	77%	28	85%	17	74%	32	73%	
No	23	23%	5	15%	6	26%	12	27%	
Pregnancy in lifetime	100								0.256
Yes	67	67%	19	58%	15	65%	33	75%	
No	33	33%	14	42%	8	35%	11	25%	
Lubricant use in last 2 mo	91								0.281
Yes	22	24%	5	19%	8	36%	9	22%	
No	69	76%	23	85%	14	64%	32	78%	
Condom use in last 2 mo	86								0.643
Often/always	38	44%	9	38%	10	48%	19	48%	
Rarely/never	48	56%	16	67%	11	52%	21	53%	
Douching	100								0.060
Ever douched yes	51	51%	12	36%	11	48%	28	64%	
Ever douched no	49	49%	21	64%	12	52%	16	36%	
Last 2 mo yes	12	12%	3	9%	2	9%	7	16%	0.674
Last 2 mo no	88	88%	30	91%	21	91%	37	84%	

*Participants were grouped into community class according to their main CST over the course of the study, determined through hierarchical clustering on CSTs. LC: mostly CST I; LG: mostly CST II; LI: mostly CST III; DA: mostly CST IV-A; DB: mostly CST IV-B, DC: mostly CST IV-C.

†*P* value was calculated using the Fisher exact test.

IV-C). Overall, there were 292 molecular-BV incidence events (for 2214 nonincidence events) and 295 molecular-BV clearance events (1877 nonclearance events) in the study.

Factors Associated With Incident Molecular-BV, Whole Cohort

Menstruations within prior 24 hours were associated with incidence of molecular-BV (adjusted odds ratio [aHR], 2.09 [1.51–2.89]), as well as menstruations in the prior 48 hours and in the prior 96 hours (Table S2, http://links.lww.com/OLQ/A837, and Fig. 2). Other factors, including time-varying intravaginal practices (douching, lubricant use), type of undergarment, and sexual behaviors (condom use, anal sex, digital, receptive oral sex), and time-fixed factors such as race, age, and hormonal contraception were not associated with incident molecular-BV. In a multivariate model using race, contraception, and antibiotic use for BV as confounders, an age between 30 and 39 years was associated with a reduced rate of transition to molecular-BV compared with age 18 to 29 years (File S3, http://links.lww.com/OLQ/A838). Participants with a CST III at the previous sample were more likely to transition to molecular-BV compared with participants with a CST I (aHR, 2.25 [1.48–3.40]. When controlling for confounders such as contraception, menstruations in prior 72 hours, douching in prior 72 hours, and antibiotic use for BV, participants with a CST III at the previous sample remained more likely to transition to molecular-BV compared with CST I (File S3, http://links.lww.com/OLQ/A838).

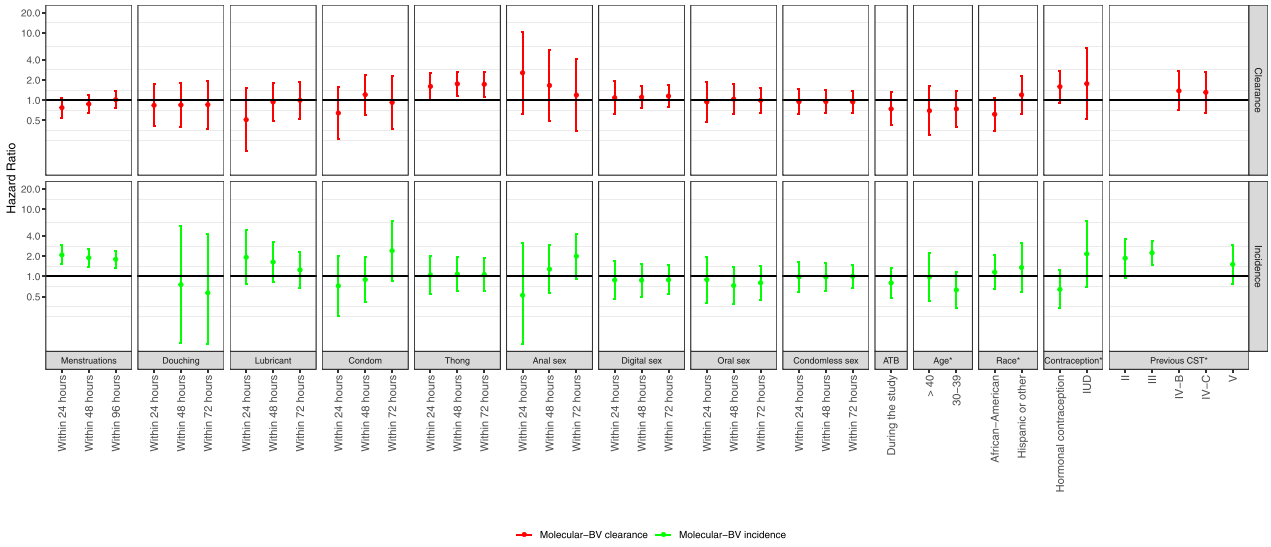

Figure 2. Time-varying and time-independent factors associated with incidence and clearance of molecular-BV (CST IV) in bivariate analyses, in 100 participants from the HMP-UMB study in Birmingham, AL. Analyses were adjusted for antibiotic treatment for BV in participants from the moment they took the antibiotic until the end of the study. ATB, antibiotics for BV. *Reference categories for age, race, contraception, and previous CST were "18–29 years old," "White," "Non-hormonal," "CST I" (for incidence), and "CST IV-A" (for clearance).

Factors Associated With Molecular-BV Spontaneous Clearance, Whole Cohort

Concerning transitions from molecular-BV to a *Lactobacillus*-dominated state (spontaneous clearance of molecular-BV), only thong undergarment use in the prior 24 hours, in the prior 48 hours, and in the prior 72 hours were associated with higher clearance rates of molecular-BV (aHR, 1.61 [1.02–2.54] in the prior 24 hours). Of note, this practice was mostly driven by 12 participants who wore thong undergarment repeatedly throughout the study. Other factors, including prior CST, hormonal contraception, sexual practices, and vaginal douching, were not statistically associated with molecular-BV clearance.

Description of CST Transitions by Community Class

Participants had a daily probability of 8% to transition to molecular-BV (incidence probability, 0.083 [95% confidence interval {CI}, 0.075–0.093]) and a daily probability of 11% to clear molecular-BV (clearance probability, 0.112 [95% CI, 0.100–0.125]). Because distinct microbiota might exhibit different resilience and stability, we investigated stability patterns by evaluating daily probabilities of transition and sojourn length in and out of molecular-BV by community class, which corresponded to the main CST detected throughout the study for an individual (Table 2). After a molecular-BV incidence event, participants from LC or LG community class persisted, on average, 2.3 days in a molecular-BV state, and participants from LI community class persisted, on average, 2.1 days in a molecular-BV state. For participants from low-*Lactobacillus* CST IV–dominated community classes (DA, DB, and DC), molecular-BV lasted for 12.2 days on average, with short-lived transitions to *Lactobacillus*-dominated states lasting 3.2 days on average.

The daily incidence probability of molecular-BV for LI participants was twice as high as that for LC/LG participants (0.082 [95% CI, 0.067–0.103] vs. 0.040 [95% CI, 0.032–0.050]). These participants also spent less time in a *Lactobacillus*-dominated state than participants in the LC/LG class: 9.2 days (95% CI, 7.3–11.8 days) versus 19.6 days (95% CI, 14.9–25.3 days).

Higher fluctuation and therefore less stable patterns in the LI community class are evident in Figure 1 and in Table 2. Participants in the LI community class had 71% of their samples corresponding to a *L. iners*–dominated CST III compared with 83% of CST I, II, and V for participants in LC/LG community class and 80% of CST IV for participants in DA/DB/DC community class (in CST IV-A, IV-B, and IV-C, respectively; Table 3).

TABLE 2. Daily Probabilities of Incidence and Clearance of Molecular-BV (CST-IV) by Community Class in the HMP-UMB Study in Birmingham, AL (N = 100)

	LC/LG*		LI*		DA/DB/DC*	
No. transitions to molecular-BV (incidence)	65		85		142	
No. nontransitions to molecular-BV (nonincidence)	1175		722		317	
No. transitions from molecular-BV (clearance)	65		90		140	
No. nontransitions to molecular-BV (nonclearance)	93		122		1662	
Daily probability of incident molecular-BV	0.040	0.032–0.050	0.082	0.067–0.103	0.257	0.222–0.296
Daily probability of clearing molecular-BV	0.347	0.281–0.426	0.357	0.301–0.424	0.068	0.058–0.080
No. consecutive days in molecular-BV	2.3	1.8–2.9	2.1	1.7–2.7	12.2	10.2–14.4
No. consecutive days out of molecular-BV	19.6	14.9–25.3	9.2	7.3–11.8	3.2	2.7–3.8

*Participants were grouped into community class according to their main CST over the course of the study, determined through hierarchical clustering on CSTs. LC: mostly CST I; LG: mostly CST II; LI: mostly CST III; DA: mostly CST IV-A; DB: mostly CST IV-B, DC: mostly CST IV-C.

TABLE 3. Number of Observations for Each Community State Type (CST) and Each Time-Dependent Variable by Community Class in the HMP-UMB Study in Birmingham, AL (N = 100)

	LC/LG/LJ*		LI*		DA/DB/DC*	
	n	%	n	%	n	%
CST						
I, II, V	1189/1431	83	86/1042	8	155/2305	7
III	81/1431	6	742/1042	71	315/2305	14
IV-A, IV-B, IV-C	161/1431	11	214/1042	21	1835/2305	80
Time-varying covariates						
Menstruations	240/1431	17	184/1042	18	518/2305	22
Rectal sex	3/1420	0	6/1042	1	32/2264	1
Douching	4/1418	0	4/1042	0	19/2263	1
Lubricant use	2/1400	0	25/1028	2	41/2247	2
Condom use	17/1420	1	48/1041	5	82/2267	4
Digital sex	39/1419	3	86/1040	8	111/2264	5
Oral sex	42/1420	3	57/1042	5	86/2265	4
Thong use	120/1415	8	243/1041	23	172/2260	8
Condomless vaginal sex	89/1420	6	93/1042	9	210/2262	9

*Participants were grouped into community class according to their main CST over the first 14 days of the study, determined through hierarchical clustering on CSTs. LC: mostly CST I; LG: mostly CST II; LI: mostly CST III; DA: mostly CST IV-A; DB: mostly CST IV-B, DC: mostly CST IV-C.

Restricted Analysis: Factors Associated With Incidence and Spontaneous Clearance of Molecular-BV Among Participants From the *L. iners*–Dominated Community Class LI

We hypothesized that participants from the *L. iners*–dominated (LI) community class may have a microbiota that was more easily disrupted by behavioral factors than participants from other community classes, thus explaining why LI community class tends to have less stable patterns. We therefore restricted analysis to LI participants and found that there were 85 molecular-BV incidence events (for 722 nonincidence events) and 90 molecular-BV clearance events (for 122 nonclearance events; Table 2). In LI participants (Table S4, http://links.lww.com/OLQ/A839, and Fig. 3), the only factor significantly associated with incidence of molecular-BV was menstruations in the prior 24 hours and in the prior 48 hours, as was also indicated in the analysis of the whole cohort previously.

Concerning molecular-BV clearance in LI participants, several factors were associated with a lower probability of clearing molecular-BV, including African American participants (aHR, 0.44 [0.26–0.75]) compared with White participants, older participants compared with those aged 18 to 29 years (ages, 40–49 and 30–39 years (aHR, 0.38 [0.23–0.61] and 0.48 [0.28–0.83], respectively), and douching in the prior 24, 48, or 72 hours (aHR, 0.45 [0.28–0.73]).

DISCUSSION

Main Findings

Epidemiologic studies have indicated that race, older age, recent new sex partner, lifetime number of partners, vaginal

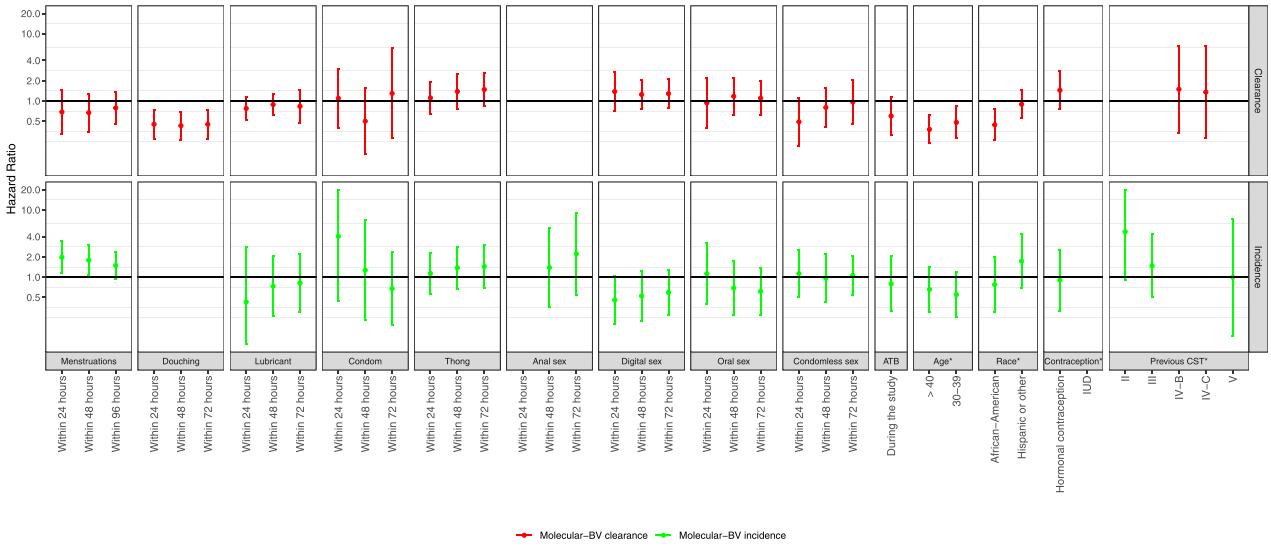

Figure 3. Time-varying and time-independent factors associated with incidence and clearance of molecular-BV (CST IV) in bivariate analyses, in 23 participants from community class LI (*L. iners*–dominated trajectory over time), from the HMP-UMB study in Birmingham, AL. Analyses were adjusted for antibiotic treatment for BV in participants from the moment they took the antibiotic until the end of the study. ATB, antibiotics for BV. *Reference categories for age, race, contraception, and previous CST were "18–29 years old," "White," "Non-hormonal," "CST I" (for incidence), and "CST IV-A" (for clearance).

douching, and smoking are among the most significant risk factors for incident BV, whereas hormonal contraception and condom use are largely considered protective.[32s–35s] However, most studies have approached this topic by evaluating time-varying factors with weeks or months between sampling and surveys. We sought to assess time-varying exposures on a frequent basis with their immediate effect on vaginal microbiota dynamics with daily diaries and samples collected daily or every 2 days. We confirmed that menstruation was statistically associated with incident molecular-BV and found that having a CST III (*L. iners* dominated) at the previous sample increased the rate of molecular-BV incidence. In addition, this study revealed that participants most often in a CST III state (LI community class) exhibited less stable patterns compared with other participants.

A focus of this analysis was also to assess the factors associated with spontaneous BV clearance, that is, transitioning to a *Lactobacillus*-dominated state regardless of antibiotic use for BV, a topic that has been understudied. In a study that followed participants every 4 to 8 weeks, Taha et al.[36s] reported *Trichomonas vaginalis* detection and multiple sex partners were associated with reduced BV clearance, whereas a pH less than 4.5 was associated with BV clearance, in a nontreated cohort. Others have approached the topic in the context of BV chronicity and patterns of recurrence.[37s,38s] In our analysis, among all participants (whatever the vaginal microbiota profiles), no factors were associated with molecular-BV spontaneous clearance, except for thong undergarment. However, in participants with a *L. iners*–dominated CST III longitudinal profile (community class LI, which demonstrate more unstable patterns), we revealed that specific risk factors were associated with lower molecular-BV clearance rates, such as African American race, age greater than 30 years, and douching. No factor was associated with improved clearance rates in *L. iners*–dominated individuals.

Finally, we used molecular characterization of the vaginal microbiota to demonstrate that molecular-BV is usually a short-lived state (lasting between 2 and 3 days for participants who present a vaginal microbiota usually dominated by *Lactobacillus* species) or an enduring pattern (12.2 days on average) in which participants had short-lived transitions to other *Lactobacillus*-dominated CSTs.

Interpretation

Menstruation is an important factor for incident molecular-BV in the whole cohort and also in the analysis restricted to participants from the LI community class. This result confirms previous studies describing a decrease of *L. crispatus* and *L. gasseri* during menstruations and a concomitant increase of *G. vaginalis* or other bacterial anaerobes.[19,31s,39s] General antibiotic use and antibiotic use for BV treatment were reported throughout the study, likely affecting the vaginal microbiota composition and dynamics. We tested the association between antibiotic use for BV from the day it was taken until the end of the study and incidence/clearance of molecular-BV in a univariate analysis and controlled for this exposure when modeling the impact of other factors (bivariate analyses). Previous studies showed that, among patients treated for BV with antibiotics, there are initial declines in the abundances and proportions of BV-associated bacteria and a rapid expansion of lactobacilli, usually *L. iners*[19,27,40s]; however, patients often quickly relapse to molecular-BV.[27] In our study, having been treated with an antibiotic during the study was not associated with molecular-BV incidence or clearance; therefore, the effect of the antibiotics taken by participants, if any, does not seem to affect transition rates in and out of molecular-BV. Interestingly, sexual practices were not associated with any type of transition, suggesting a limited contribution to vaginal microbiota dynamics. Additional studies on factors associated with BV incidence and clearance are needed to inform how sexual practices, which are intertwined with issues such as sexual transmission of bacteria, partner concordance, or behavior substitution, affect the vaginal microbiota.

Interestingly, among the whole cohort, the CST of the previous sample was an important risk factor, with a CST III (*L. iners* dominated) increasing the likelihood of a transition to molecular-BV. Because *L. iners*–dominated communities exhibited highly fluctuating patterns suggesting low resiliency,[41s] we evaluated separately the factors associated with transitions in LI individuals. This restricted analysis is consistent with prior work demonstrating that *L. iners*–dominated CST III is associated with *Chlamydia trachomatis* infection,[11,42s] and that *L. iners* displays different properties than other *Lactobacillus* species, in particular an inability to produce D-lactic acid.[12] With this analysis, it is not yet possible to know if *L. iners*–dominated vaginal microbiota is detrimental in itself or if it is associated with negative outcomes because it fluctuates more easily in and out of CST IV. In any case, our analysis suggested that individuals with *L. iners*–dominated communities had specific risk factors, in particular regarding the persistence of a molecular-BV state (defined as an absence of spontaneous clearance of molecular-BV in our time-to-event analysis). Among these specific risk factors, douching was associated with lower rates of molecular-BV clearance events in participants from the LI community class. There are few data available on the effect of douching on BV clearance, although both Onderdonk et al.[43s] and Pavlova et al.[44s] demonstrated with in vivo and in vitro studies, respectively, reductions in the abundance of bacteria after douching. Sabo et al.[45s] demonstrated in a US cohort a higher likelihood of detection of BV-associated bacteria among participants who reported vaginal washing (7 of 26 participants). However, Brown et al.[46s] recently found that douching cessation was not associated with major changes in vaginal microbiota in a pilot study of 34 participants.

Studying factors related to the temporal dynamics of the vaginal microbiota is challenging because rapid fluctuation between *Lactobacillus*-dominated CSTs and molecular-BV CST IV is common.[31s] The approach of assessing factors associated with molecular-BV incidence and clearance in a particular community class may serve as a starting point for future research to better understand the temporal profiles and resilience of certain communities of vaginal microbiota, because these factors may be different depending on a woman's community class. To our knowledge, this analysis is the first to report on factors associated with both incidence and spontaneous clearance of molecular-BV.

CONCLUSIONS

Most CST transitions identified in our study were short-lived. We highlighted distinct factors associated with molecular-BV incidence, such as menstruations and an *L. iners*–dominated CST III at the previous sample. Furthermore, participants' vaginal microbiota dynamics can be classified into community classes based on the most common CST over time. *Lactobacillus iners*–dominated participants had specific factors associated with a reduced clearance of molecular-BV, such as African American race, age 30 to 49 years, and douching, indicating that these individuals may have less resilient vaginal microbiota and may experience acute transitions in and out of molecular-BV. Defining vaginal microbiota profiles may aid future studies in identifying risk factors and responses to treatment, as resilience of stable versus fluctuating patterns may vary.

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