Characteristics associated with *Lactobacillus iners*-dominated vaginal microbiota

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Received 6 October 2020
Accepted 29 August 2021
Published Online First 8 September 2021

To cite: Novak J, Ravel J, Ma B, *et al*. *Sex Transm Infect* 2022;**98**:353–359.

ABSTRACT

Objective The protective role of *Lactobacillus iners* in the vaginal microbiota has been questioned. Recent studies have shown that *L. iners* is the dominating taxon in a large subset of women worldwide. The aim of this study was to identify sociodemographic, behavioural and clinical variables associated with *L. iners*-dominated community state type (CST) III in Brazilian women of reproductive age.

Participants and methods This study leveraged microbiota compositional data generated by sequencing of the V3-V4 16S rRNA gene from vaginal samples collected from 442 participants enrolled in a previous cross-sectional study that included 609 women in five geographical regions of Brazil. A total of 167 (27.4%) participants were excluded from the current study as they did not present a *Lactobacillus*-dominated vaginal microbiota. Data on sociodemographic and behavioural characteristics of the study population were obtained through face-to-face interviews. Participants were assigned to two study groups: those with *L. iners*-dominated CST III (n=222) and those with three distinct CSTs (I, II or V) dominated by another *Lactobacillus* spp. (n=220). Logistic regression analysis using a stepwise method was performed to test association between CST III and participants' characteristics, considering their OR and 95% CIs.

Results Among the population characteristics assessed, *L. iners*-dominated CST III was independently associated with having two or more sexual partners (OR 3.27; 95% CI 1.50 to 7.11) and microscopic detection of *Candida* sp. on vaginal smears (OR 2.24; 95% CI 1.02 to 4.89). Other characteristics were inversely associated with CST III, including condom use (OR 0.59; 95% CI 0.38 to 0.91), higher educational level (OR 0.61; 95% CI 0.41 to 0.91) and diet containing milk/dairy intake (OR 0.43; 95% CI 0.20 to 0.90).

Conclusion Unprotected sex practices, number of sexual partners and lower educational levels may be useful for identifying women with *L. iners*-dominated microbiota and its suboptimal protective properties. *L. iners* microbiota does not seem to provide optimal protection against *Candida* sp. colonisation, warranting further investigation.

INTRODUCTION

Previous studies have shown that there are different types of vaginal microbiota in women of reproductive age, and they differ in terms of species composition and dynamics.[1–3] At least five major types of vaginal microbiota or community state types (CSTs) have been identified.[1] Four of these CSTs are dominated by one of four species of *Lactobacillus* (CST I: *L. crispatus*; CST II: *L. gasseri*; CST III: *L. iners*, CST V: *L. jensenii*). Microbiota dominated by *Lactobacillus* species are characterised by a low vaginal pH (<4.5), which is driven by lactic acid produced by these microorganisms.[4] In contrast, CST IV is characterised by a paucity of *Lactobacillus* spp. and a diverse array of strict and facultative anaerobes such as *Gardnerella vaginalis*, *Atopobium vaginae* and *Prevotella*, among others.[1 3] Interestingly, CST IV microbiota is frequent in women who are asymptomatic and otherwise healthy, but reminiscent of those in women diagnosed with bacterial vaginosis (BV). They are also characterised by relatively high pH.[1] The Nugent score, a microscopic evaluation of bacterial morphotypes of a Gram stain vaginal smear, is routinely used to assess BV, referred to as Nugent-BV.[5] Nugent-BV is associated with an increased risk for the acquisition of several STIs, including the human papillomavirus (HPV) and human immunodeficiency virus (HIV).[6–8] Studies have shown that Nugent-BV is also associated with several population characteristics, such as ethnicity, sexual behaviour and certain contraceptive methods.[9–11] Nugent-BV is closely associated with CST IV, and as expected, they share similar associations with women's sociodemographic and behavioural characteristics.[12–14]

Interestingly, women with microscopically detected BV may also present with *L. iners*-dominated vaginal microbiota, often referred to as CST III.[3 14] The association between Nugent-BV and CST III might be driven by the polymorphic nature of *L. iners* which can present as small Gram-negative coccobacilli due to its thinner peptidoglycan layer when compared with other *Lactobacillus* spp.[15] Such microscopic features of *L. iners* are similar to *G. vaginalis* which contributes to Nugent score.[5] The protective role of *L. iners* in the vaginal environment has been considered as suboptimal, as it offers limited protection against *Chlamydia trachomatis*, HPV and HIV.[16–19] In addition, *L. iners*-dominated microbiota is associated with instability and frequently shifts to *Lactobacillus*-deprived CST IV and vice versa.[2 16–20] Of the four most frequent vaginal *Lactobacillus* spp., *L. iners* is unique. It has a smaller genome size,[21] does not produce hydrogen peroxide, nor D-lactic acid, producing only L-isomer of lactic acid,[22] has cytotoxic capabilities through the production of inerolysin, a pore-forming cholesterol-dependent cytolysin.[23 24]

Considering the high prevalence rates reported for *L. iners*-dominated CST III in many populations worldwide, including Brazil,[14] these observations are concerning. Thus, given the potential role for *L. iners* in vaginal microbiota instability and increase risk for STIs, we aimed to identify sociodemographic, behavioural and clinical characteristics in Brazilian women that are associated with *L. iners*-dominated CST III.

METHODS

Study design and population

The study leveraged data from a subset of participants from a prior study that aimed to characterise the vaginal microbiota of Brazilian woman of reproductive age and to identify the characteristics associated with *Lactobacillus*-depleted microbiota, that is, CST IV.[14] In the parent study, we cross-sectionally enrolled 609 participants from five geographical regions in Brazil (South, n=109; Southeast, n=140; Midwest, n=119; North, n=133; and Northeast, n=108), from 2012 to 2014.[14] These women were approached when attending primary healthcare clinics, except for in the South region where enrolment was performed through a referral hospital. Only women presenting for routine cervical cancer screening (Pap testing) were considered for enrolment.

Participants were enrolled if they were between 18 and 50 years old, were not pregnant and did not report use of intrauterine device, immunosuppressive therapy or current urinary tract infection. Samples were collected at a minimum of 5 days after the end of last menstrual period, at least 72 hours since last sexual intercourse. Participants did not report use of antimicrobial drugs in the 45 days prior to enrolment.

Data collection and sampling procedures

Data and biological sample collection were as previously described in the parent study.[14] Briefly, before the clinical examination, a face-to-face interview was administered using a structured questionnaire that comprised information regarding sociodemographic, behavioural characteristics and clinical history.[14]

After insertion of a non-lubricated speculum, two sterile swabs were rolled on the middle third of the vaginal wall. The first swab was stored in Amies liquid transport medium (Copan, Brescia, Italy) at −80°C for the characterisation of the vaginal microbiota composition. The other vaginal swab was smeared onto glass slides for Nugent scoring.[5] The same swab was used for KOH testing, adding two drops of KOH solution with results reported as positive, inconclusive or negative by the nurse/physician depending on the presence of volatile amines ('whiff test'). Assessment of vaginal pH was performed by applying a pH strip to the mid-vaginal wall for 1 min and comparing with the colour scale provided by the manufacturer (Merck, Darmstadt, Germany). Cervical brush samples were taken for *C. trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* testing by PCR according to methods previously described.[14]

Nugent scoring and microbiota analysis

Vaginal smears were Gram-stained and further classified according to Nugent *et al*. (1991)[5] The presence of presumptive *Candida* spp. morphotypes (pseudohyphae and/or hyphae) was recorded. The number of polymorphonuclear (PMN) inflammatory cells were counted in at least 20 fields for each smear, and results were expressed as average number of cells per field.

Total bacterial DNA was extracted from swabs stored in Amies transport medium using MoBio Powersoil Kit (MoBio Lab, Carlsbad, California, USA) according to previous published and validated procedure.[25 26] Microbiota characterisation was performed through the Microbiome Service Laboratory at the Institute for Genome Sciences, University of Maryland School of Medicine in Baltimore, Maryland, using the methodology developed by Fadrosh *et al*. (2014)[25] The V3-V4 regions of the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq platform (Illumina). Sequence analysis details were previously reported by Marconi *et al*. (2020)[14] Study samples were then clustered into CSTs based on their taxonomic composition, taxa relative abundances and Jensen-Shannon divergence metrics, according to methods previously described.[1 3] As previously detailed,[14] CST III was dominated by *L. iners* over other bacterial taxa, while the dominating taxa in CSTs I, II and V were, respectively, *L. crispatus*, *L. gasseri* and *L. jensenii*. For the analysis of this study, participants with *L. iners* CST III (n=222) were compared with those with non-*L. iners* dominated CSTs (CSTs I, II and V) (n=220).

Statistical analysis

Descriptive statistical analyses were performed for sociodemographic, behavioural and clinical variables between CST III and others *Lactobacillus* spp.-dominated CSTs using χ^2 and Mann-Whitney tests for categorical and continuous variables, respectively, with a p value <0.05 considered as statistically significant.

We performed univariate logistic regression analysis to test the association between CST III and participant characteristics. Crude and age-adjusted and region-adjusted OR were estimated, as well as the corresponding 95% CIs. A multivariable logistic regression analysis was also performed using a forward stepwise model (variables were retained at p value ≤0.15) with CST III set as dependent variable. All analyses were performed in Stata/SE, V.15.1 (Stata Corp, College Station, Texas, USA).

RESULTS

Sociodemographic and behavioural data of the 442 women included in the two study groups are displayed in table 1. For both groups, median age of participants was 34 years old and slightly more than half of them self-reported as having a black or other alike skin colour. The proportion of women with CST III with completed high school was lower (52.70%), when compared with other CSTs (66.36%) (p=0.003). Regarding the behavioural characteristics, nearly all (94.55%) women with CSTs I/II/V reported intake of milk and dairy products. Condom use as contraceptive method was reported by less women with CST III (29.28%) when compared with other CSTs (40.00%) (p=0.018). Participants who reported having two or more sexual partners in the years prior to enrolment were frequent in CST III (11.26%) when compared with other CSTs (5.00%) (p=0.009). In relation to the clinical characteristics displayed in table 2, frequency of Nugent-BV (12.16%), increased number of inflammatory cells (10.81%) and presence of *Candida* morphotypes (pseudohyphae and/or hyphae) (9.91%) on vaginal smears were superior in CST III when compared with other vaginal microbiota types (p<0.05). The overall prevalence rates for *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* were, respectively, 4.1% (n=18), 0.4% (n=2) and 0.2% (n=1). The prevalence of these infections was similar in the two study groups (table 2).

Crude logistic regression analysis (table 3) showed that CST III was over-represented among women enrolled in the northeast region (OR 1.98; 95% CI 1.09 to 3.60). Participants with CST III showed lower education level (OR 0.56; 95% CI 0.38 to 0.82), less consumption of milk and derivatives (OR 0.43; 95% CI

Table 1 Sociodemographic and behavioural variables grouped into CST I, II and V vs CST III

Characteristics	CST III (n=222)	CST I, II and V (n=220)	P value*
Sociodemographics			
Region			0.132
South	41 (18.47)	59 (26.82)	
Southeast	51 (22.97)	45 (20.45)	
Central	44 (19.82)	36 (16.36)	
Northeast	47 (21.17)	34 (15.45)	
North	39 (17.57)	46 (20.91)	
Residence			0.991
Urban	219 (98.65)	217 (98.64)	
Rural	3 (1.35)	3 (1.36)	
Age, median (range)	34 (18–51)	34 (18–50)	0.917
Age			0.773
18–35 years	113 (50.90)	115 (52.27)	
≥35 years	109 (50.90)	105 (47.73)	
Skin colour			0.793
Black and other†	117 (52.70)	112 (50.91)	
White	97 (43.70)	96 (43.64)	
Other	8 (3.60)	12 (5.45)	
Living with partner			0.532
No	82 (36.94)	75 (34.09)	
Yes	140 (63.06)	145 (65.91)	
Completed high school			**0.003**
No	105 (47.30)	74 (33.64)	
Yes	117 (52.70)	146 (66.36)	
Has personal income?			0.406
No	81 (36.49)	72 (32.73)	
Yes	141 (63.51)	148 (67.27)	
Behavioural			
Milk and dairy intake			**0.019**
No	26 (11.71)	12 (5.45)	
Yes	196 (88.29)	208 (94.55)	
Alcohol consumption			0.562
No	158 (71.17)	162 (73.64)	
Yes	64 (28.83)	58 (26.36)	
Smoking			0.546
No	198 (89.19)	200 (90.91)	
Yes	24 (10.81)	20 (9.09)	
Intimate soap, daily use			0.777
No	115 (51.80)	111 (50.45)	
Yes	107 (48.20)	109 (49.55)	
Douching			0.553
No	194 (87.39)	188 (85.45)	
Yes	28 (12.61)	32 (14.55)	
Sitz bathing			0.627
No	202 (90.99)	203 (92.27)	
Yes	20 (9.01)	17 (7.73)	
Daily use of pantyliners			0.447
No	215 (96.85)	210 (95.45)	
Yes	7 (3.15)	10 (4.55)	
Number of sexual partners‡			**0.009**
0	15 (6.76)	7 (3.18)	
1	182 (81.98)	202 (91.82)	
2+	25 (11.26)	11 (5.00)	
New sexual partner§			0.505
No	202 (90.99)	204 (92.73)	
Yes	20 (9.01)	16 (7.27)	
Number of sexual intercourses/ week			0.301
0	43 (19.37)	31 (14.10)	
1–2	102 (45.94)	112 (50.90)	
3+	77 (34.69)	77 (35.00)	
Hormonal contraceptive			0.181
No use	131 (59.01)	120 (54.55)	
Oral	68 (30.63)	84 (38.18)	
Injectable	23 (10.36)	16 (7.27)	
Condom use			**0.018**
No	157 (70.72)	132 (60.00)	
Yes	65 (29.28)	88 (40.00)	

Values with significance of P<0.05 are in bold.
*P<0.05 considered as statistically significant, continuous and categorical variables compared using, respectively, Mann-Whitney χ^2 tests.
†Another self-reported skin colour (ie, brown 'pardo').
‡Twelve months prior to enrolment.
§Two months prior to enrolment.
CST, community state type.

0.21 to 0.88) and lower rates of condom use (OR 0.62; 95% CI 0.41 to 0.92). Further, CST III was associated with having two or more sex partners in the past 12 months (OR 2.52; 95% CI 1.20 to 5.27). The age-adjusted and region-adjusted logistic regression analysis showed very similar associations than those obtained with the crude analysis, except that this model identified a positive association between CST III with report of prior episode of BV (OR 1.62; 95% CI 1.08 to 2.43).

Multivariable analysis (table 3) showed that CST III was independently associated with having two or more sex partners when compared with one sex partner (OR 3.27; 95% CI 1.50 to 7.11) and with the presence of *Candida* on vaginal smears (OR 2.24; 95% CI 1.02 to 4.89). Some sociodemographic and behavioural characteristics such as higher education level (OR 0.61; 95% CI 0.41 to 0.91), consumption of milk and derivatives (OR 0.43; 95% CI 0.20 to 0.90) and condom use (OR 0.59; 95% CI 0.38 to 0.91) were inversely associated with CST III vaginal microbiota.

DISCUSSION

Data from previous clinical and laboratory studies suggest that *L. iners*-dominated vaginal microbiota, often referred to as CST III, may not be optimal for women's reproductive health.[16–18] Understanding population characteristics associated with vaginal microbiota dominated by *L. iners* is thus critical for the management of adverse outcomes associated with this type of microbiota. In this study, we identified several characteristics associated with *L. iners*-dominated CST III in a cohort of Brazilian women. The proportion of women with CST III was lower among those who reported having a high school degree, consuming milk/dairy and using condom during sex, while CST III was significantly more frequent among participants with more than one sex partner in the year prior to enrolment. Interestingly, another factor associated with *L. iners*-dominated CST III was the presence of pathogenic forms of *Candida* spp. on vaginal smears by microscopic observation. However, we should consider that this is a cross-sectional study, and this study limitation does not allow to address any causal links between participants' characteristics and type of vaginal microbiota.

Table 2 Clinical variables grouped into CST I, II and V vs CST III

Characteristics	CST III (n=222)	CST I, II and V (n=220)	P value*
Clinical findings			
Body mass index†			0.923
Underweight/normal	115 (51.80)	110 (50.00)	
Overweight	68 (30.63)	69 (31.36)	
Obese	39 (17.57)	41 (18.64)	
Prior pregnancy			0.470
No	50 (22.52)	56 (25.45)	
Yes	172 (77.48)	164 (74.55)	
STD history‡			0.970
No	192 (86.49)	190 (86.36)	
Yes	30 (13.51)	30 (13.64)	
BV history‡			0.085
No	109 (49.10)	126 (57.27)	
Yes	113 (50.90)	94 (42.73)	
Dyspareunia			0.743
No	142 (63.96)	144 (65.45)	
Yes	80 (36.04)	76 (34.55)	
Abnormal discharge			0.280
No	125 (56.31)	135 (61.36)	
Yes	97 (43.69)	85 (38.64)	
Pruritus			0.213
No	181 (81.53)	189 (85.91)	
Yes	41 (18.47)	31 (14.09)	
Phase cycle			0.340
Luteal	95 (42.79)	102 (46.37)	
Follicular	108 (48.65)	93 (42.27)	
Suppressed	19 (8.56)	25 (11.36)	
Suggestive of cervicitis§			0.140
No	205 (92.34)	194 (88.18)	
Yes	17 (7.66)	26 (11.82)	
Vaginal pH			0.281
<4.5	124 (55.86)	134 (60.91)	
≥4.5	98 (44.14)	86 (39.09)	
KOH test			0.179
Negative	151 (68.02)	166 (75.45)	
Inconclusive	25 (11.26)	16 (7.27)	
Positive	46 (20.72)	38 (17.27)	
Nugent microscopic categories			**0.001**
Normal (scores 0–3)	160 (72.07)	192 (87.27)	
Intermediate (scores 4–6)	35 (15.77)	14 (6.36)	
BV (scores 7–10)	27 (12.16)	14 (6.36)	
Microscopic *Candida*-positivity¶			**0.050**
No	200 (90.09)	209 (95.00)	
Yes	22 (9.91)	11 (5.00)	
Inflammatory PMN cells (≥5 per field)			**0.007**
No	198 (89.19)	211 (95.91)	
Yes	24 (10.81)	9 (4.09)	
CT infection			0.984
No	213 (95.94)	211 (95.90)	
Yes	9 (4.06)	9 (4.10)	
NG infection			0.158
No	220 (99.10)	220 (100.00)	
Yes	2 (0.90)	0 (0.00)	
TV infection			0.315
No	222 (100.00)	219 (99.55)	
Yes	0 (0.00)	1 (0.45)	

*P<0.05 considered as statistically significant, continuous and categorical variables compared using, respectively, Mann-Whitney χ^2 tests.
†Categorised according to the WHO into underweight/normal (<25.0 unit), overweight (25.0–29.9) and obese (≥30.0).
‡Self-reported but not cross-checked in participants' medical records.
§Mucopurulent secretions and/or bleeding cervix.
¶Presence of pseudohyphae and/or hyphae.
BV, bacterial vaginosis; CT, *Chlamydia trachomatis*; KOH, potassium hydroxide; NG, *Neisseria gonorrhoeae*; PMN, polymorphonuclear; TV, *Trichomonas vaginalis*.

The current findings demonstrated a novel association between a CST III microbiota and the lower scholar degree, that is, participants who did not complete high school. Education is one of the main socioeconomic indicators and is well acknowledged as predisposing to several STIs.[27] Other studies have shown that STI acquisition is increased in the presence of vaginal microbiota associated with BV, which, combined with our results, indicates an association between socioeconomic characteristics and the composition of the microbiota.[8–10] Because *L. iners*-dominated CST III have been associated with shifts to *Lactobacillus*-deprived CST IV types and vice versa,[3 16 20] these two types of microbiota are not completely independent, and it is not surprising that we also identified lower education as a factor associated with CST III vaginal microbiota.

Similarly, sexual behaviour was shown to be significantly associated with CST III. This factor is also associated with BV including high number of sexual partners and inconsistent condom use.[28] Current data showed the association between having two or more sex of partners in the previous year with CST III, in relation to one sex partner that was used as reference category due most of women included in this study referred having one sex partner. Although only 8.1% of the study participants reported having two or more sex partner, current observation agrees with previous study that reported increased prevalence of *L. iners* among African women with two or more sexual partners within 3 months prior to enrolment.[12] Regarding the use of condom, our data are in agreement with findings by Vodstrcil *et al.* (2017) who showed that unprotected sex is associated with vaginal microbiota dominated by *L. iners* or BV-associated *G. vaginalis*.[29] Interestingly, *L. crispatus*-dominated CST I was significantly associated with reporting consistent condom use.[30] Despite the significant associations reported herewith, our understanding of the role of a woman's sexual behaviour on the composition of the vaginal microbiota is not complete and should be explored further. A study limitation was that sexual behaviours were assessed through face-to-face interviews, and due to the sensitive nature of the questions, some participants may have omitted some information. This might explain the lack of association between CST III and other behaviours such as new sex partner and the frequency of sexual intercourse, which have been previously shown to be associated with CST IV.[14]

Studying diet and the vaginal microbiota is emerging, as diet is a modifiable factor that is thought to modulate the composition of the vaginal microbiota.[31 32] Here we found an inverse association between milk and dairy intake and *L. iners*-dominated microbiota. Recently, our study group showed inverse association between milk and dairy intake with *Lactobacillus*-depleted

Table 3 ORs and 95% CIs for the association between sociodemographic and behavioural factors *Lactobacillus iners*-dominated vaginal microbiota (community state type III)

	Crude	Age-adjusted and region- adjusted	Multivariable
Region			
South	1.00	1.00	–
Southeast	1.63 (0.92 to 2.87)	1.62 (0.92 to 2.86)	
Central	1.75 (0.97 to 3.18)	1.76 (0.97 to 3.19)	
Northeast	1.98 (1.09 to 3.60)	1.98 (1.09 to 3.60)	
North	1.22 (0.68 to 2.18)	1.22 (0.68 to 2.18)	
Age			
<35 years	1.00	1.00	–
≥35 years	1.05 (0.72 to 1.53)	1.31 (0.66 to 2.63)	
Skin colour			
Black and other*	1.00	1.00	–
White	0.96 (0.65 to 1.41)	1.09 (0.71 to 1.69)	
Other	0.63 (0.25 to 1.61)	0.58 (0.22 to 1.50)	
Living with partner			
No	1.00	1.00	–
Yes	0.88 (0.59 to 1.30)	0.85 (0.56 to 1.29)	
Completed high school			
No	1.00	1.00	1.00
Yes	0.56 (0.38 to 0.82)	0.58 (0.39 to 0.87)	0.61 (0.41 to 0.91)
Has personal income			
No	1.00	1.00	–
Yes	0.84 (0.57 to 1.25)	0.87 (0.58 to 1.31)	
Milk and dairy intake			
No	1.00	1.00	1.00
Yes	0.43 (0.21 to 0.88)	0.46 (0.22 to 0.97)	0.43 (0.20 to 0.90)
Alcohol consumption			
No	1.00	1.00	–
Yes	1.13 (0.74 to 1.71)	1.23 (0.80 to 1.90)	
Smoking			
No	1.00	1.00	–
Yes	1.21 (0.64 to 2.26)	1.18 (0.62 to 2.24)	
Intimate soap daily use			
No	1.00	1.00	–
Yes	1.08 (0.70 to 1.65)	1.03 (0.69 to 1.52)	
Douching			
No	1.00	1.00	–
Yes	0.84 (0.49 to 1.46)	0.89 (0.51 to 1.55)	
Sitz bathing			
No	1.00	1.00	–
Yes	1.18 (0.60 to 2.32)	1.32 (0.62 to 2.80)	
Daily pantyliner use			
No	1.00	1.00	–
Yes	0.68 (0.25 to 1.82)	0.92 (0.32 to 2.62)	
Number of sexual partners†			
0	2.37 (0.94 to 5.96)	2.51 (0.98 to 6.39)	2.34 (0.90 to 6.07)
1	1.00	1.00	1.00
2+	2.52 (1.20 to 5.27)	2.58 (1.21 to 5.49)	3.27 (1.50 to 7.11)
New sexual partner‡			
No	1.00	1.00	–
Yes	1.26 (0.63 to 2.50)	1.42 (0.69 to 2.91)	
Number of sexual intercourses/week			
0	1.52 (0.89 to 2.59)	1.65 (0.95 to 2.85)	–
1–2	1.00	1.00	
3 +	1.09 (0.72 to 1.66)	1.04 (0.68 to 1.59)	
Hormonal contraceptive			
No use	1.00	1.00	–
Oral	0.74 (0.49 to 1.11)	0.74 (0.47 to 1.15)	
Injectable	1.31 (0.66 to 2.61)	1.20 (0.59 to 2.44)	
Condom use			
No	1.00	1.00	1.00
Yes	0.62 (0.41 to 0.92)	0.59 (0.39 to 0.90)	0.59 (0.38 to 0.91)
Body mass index§			
Underweight/normal	1.00	1.00	–
Overweight	0.94 (0.61 to 1.44)	0.91 (0.59 to 1.42)	
Obese	0.90 (0.54 to 1.51)	0.82 (0.48 to 1.41)	
Phase cycle			
Luteal	1.24 (0.84 to 1.84)	1.31 (0.88 to 1.96)	–
Follicular	0.81 (0.42 to 1.57)	0.84 (0.42 to 1.64)	
STD history¶			
No	1.00	1.00	
Yes	0.98 (0.57 to 1.70)	1.02 (0.59 to 1.78)	–
BV history¶			
No	1.00	1.00	1.00
Yes	1.38 (0.95 to 2.02)	1.62 (1.08 to 2.43)	1.45 (0.98 to 2.15)
Microscopic *Candida*-positivity**			
No	1.00	1.00	1.00
Yes	2.09 (0.98 to 4.42)	1.98 (0.92 to 4.25)	2.24 (1.02 to 4.89)
Suggestive of cervical infection††			
No	1.00	1.00	
Yes	1.09 (0.45 to 2.63)	1.09 (0.44 to 2.68)	–

*Another self-reported skin colour (ie, brown 'pardo').
†Twelve months prior to enrolment.
‡Two months prior to enrolment.
§Categorised according to the WHO into underweight/normal (<25.0 units), overweight (25.0–29.9) and obese (≥30.0).
¶Self-reported.
**Presence of pseudohyphae and/or hyphae.
††Infection by *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and/or *Trichomonas vaginalis* detected by PCR.
BV, bacterial vaginosis.

CST IV.[14] Other studies have shown that BV is associated with a low intake of vitamins and minerals often present in milk such as vitamin D, vitamin E and calcium,[33 34] but no study has investigated these factors and the composition of the vaginal microbiota. It is well acknowledged that the composition of the gut microbiota is impacted by diets.[35] Although a few studies point to an association between vaginal microbiota and host's diet, this topic has not been as investigated as the relation between diet and gut microbiome. One of the few studies available was

performed by Laue *et al*. (2018) and showed that the intake of a probiotics-containing yoghurt improved the remission rate and symptoms of BV.[36] Another study using a mouse model showed that a *Saccharomyces cerevisiae*-based probiotic decreased vaginal loads of *G. vaginalis*.[37] Thus, the impact of diet on vaginal microbiota suggests a potential association between its components with those colonising the gut. However, the underlying mechanisms of the association between vaginal and gut microbiota are poorly understood and should be investigated in detail by further studies. In fact, we are aware of this study limitation, as it fails to provide more detailed information regarding milk/dairy intake such as quantities and type of products more frequently consumed (yoghurt, cheese, fermented milk) by the study population.

Regarding the clinical characteristics, the association between microbiota dominated by *L. iners* and increased number of inflammatory cells on vaginal smears had not been reported previously. Thus, we have hypothesised that increased PMN cells in CST III could be due to a concurrent cervical infection. However, we have not observed any association between CST III and infections caused by *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* infections in the present study. Recently, van Houdt *et al*. (2018) showed that women with *L. iners*-dominated microbiota have an increased susceptibility to *C. trachomatis* infection,[18] which could be due to the incapability of D-lactic acid production by *L. iners*.[19] However, we should consider *L. iners* produces the L-isomer of lactic acid, which may also play a protective role against infection.[38] We recognise that the increased PMN cells on vaginal smears could also be due to the presence of other agents of cervical infection (eg, *Mycoplasma genitalium*) or other vaginal colonisers that were not addressed by this study. However, because of the low frequency of cervicitis in the population and the association shown between CST III and *Candida* spp. morphotypes on vaginal smears, we find it reasonable to attribute to the latter agents the most probable cause of the increased number of PMN cells in CST III.

In fact, a study conducted in three African countries demonstrated a higher prevalence of vaginal candidiasis, diagnosed by wet mount microscopy, in *L. iners*-dominated vaginal microbiota, although this finding was not statistically significant.[13] Recently, Tortelli *et al*. (2019) showed that *L. iners*-dominated microbiota were more likely to test positive for *Candida* sp. using quantitative real-time PCR than *L. crispatus*-dominated community.[39] On the other hand, a study by Brown *et al*. (2019) showed that CST dominated by *L. iners* had lower number of samples with *C. albicans* detected by PCR, while CST dominated by *L. crispatus* were most likely to present *C. albicans*.[40] It is worth mentioning that PCR detects presence of *Candida* in minimum amounts, while microscopy is less sensitive. Thus, we hypothesised that *L. crispatus* could allow the colonisation by *Candida* sp. but might inhibit its growth and development. Alternatively, *L. iners* may not be as protective and could allow for the progression of *Candida* infection, thus explaining the presence of higher number of inflammatory PMN cells on vaginal smears of CST III. This hypothesis is supported by the study of Tortelli *et al*. (2019) that showed an increased in vitro inhibition of *C. albicans* growth by *L. crispatus* when compared with *L. iners*.[39]

CONCLUSION

L. iners-dominated CST III is associated with behavioural and socioeconomic characteristics (number of sexual partners, regular use of condoms and education level) that could be easily assessed in gynaecology clinics. Microscopic observation of *Candida* morphotypes on vaginal smears may also be helpful for identifying women with CST III. Considering that the protective role of *L. iners* has been questioned, the identification of women presenting this type of microbiota might be of clinical interest. However, there is no therapeutic option that target *L. iners*, and thus the information is not actionable currently. Further studies are necessary to identify novel strategies to modulate the vaginal microbiota for maintenance of a protective and beneficial environment to women's reproductive health.

Key messages

⇒ Dominance of *Lactobacillus iners* in the vaginal microbiota is associated with low dairy intake and sex behaviour.
⇒ *L. iners* may offer a suboptimal protection against vaginal colonisation by *Candida* sp.
⇒ Easily available sociodemographic/behavioural characteristics may help in identifying women with a potentially more vulnerable state of the vaginal microbiota.

Handling editor Francesca Ceccherini-Silberstein

Contributors CM and MGS designed the study. CSTF, ART and CM lead the clinical study and biological sample collection. JN, CM and CSTF performed laboratory analysis. JR and BM obtained and processed sequencing data. JN and CM conducted the statistical analysis and interpreted the data. JN drafted the manuscript under the supervision of CM and MGS. JR and AdRT edited the manuscript. All authors reviewed and approved the submitted version of the manuscript.

Funding This study has received funding by developmental funds granted by São Paulo Research Foundation – FAPESP (grant number 2012/16800-3) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil – CAPES (Master's scholarship number 1680048).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was reviewed and approved by the Ethics Committee of Botucatu Medical School (approval numbers: 3.094.514 and 294.202). After being informed of the aims and procedures of the study, all participants signed a written informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available. Not available.

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Introdução: O papel protetor do *Lactobacillus iners* para a microbiota vaginal tem sido questionado e a microbiota dominada por esta espécie apresenta alta taxa de prevalência ao redor do mundo. Neste sentido, o objetivo deste estudo foi identificar variáveis sociodemográficas, comportamentais e clínicas associadas a comunidade com predomínio de *L. iners* (CST III) em mulheres brasileiras de idade reprodutiva.

Participantes e métodos: Este estudo utilizou os dados de composição da microbiota gerados pelo sequenciamento das regiões V3-V4 do gene rRNA 16S de amostras vaginais coletadas de 442 participantes inscritas em um estudo transversal anterior que incluiu 609 mulheres em cinco regiões geográficas do Brasil. Participantes que não apresentaram microbiota dominada por *Lactobacillus sp.* (n=167, 27,4%) não foram incluídas nas análises estatísticas do presente estudo. Os dados sobre as características sociodemográficas e comportamentais da população estudada foram obtidos por meio de entrevistas presenciais. As participantes foram divididos em dois grupos de estudo: aquelas com predomínio de *L. iners*, CST III, (n=222) e aquelas predomínio de outras espécies de *Lactobacillus*, CSTs I, II e V, (n=220). Uma análise de regressão logística pelo método *stepwise* foi realizada para testar a associação entre a CST III e as características dos participantes, considerando odds ratios (OR) e intervalos de confiança de 95% (IC 95%).

Resultados: Entre as características populacionais avaliadas, CST III foi independentemente associada a ter dois ou mais parceiros sexuais (OR: 3,27; IC 95%: 1,50-7,11) e detecção microscópica de *Candida sp*. em esfregaços vaginais (OR: 2,24; IC 95%: 1,02-4,89). Outras características foram inversamente associadas com CST III, incluindo uso de preservativo (OR: 0,59; IC 95%: 0,38-0,91), maior nível de escolaridade (OR: 0,61; IC 95%: 0,41-0,91) e dieta contendo leite ou derivados (OR: 0,43; IC 95%: 0,20-0,90).

Conclusão: Práticas sexuais desprotegidas, número de parceiros sexuais e níveis educacionais mais baixos podem ser úteis para identificar mulheres com microbiota dominada por *L. iners*. A microbiota com predomínio de *L. iners* parece não fornecer proteção ideal contra a colonização por *Candida sp*., justificando uma investigação mais aprofundada.