Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP
Faculdade de Medicina de Botucatu

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Estudo da associação entre o microbioma vaginal com variáveis sociodemográficas e de hábitos comportamentais de mulheres brasileiras em idade reprodutiva

Dissertação apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, para obtenção do título de Mestre em Patologia.

Orientadora: Profa. Dra. Camila Marconi
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Palavras-chave: Lactobacillus iners; Microbioma vaginal; infecções sexualmente transmissíveis; sequenciamento de nova geração; vaginose bacteriana.

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“Aqueles que passam por nós, não vão sós, não nos deixam sós. Deixam um pouco de si, levam um pouco de nós”. Antoine de Saint-Exupéry

SUMÁRIO

1. RESUMO

A microbiota vaginal normal é composta predominantemente por *Lactobacillus* spp. que conferem proteção contra infecções por patógenos, por meio da produção de ácido lático, peróxido de hidrogênio e bacteriocinas. Diferentemente, a vaginose bacteriana (VB) é caracterizada pela substituição da microbiota de *Lactobacillus* spp. por bactérias anaeróbias em sua maioria. A VB é a alteração de microbiota vaginal mais comum em mulheres de idade reprodutiva, acometendo aproximadamente 30% dessa população. Além disso, a VB é fator de risco para aquisição de infecções sexualmente transmissíveis (IST). Diversas características da população já foram associadas à VB, como idade, etnia, comportamentos sexual e de higiene. Entretanto, a real composição da microbiota vaginal só foi possível em 2011 com estudo utilizando o sequenciamento de nova geração do gene bacteriano RNA ribossômico 16S. Foi demonstrado que o microbioma vaginal pode ser classificado em cinco tipos de comunidades bacterianas (community-state types, CST). Quatro dessas CSTs tem predomínio de *Lactobacillus*: *L. crispatus* (CSTI), *L. gasseri* (CST II), *L. iners* (CST III) e *L. jensenii* (CST V), enquanto que a CST IV apresenta grande diversidade bacteriana e engloba a maioria dos casos de VB. Apesar de quatro CSTs apresentarem predomínio de *Lactobacillus*, o papel protetor da CST III, dominada por *L. iners,* contra aquisição de IST tem se demonstrado menor que os demais. Embora os estudos de microbioma tenham possibilitado conhecer melhor a relação entre as espécies bacterianas com as IST, não existe na literatura informações acerca dos fatores sociodemográficos, comportamentais e clínicos das mulheres associados às CSTs. Desta forma, o objetivo desse estudo foi identificar as características sociodemográficas, comportamentais e clínicas da população associadas à CST III. Incluímos neste estudo transversal 609 mulheres brasileiras, que buscaram unidades de saúde para realização do exame preventivo do câncer de colo do útero. As participantes responderam a um questionário estruturado para obtenção de dados sociodemográficos, comportamentais e clínicos. Para caracterização do microbioma vaginal, amostras vaginais foram submetidas ao sequenciamento das regiões V3-V4 do RNA ribossômico16S em plataforma Miseq (Illumina, San Diego, CA). Um modelo de

regressão logística utilizando o método *stepwise* foi utilizado para testar a associação entre o microbioma dominado por *L. iners* com todas as variáveis avaliadas, considerando suas razões de chances (OR) e intervalos de confiança de 95% (IC95%) em *software* Stata (Stata Corp, College station, TX). A maior prevalência foi de CST III foi de 36,5% (n = 222), das demais CST foram: CST I, 30,5% (n=186), CST II 4,4% (n = 27) e CST V 1,2% (n = 7). As demais 167 (27,4%) participantes apresentaram CST IV e não foram considerados na análise. A análise multivariada mostrou que as participantes que relataram ter dois ou mais parceiros sexuais no ano anterior ao estudo apresentaram um risco aumentado para CST III (OR: 3,27; IC 95% 1,50-7,11), bem como o achado microscópico de *Candida* spp. em esfregaços vaginais (OR: 2,24; IC 95% 1,02-4,89). Além disso, três fatores foram protetores para esse tipo de microbioma: uso de preservativo (OR: 0,59; IC 95% 0,38-0,91), ensino médio completo (OR: 0,61; IC 95% 0,41-0,91) e consumo diário de leite ou derivados (OR: 0,43; IC 95% 0,20-0,90). Dessa forma, concluímos que o microbioma vaginal dominado por *L. iners* é independentemente associado a características da população anteriormente associadas à VB como baixa escolaridade, múltiplos parceiros sexuais e sexo desprotegido.

Palavras-chave: Microbioma vaginal; *Lactobacillus iners*; Infecções sexualmente transmissíveis; Vaginose bacteriana; Sequenciamento de nova geração.

2. ABSTRACT

The normal vaginal microbiota is predominantly composed of *Lactobacillus* spp. which confer protection against pathogen infections through the production of lactic acid, hydrogen peroxide and bacteriocins. Differently, bacterial vaginosis (BV) is characterized by the replacement of the microbiota of *Lactobacillus* spp. by anaerobic bacteria for the most part. BV is the most common vaginal microbiota alteration in women of reproductive age, affecting approximately 30% of this population. In addition, BV is a risk factor for the acquisition of sexually transmitted infections (STIs). Several characteristics of the population have already been associated with BV, such as age, ethnicity, sexual and hygiene behaviors. However, the actual composition of the vaginal microbiota was only possible in 2011 with study using the new generation sequencing of the bacterial 16S ribosomal RNA gene. It has been shown that the vaginal microbiome can be classified into five types of community-state types (CST). Four of these CSTs have a predominance of *Lactobacillus*: L. *crispatus* (CSTI), L. *gasseri* (CST II), *L. iners* (CST III) and *L. jensenii* (CST V), while CST IV shows great bacterial diversity and involve most cases of BV. Although four CSTs have a predominance of *Lactobacillus*, the protective role of CST III, dominated by *L. iners*, against IST acquisition has been shown to be lower than the others are. Although microbiome studies have made it possible to know better the relationship between bacterial species and STIs, there is no information in the literature about the socio-demographic, behavioral and clinical factors of women associated with STIs. Thus, the objective of this study was to identify the sociodemographic, behavioral and clinical characteristics of the population associated with CST III. We included in this cross-sectional study 609 Brazilian women, who sought health units for the cervical cancer screening. Participants answered a structured questionnaire to obtain sociodemographic, behavioral and clinical data. To characterize the vaginal microbiome, vaginal samples were submitted to the sequencing of the V3-V4 regions of 16S ribosomal RNA in Miseq platform (Illumina, San Diego, CA). A logistic regression model using the stepwise method was used to test the association between the *L. iners* dominated microbiome with all the variables evaluated, considering its odds ratios (OR) and 95% confidence intervals (95% CI)

in software Stata (Stata Corp., College Station, TX). The highest prevalence of CST III was 36.5% (n = 222); the other CSTs were CST I, 30.5% (n = 186), CST II 4.4% (n = 27) and CST V 1.2% (n = 7). The remaining 167 (27.4%) participants presented CST IV and were not considered in the analysis. Multivariate analysis showed that participants who reported having two or more sex partners in the year prior to the study had an increased risk for CST III (OR: 3.27; 95% CI 1.50-7.11), as well as the finding microscopic study of Candida spp. in vaginal smears (OR: 2.24; 95% CI 1.02-4.89). In addition, three factors were protective for this type of microbiome: condom use (OR: 0.59, 95% CI 0.38-0.91), complete high school (OR: 0.61, 95% CI, 41-0.91) and daily consumption of milk or derivatives (OR: 0.43; 95% CI 0.20-0.90). Thus, we conclude that the vaginal microbiome dominated by *L. iners* is independently associated with characteristics of the population previously associated with BV such as low education level, multiple sexual partners and unprotected sex.

Keywords: Vaginal microbiome; *Lactobacillus iners*; Sexually transmitted infections; Bacterial vaginosis; Next generation sequencing.

Revisão de Literatura

2. REVISÃO DE LITERATURA

A microbiota vaginal foi descrita pela primeira vez em 1892 por Albert Döderleine, já neste momento, foi considerada como saudável quando encontrada a predominância de espécies do gênero *Lactobacillus*[1]. Ao longo dos anos, a literatura constantemente tem confirmado a importância da colonização por tais microrganismos[2]. Espécies de *Lactobacillus* são responsáveis pela manutenção do ambiente vaginal equilibrado, visto que conferem defesa contra patógenos[3]. Dentre as linhagens de *Lactobacillus* spp., grande parte é capaz de liberar peróxido de hidrogênio e bacteriocinas, conferindo importante capacidade antimicrobiana[4,5]. Outro importante fator se dá pela capacidade que os *Lactobacillus* spp. possuem de produzir ácido lático utilizando o metabolismo do glicogênio armazenado nas células epiteliais vaginais sob a influência do estrogênio do hospedeiro, dessa forma esses microrganismos contribuem para a manutenção do pH vaginal normal ácido, que também é considerado importante fator na manutenção da microbiota vaginal normal[6,7]. Além disso, ao romper membranas celulares bacterianas o ácido lático estimula a imunidade do hospedeiro na presença de lipopolissacarídeo presente na parede bacteriana de algumas espécies[8]. Curiosamente, há uma pequena porcentagem de mulheres que podem apresentar microbiota vaginal diversa com pH local ácido, composta por espécies também produtoras de ácido lático[9,10], embora os métodos utilizados para identificação bacteriana nesses casos sejam atualmente questionáveis.

Devido à capacidade protetora das diferentes espécies de *Lactobacillus,* vários trabalhos tiveram por objetivo realizar avaliação individual de cada espécie. Um dos primeiros estudos, ao avaliar a capacidade de produção de peróxido de hidrogênio demonstrou que apenas 9% dos isolados de *L. iners* apresentaram

essa capacidade, enquanto que 95% dos isolados de *L. crispatus* foram capazes de produzi-lo[11]. Com relação à produção de ácido lático, já foi demonstrado que *L. iners* produz majoritariamente a isoforma L-lactato, enquanto que *L. crispatus* produz maiores quantidades de D-lactato[12]. A contribuição da isoforma L-lactato no ambiente vaginal ainda não é completamente compreendida, no entanto estudos têm mostrado que *L. iners* tem a capacidade de manter o pH vaginal relativamente baixo, mas não tão eficientemente quanto *L. crispatus*[13-15]. Ainda com relação ao pH, recentemente foi demonstrado que somente o pH baixo não é suficiente para proteção contra a infecção pelo vírus da imunodeficiência humana (HIV), mas a isoforma mais produzida pelo *L. crispatus* é a que oferece melhor proteção contra esse vírus por dificultar a permeabilidade ao muco cérvico-vaginal[16].

A literatura é consistente ao demonstrar que mulheres com predomínio de *Lactobacillus* spp. estão menos propensas à aquisição de infecções sexualmente transmissíveis (IST), sejam as bacterianas por *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, bem como as infecções virais, como pelo papilomavírus humano (HPV) e HIV[1-3; 16].

Quando há depleção de espécies de *Lactobacillus*, tem-se a principal alteração de microbiota vaginal, denominada vaginose bacteriana (VB), neste caso *Lactobacillus* spp. são parcialmente ou totalmente substituídos por outras espécies bacterianas, principalmente anaeróbias[17]. Esta alteração foi descrita pela primeira vez em 1954 por Gardner e Dukes, como sendo causada por um único agente bacteriano, o bacilo Gram-variável e anaeróbio facultativo, denominado *Gardnerella vaginalis*[18]. No entanto, os estudos posteriores demonstraram que na verdade a VB está associada ao aumento de várias outras

espécies bacterianas como *Mobiluncus* sp., *Mycoplasma* sp., *Peptostreptococcus* sp., *Prevotella* sp., *Bacteroides fragilis*, *Ureaplasma* sp.,*Staphylococcus aureus* e *Streptococcus* sp., e, portanto, tal alteração passou a ser considerada como de etiologia polimicrobiana[17, 19-20].

A prevalência de VB é de aproximadamente 30% em mulheres em idade reprodutiva em todo o mundo, incluindo estudos realizados no Brasil, mas pode variar conforme características populacionais como idade, etnia e características comportamentais[21-22]. Deste modo, os estudos já demonstraram a associação entre VB e fatores como hábitos sexuais como histórico de mais de um parceiro sexual na vida, relato de novo parceiro sexual ou início de atividade sexual em idade mais jovem, além do uso de duchas vaginais e estresse crônico[22-25].

É bem estabelecido que há associação entre a VB e diversas complicações obstétricas e ginecológicas levando a efeitos deletérios para a saúde, visto que, mulheres que apresentam essa alteração de microbiota têm risco aumentado para desenvolver doença inflamatória pélvica (DIP) e aquisição e transmissão de IST. No contexto da associação negativa entre *Lactobacillus* spp. e IST, a VB já foi associada à infecção por *C. trachomatis*, *N. gonorrhoeae*, HPV e HIV. Além destas, a instalação dessa condição também aumenta o risco de infecção pelo parasita *Trichomonas vaginalis*[26-30]. Com relação à infecção pelo HPV, sabe-se que a persistência desta infecção é condição necessária para o desenvolvimento das lesões neoplásicas no colo uterino e nesse sentido já foi demonstrado que a VB aumenta o tempo de *clearance* viral[31].

Dentre as mulheres grávidas, tal condição é particularmente deletéria, visto que a VB durante a gestação pode levar a complicações como aborto

espontâneo, corioamnionite, trabalho de parto pré-termo, além de ser um fator de risco para apresentar baixo peso ao nascimento[32-35].

O padrão-ouro para o diagnóstico da VB se dá pela observação microscópica dos morfotipos bacterianos presentes no esfregaço do conteúdo vaginal após coloração pela técnica de Gram desenvolvida por Nugent et al.[13] Desta forma, baseando-se na semiquantificação dos morfotipos presentes, são atribuídos escores de 0 a 10, onde a microbiota normal recebe escores de 0 a 3, a intermediária de 4 a 6 e escores superiores são compatíveis com VB[36]. Devido às diversas complicações ocasionadas pela VB e as limitações das técnicas microscópicas, vários estudos direcionaram seus esforços a fim de se conhecer a composição bacteriana exata do ambiente vaginal, bem como as interações existentes entre as espécies. Desta forma, os primeiros estudos realizados com métodos de cultura bacteriana contribuíram para o conhecimento da composição bacteriana do ambiente vaginal, no entanto tais métodos não permitiam cultivar bactérias fastidiosas ou não-cultiváveis, com o avanço das técnicas de biologia molecular, inicialmente pela reação em cadeia da polimerase (PCR) e depois por clonagem pôde-se ter conhecimento das espécies presentes nesta alteração, embora houvesse limitação com relação ao número de clones recuperados[37-40].

Embora as técnicas acima citadas já tivessem permitido avanços no conhecimento da composição bacteriana da microbiota vaginal, o marco para a determinação não só do microbioma vaginal, bem como do microbioma humano como um todo foi o desenvolvimento das técnicas de sequenciamento de nova geração. Neste sentido, tais técnicas permitiram conhecer a real composição bacteriana no ambiente vaginal bem como os estudos tornaram possível avaliar as transições de microbiota vaginal saudável e VB[13, 41]. Tal técnica consiste no

sequenciamento de porções do gene bacteriano RNA ribossômico 16S, permitindo a identificação das espécies bacterianas presentes, além de determinar qual a participação de cada espécie na comunidade bacteriana total[42]. A partir da utilização dessa técnica demonstrou-se que grande parte da microbiota vaginal é composta por organismos fastidiosos e até então nunca cultivados em laboratório[13, 43].

Empregando o pirosequenciamento do gene bacteriano RNAr 16S, Ravel et al. (2011)[41], classificaram pela primeira vez o microbioma vaginal. Este estudo demonstrou que 75% das mulheres norte-americanas apresentaram microbiota vaginal constituída predominantemente por uma ou mais espécies de *Lactobacillus*, além do fato de que apesar da grande diversidade microbiana vaginal, o microbioma vaginal de mulheres em idade reprodutiva pode ser agrupado em cinco principais comunidades bacterianas (CST, *Community-state type*) conforme predominância de determinadas espécies. Destas cinco comunidades, quatro são caracterizadas pela prevalência de uma das espécies de *Lactobacillus*: *L. iners*, *L. crispatus*, *L gasseri* e *L. jensenii*. Enquanto que a comunidade remanescente não apresenta predomínio de *Lactobacillus* spp., mas sim grande diversidade de espécies, embora em alguns casos possa existir a presença de *Lactobacillus* spp. Neste ponto, vale destacar que é justamente nesta comunidade onde há diversidade e heterogeneidade bacteriana que estão incluídos a maioria dos casos de VB, quando a microbiota é classificada pelo método de Nugent, além do fato de que a microbiota vaginal intermediária pode estar distribuída entre as cinco comunidades descritas, mas a maior parte delas concentra-se naquela dominada por *L. iners*.

Lactobacillus iners foi descrito pela primeira vez em 1999 sendo isolado do trato genital inferior e urinário[44]. Este microrganismo foi descrito como bacilo Gram-positivo, com arranjos individuais, em pares ou ainda em cadeias curtas. O crescimento é lento, aproximadamente 24 horas, e as colônias são pequenas, lisas e não pigmentadas[44]. Posteriormente foi demonstrado que a espécie nem sempre é Gram-positiva, visto que alguns isolados se apresentam como cocobacilo e coloração Gram-negativa. Tal fator possivelmente dificultou seu reconhecimento em esfregaços vaginais [45]. Essa diferença na morfologia celular e nas propriedades de coloração de Gram do *L. iners* deve ser ainda explorada, visto que muitas determinações diagnósticas dependem dessas características, por exemplo, o escore de Nugent, que é amplamente usado na rotina diagnóstica de alterações da microbiota vaginal. Tal método se baseia na coloração pelo método de Gram do conteúdo vaginal permitindo diferir *Lactobacillus* spp. como bastonetes Gram-positivos de outros morfotipos bacterianos proporcionando o diagnóstico, principalmente da VB, sendo um método de diagnóstico dependente da morfologia vista ao microscópio, o comportamento diferente do *L. iners* comparado as outras espécies lactobacilar, pode ser um fator confundidor[36].

O microbioma com predomínio de *L. iners* corresponde a um dos mais prevalentes em mulheres em todo o mundo, chegando a quase 40% dependendo da população estudada[46-49] sendo que em gestantes essa prevalência pode ser ainda maior, visto que estudo encontrou predomínio desta espécie em 51% da população avaliada[49].

Visto que *L. iners* figura entre as espécies mais prevalentes colonizando o ambiente vaginal, estudos direcionaram seus objetivos a entender melhor a sua participação na microbiota vaginal. Desta forma, alguns autores apontam que a

comunidade com predomínio de *L. iners* é mais instável e variável com relação à variedade de outras espécies co-habitando o mesmo ambiente, dessa forma pode-se assumir que o microbioma com predomínio de *L. iners* favorece o aumento da beta-diversidade, enquanto que as comunidades com predomínio de *L. crispatus* e *L. gasseri* tendem a ser mais estáveis ao longo do tempo[13, 50]. De fato, estudos prévios já demonstraram associação entre o *L. iners* e a VB[39-40,51-52].

Além da associação com VB, o microbioma vaginal dominado por *L. iners* já demonstrou ter associação com ISTs, como no caso da infecção pelo HPV, além disso, este estudo demonstrou que o tempo do *clearance* viral foi maior, diferente do que ocorreu no microbioma dominado por *L. gasseri*[50]. No caso da infecção bacteriana por *C. trachomatis*, o microbioma dominado por *L. iners* é fator de risco para aquisição, enquanto que o microbioma dominado por *L. crispatus* apresenta associação contrária, diminuindo as chances de se adquirir essa infecção[53]. De fato, estudo *in vitro* demonstrou que *L. crispatus* é capaz de diminuir a adesão e a infectividade da *C. trachomatis* devido a sua capacidade de produção de D-lactato[54-55].

Pouco se sabe sobre a interação entre microbioma vaginal e presença de *Candida* spp., causadora de vaginites como a candidíase vulvovaginal, que acomete entre 30 a 50% de mulheres ao menos uma vez durante a vida[56-57], e compartilha de fatores de risco já associados ao microbioma, como frequência de relação sexual, ducha vaginal e uso de contraceptivos[58-59]. Neste sentido, estudo apontou que embora não houvesse associação estatisticamente significativa, houve maior prevalência de mulheres com candidíase vulvovaginal na comunidade com predomínio de *L. iners* definida pelo microbioma[60].

Até o presente momento existem diversos estudos associando fatores sociodemográficos, comportamentais e clínicos à VB, possibilitando saber quais características populacionais são fatores de risco para o desenvolvimento desta alteração que pode levar a efeitos deletérios a saúde da mulher[22-25]. Sabe-se que a composição do microbioma humano está relacionada a fatores ambientais, de comportamento e dieta do hospedeiro. No entanto, a literatura ainda carece de dados acerca da relação entre o microbioma vaginal e variáveis sociodemográficas, comportamentais e clínicas. Principalmente, no que se refere ao microbioma vaginal com predomínio de *L. iners*, que embora seja o mais frequente em diversas populações e apresente similaridades com àquelas observadas na alteração da microbiota vaginal, não existem informações sobre sua relação com características da população.

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Artigo científico

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4. ARTIGO CIENTÍFICO

FACTORS ASSOCIATED TO *Lactobacillus iners*-DOMINATED VAGINAL MICROBIOME

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ABSTRACT

Objective: Given the high prevalence of *Lactobacillus iners*-dominated microbiome and the instability of this type of vaginal microbiota, we aimed to test the association between the *L. iners*-microbiome with sociodemographic, behavioral and clinical variables in Brazilian women of reproductive age.

Participants and Methods: This cross-sectional study included 609 Brazilian women attending clinics in five cities throughout the country for routine Pap-testing. Participants answered a structured questionnaire for assessment of sociodemographic, behavioral and clinical data. For characterization of vaginal microbiota, vaginal samples were evaluated by microscopy using Nugent criteria and sequenced forV3-V4 16S rRNA genes in Miseq platform (Illumina, San Diego, CA) for microbiome analysis. A logistic regression model using stepwise method was used to test association between *L. iners*-dominated microbiome with all assessed variables, considering their odds ratios (OR) and 95% confidence intervals (95% CI).

Results: The prevalence of microbiome dominated by *L. iners* was 36.5% (n=222), while the remaining participants had their microbiome dominated by *L. crispatus* (30.5%, n=186), *L. gasseri* (4.4%, n=27) and *L. jensenii* (1.2%, n=7). The 167 (27.4%) participants with *Lactobacillus*-depleted microbiome were excluded of the analysis. The multivariable analysis showed that participants who reported having two or more sexual partners in the year prior to enrollment had an increased risk of presenting *L. iners* microbiome (OR: 3.27; CI 95% 1.50-7.11), as well as the microscopic finding of *Candida* spp. on vaginal smears (OR: 2.24; CI 95% 1.02-4.89). Three variables showed protective against having *L. iners*-dominated: condom use (OR: 0.59; CI 95%0.38-0.91), high school degree (OR:

0.61; CI 95% 0.41-0.91) and daily intake of milk or dairy (OR: 0.43; CI 95%0.20-0.90).

Conclusion: The vaginal microbiome dominated by *L. iners* is independently associated with several population characteristics, such as low education level, multiple sex partners and unprotected sex. Such characteristics were already associated with *Lactobacillus*-depleted microbiota.

INTRODUCTION

The studies concerning the structure of vaginal microbiome into community-state types (CST) were initiated by Ravel et al. (2011) and allowed to determine the exact composition of vaginal microbiota. It has been widely accepted that vaginal microbiome may be classified into five CSTs, according to the dominance and diversity of bacterial species found in this niche. Four out of the five CSTs are dominated by *Lactobacillus* spp.: *L. crispatus* (CST I), *L. gasseri* (CST II), *L. iners* (CST III) and *L. jensenii* (CST V). Women with the remaining CST (IV) have partial or total depletion of *Lactobacillus*, accompanied by an increased microbial beta-diversity. In this latter community, most of bacterial vaginosis (BV) cases stand [1]. This condition is the main type of abnormal vaginal microbiota that affects around 30% of reproductive aged-women, which main complains are genital malodor and increased vaginal discharge [2].

It is widely recognized that BV increases the risk for prevalence and incidence of sexually transmitted infections (STI) including, but not limited to *Chlamydia trachomatis,* human papillomavirus (HPV) and human immunodeficiency virus (HIV) [3-5]. Several characteristics of the population were already associated with microscopically-detected BV, such as ethnicity, sexual behavior, use of hormonal contraceptives, among others [2; 6-7]. The recent studies on vaginal microbiome have confirmed such associations with *Lactobacillus*-depleted microbiome [7].

Despite most of microscopic BV cases are placed into CST IV, many of them are categorized as CST III [8-11]. In addition, longitudinal studies on vaginal microbiome have shown that *L. iners*-dominated CST III frequently shifts to CST IV and vice-versa, suggesting the instability of this type of microbiome [12-13].

The first description of *L. iners* dates to 1999 [14], thus despite the studies conducted since still much remains to be established regarding its role for vaginal microbiota. Recent studies have suggested that *L. iners* may offer diminished protection against important pathogens as *C. trachomatis*, HPV and HIV [13; 15-16]. This is particularly troublesome given the high prevalence of *L. iners*-dominated microbiome, which is found in more than one-third of women in reproductive age [1; 17-19] and is even higher in pregnant women (reaching up to 51.8% prevalence) [20-21].

Considering the high prevalence of women with a *L. iners*-dominated microbiome and the fact that it was already shown to be rather unstable and associated with STI, our aim was to identify sociodemographic, behavioral and clinical characteristics of the population that is associated with this type of microbiome.

METHODS

Study design and population

Recently, our group characterized the vaginal microbiome of Brazilian woman in reproductive age [22]. Briefly, we cross-sectionally included 609 Brazilian women belonging to the five geographic regions of country (South = 109; Southeast = 140; Midwest = 119; North = 133; Northeast = 108). Women enrolled were aged between 18 and 50 years-old, were not pregnant, and did not report use of intrauterine device, infection or urinary loss, less than five days after the end of the menstrual period, less than 72 hours of last sexual intercourse use of local or systemic antibiotics or antifungal drugs within 45 days prior to enrollment. These women were approached when attending primary healthcare clinics for routine screening of cervical cancer (Pap-testing), except for one center (South) where enrollment was taken place at a referral University hospital. The study was reviewed and approved by the Ethics Committee of Botucatu Medical School (Approval number: 3.094.514). After being informed of the aims and procedures of the study, all participating women signed an informed consent term.

Data collection and sampling procedures

Upon enrollment, women answered a questionnaire that included information regarding sociodemographics, behavioral characteristics, and clinical history. Women answered the questionnaire individually during an interview with one training member of the research team.

During physical examination, a non-lubricated speculum was used and two sterile swabs were used for sampling the middle third of the vaginal wall. The first swab was stored in Amies liquid transport medium (Copan, Brescia, Italy) at -80^{0}C until microbiome analysis. The other swab was smeared onto glass slides for microscopic analysis and after wards used for KOH testing that was reported as positive, inconclusive or negative by the nurse/physician. Vaginal pH was assessed by allowing the contact of a pH strip with vaginal wall (Merck, Darmstadt, Germany) for one minute.

Cervical brush samples were taken for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* testing by PCR according to methods previously described [23-25].

Microbiota analysis and study groups

For microbiota assessment and characterization, both microscopic analysis and bacterial DNA sequencing were performed. Using Nugent criteria, vaginal smears were Gram-stained and scored from 0 to 10, according to the semiquantification of bacterial morphotypes observed by microscopy. They were further classified in normal (scores 0-3), intermediate (scores 4-6) and BV (scores >6) [26]. The presence of *Candida* pseudohyphae/hyphae was also evaluated using the Gram-stained smears, as well as the number of polymorphonuclear inflammatory cells by field at 1000x magnification.

Analysis of the bacterial DNA was performed for microbiome characterization. Aimes medium-containing samples were subjected to DNA extraction using MoBio Powersoil Kit (MoBio Lab Inc, Carlsbad, CA) according to manufacturer's instructions. The microbiome analyzes were carried out at the Institute for Genome Sciences of the University of Maryland, Baltimore, MD, using methodology developed by Fadrosh et al. (2014) [27]. In brief, such samples were submitted to sequencing of V3 to V4 region of the bacterial 16S rRNA gene on

Illumina MiSeq platform (Illumina Inc.) using barcoded primers. Sequences other than bacterial were trimmed and demultiplexed using QIIME software (version 1.8.0) [28] (for details, please refer to Fadrosh et al. [27]). Taxonomic assignments were performed using UCHIME (v5.1) [29] and the greengenes database of 16S rRNA sequences (Aug, 2013 vers.) [30]. Each participant's sequences were further clustered Markov Chain model according to similarity in terms of composition and relative abundance of species, in order to obtain the five CSTs.

Statistical analysis

Descriptive statistical analyses were calculated for sociodemographic, behavioral and clinical variables. These characteristics were compared by CST using Chi-squared and Mann-Whitney tests for categorical and continuous variables, respectively, with P-value<0.05 considered as statistically significant.

We performed univariate logistic regression models to assess the association between CST III with population characteristics. For that, crude and age- and region-adjusted odds ratios (OR) were estimated, as well as their corresponding 95% confidence intervals (CI). A multivariable logistic regression analysis was carried out using a forward stepwise model selection process (variables retained at p-value ≤0.15) to identify variables independently associated with CST III. All analyses were performed using Stata/SE, version15.1 (Stata Corp, College Station, TX).

RESULTS

Of the 609 women recruited, 167 (27.4%) did not have *Lactobacillus*-dominance (CST IV), and therefore were excluded of the analysis. Thus, this study population consisted of the 442 with *Lactobacillus*-dominated microbiome. Among them 186 (30.5%) belonged to CST I (*L. crispatus*), 27 (4.4%) had CST II (*L. gasseri*), 222 (36.5%) had CST III (*L. iners*) and 7 (1.2%) had CST V (*L. jensenii*).

The sociodemographic and behavioral data of the 442 women included in the study are displayed in Table 1. We compared these variables between the two study groups: (1) participants that had CST III (n=222) and (2) those with the other *Lactobacillus*-dominated CST (n=220).The mean age was 34 years at both study

groups most of the women self-reported as black or pardo (n=229), not differing between the groups. Having completed the high school, daily intake milk and dairy, and condom use were more frequent in women with dominance of other *Lactobacillus* spp. than *L. iners*, while having two or more sexual partners were more frequent in CST III group. The microbiota classified by microscopy and the presence of 5 or more inflammatory cells and *Candida* morphotypes also differed between the groups (Table 1). Table 2 shown the distribution of normal microbiota, intermediate and bacterial vaginosis according to the CST defined by the microbiome analysis and highlights that the majority of women with abnormal vaginal microbiota (i. e. intermediate or BV) belonged to CST III group. The prevalence of *C. trachomatis, N. gonorrhoeae* and *T. vaginalis* among the groups *Lactobacillus*-dominated was 4.1% (n=18), 0.4% (n=2) and 0.2 (n=1), respectively.

Odds ratios (OR) for the association between sociodemographic, behavioral and clinical factors and the vaginal microbiome dominated by *L. iners* (CST III) are shown in Table 2. Crude analysis showed that education level, milk and dairy intake, number of sexual partners and condom use were significantly associated with CST III. When adjusting for age and region, all previous variables remained associated with CST III. Finally, the multivariable analysis showed having a CST III-microbiome was independently associated with reporting two or more sexual partners in the previous year and the detection of *Candida* morphotypes on vaginal smears. On the other hand, some sociodemographics and behavioral characteristics such as having at least the high school degree, milk and derivates consumption and regular condom use were protective against *L. iners*-dominated microbiome.

DISCUSSION

Literature findings on the vaginal colonization by *L. iners* have demonstrated that this species can be found in the microbiota of healthy women and also in those with BV, even in the presence of BV-related symptoms [1, 8-11]. In fact, studies have demonstrated that *L. iners* colonization persists during the temporal shifts of the normal vaginal microbiota to BV [12, 31]. As well as in BV, *L. iners*-dominance in the vaginal microbiome may have serious consequences for

women's health, such as increased susceptibility to STI [13, 15-16]. Thus, identifying the population characteristic associated with this condition is of particular importance and, up to our knowledge, this was the first study carried out with this goal. For that, we limited our study population in women regularly menstruating in order to avoid possible bias due to hormonal influences.

Research on microbiome has pointed out that the influences environment has influence on the microbial composition [32]. Factors seen in poverty, diet and social conditions may contribute to a less healthy vaginal environment, such as STI [33]. For the first time, we demonstrate that education level, one of mainly social indicators, was a factor that may influence the composition of the microbiome. In this way, our group of women who had completed high school presented lower odds of having vaginal microbiome dominated by *L. iners*.

When looking at our data regarding the sexual behavioral characteristics, we found a significant association of microbiome dominated by *L. iners* with sexual habits previously associated with BV [34]. Condom use had a protective factor against CST III, while having two or more sexual partner per year increased the risk of having this type of microbiota. Literature is consistent in demonstrating that women with two or more sexual partners per year have higher chances to have BV and there is a strong inverse association between bacterial vaginosis and condom use [34]. However, up to now little is known about how a woman's sexual and habits may influence the microbiome, or *vice versa*. Similarly to our results, Vodstrcil et al. (2017) also showed that young women who had unprotected sex were more likely to have vaginal microbiome dominated by *L. iners* or the BV-associated *Gardnerella vaginalis* [35]. Corroborating with this findings, other study demonstrated that the consistent condom use increases the number of *L. crispatus* colonizing the vaginal environment [36]. When adjusted by age and region, BV history was a risk factor for having CST III, but the multivariate analysis did not show this association. As it has already been shown that in the vaginal microbiome dynamics, CST IV frequently shift to CST dominated by *L. iners*, the same situation occurs inversely [13], and beyond this transition, we now demonstrate that these communities share the same associations related to sexual behavior.

In relation to dietary behavioral characteristics studied, we found inverse association between the milk and dairy intake with *L. iners*-dominated microbiome. To our knowledge, this is the first time that association is described in studies on vaginal microbiome. Some studies have already shown the interaction between some vitamins and minerals, which are found in milk, and BV. Bodnar et al. [37] shown that maternal vitamin D deficiencies associated with BV in the first trimester of pregnancy, although a longitudinal study have no found this association [38]. Neggers et al. [39] demonstrated inverse association between bacterial vaginosis and an increased intake of vitamin E and calcium, both milk components. Prior study with the microbiome gut, which has been more studied as the relation between bacterial composition and host demonstrated that diet may be common determinants of microbial community composition [40]. This away, studies about vaginal microbiome also demonstrated this association, although the direct relationship between diet and cervicovaginal microbial composition is not as clear as in the gut, thus one study showed the influence of diet as well as probiotics in the treatment of BV, in this sense, Laue et al. [41] indicate that the administration of a yoghurt containing probiotics had better effects on the treatment of BV. Another study showed that *Saccharomyces cerevisiae*-based probiotic had antagonistic effect against *G. vaginalis* colonization in vaginal environment, such effect was associated with the fact that it inhibited sialidase, an important virulence factor of *G. vaginalis* [42].

Among the clinical characteristics we found significant difference between the CSTs when the number of polymorphonuclear cells per field was evaluated, being the microbiome dominated by *L. iners* statistically associated with increased number of cells. The increase of polymorphonuclear cells is seen in aerobic vaginitis, which was first characterized in 2002 by Donders et al., this condition is accompanied by more inflammatory changes than BV and the presence of mainly aerobic enteric commensals or pathogens [43]. Until now, there are no studies reporting the relationship between *L. iners* and increased polymorphonuclear cells and even aerobic vaginitis, as all studies have focused in the relation between *L. iners* and BV.

A previous study demonstrated that the rupture or depletion of *Lactobacillus* was not associated with vulvovaginal candidiasis [44], for the first time, we shown

association between vaginal microbiome *L. iners*-dominated and presence of *Candida* morphotypes. In fact, a study conducted in three African countries with heterogeneous group of women, including pregnant and not pregnant, has already demonstrated only high prevalence of vaginal candidiasis, detected on wet mount, in the community with a predominance of *L. iners*, although did not reach statistical significance [45]. Little is known about the interaction between the bacterial species found in the vagina and colonization by *Candida* species, this way, the association showed may be elucidate in future studies in order to get better results in the treatment of candidiasis, such as alternatives treatment with probiotics, as emerging experimental studies have shown promise [46].

CONCLUSION

Lactobacillus iners-dominated microbiome and bacterial vaginosis share the same association with some sociodemographic, behavioral characteristics. Considering that the protective role of *L. iners* has been questioned, the identification of women with this type of microbiota might be of clinical interest. Further studies are also necessary to address which factors are associated with the transition between *L. iners* and *Lactobacillus* depletion. This further knowledge may contribute for identifying strategies of vaginal microbiota stabilization.

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Table 1. Sociodemographic, behavioral and clinical variables grouped into CST I, II and V versus CST III.

	CST I, II and V (n=220)	CST III (n=222)	P value*
SOCIODEMOGRAPHIC			
Region			
South	59 (26.82)	41 (18.47)	0.13
Southeast	45 (20.45)	51 (22.97)	
Central	36 (16.36)	44 (19.82)	
Northeast	34 (15.45)	47 (21.17)	
North	46 (20.91)	39 (17.57)	
Residence			
Urban	217 (98.64)	219 (98.65)	0.991
Rural	3 (1.36)	3 (1.35)	
Age[+] (mean)	34 (18-50)	34 (18-51)	0.91
Age[++]			
18-35 years	115 (52.27)	113 (50.90)	0.773
≥35 years	105 (47.73)	109 (50.90)	
Ethnicity			
Black/pardo	112 (50.91)	117 (52.70)	0.793
White	96 (43.64)	97 (43.70)	
Other	12 (5.45)	8 (3.60)	
Living with partner			
No	75 (34.09)	82 (36.94)	0.532
Yes	145 (65.91)	140 (63.06)	
Completed high school[a]			
No	74 (33.64)	105 (47.30)	0.003*
Yes	146 (66.36)	117 (52.70)	
Has personal income?			
No	72 (32.73)	81 (36.49)	0.406
Yes	148 (67.27)	141 (63.51)	
BEHAVIORAL			
Milk and dairy intake			
No	12 (5.45)	26 (11.71)	0.019*
Yes	208 (94.55)	196 (88.29)	
Smoking			
No	200 (90.91)	198 (89.19)	0.546
Yes	20 (9.09)	24 (10.81)	
Alcohol consumption			
No	162 (73.64)	158 (71.17)	0.562
Yes	58 (26.36)	64 (28.83)	
Intimate soap, daily use			
No	111 (50.45)	115 (51.80)	0.777
Yes	109 (49.55)	107 (48.20)	
Douching			
No	188 (85.45)	194 (87.39)	0.553
Yes	32 (14.55)	28 (12.61)	
Sitz bathing			
No	203 (92.27)	202 (90.99)	0.627
Yes	17 (7.73)	20 (9.01)	
Daily use of pantyliners			
No	210 (95.45)	215 (96.85)	0.447
Yes	10 (4.55)	7 (3.15)	
Number of sexual partners[b]			
0	7 (3.18)	15 (6.76)	0.009*
1	202 (91.82)	182 (81.98)	

	CST I, II and V (n=220)	CST III (n=222)	P value*
2+	11 (5.00)	25 (11.26)	
New sexual partner[c]			
No	204 (92.73)	202 (90.99)	0.505
Yes	16 (7.27)	20 (9.01)	
Number of sexual intercourses/week			
0	31 (14.10)	43 (19.37)	0.301
1-2	112 (50.90)	102 (45.94)	
3+	77 (35.00)	77 (34.69)	
Hormonal contraceptive			
No use	120 (54.55)	131 (59.01)	0.181
Oral	84 (38.18)	68 (30.63)	
Injectable	16 (7.27)	23 (10.36)	
Condom use			
No	132 (60.00)	157 (70.72)	0.018*
Yes	88 (40.00)	65 (29.28)	
CLINICAL			
Body mass index[+]			
Median	25.1 (17.97- 50.64)	24.84 (16.18 - 47.94)	
Body mass index[++d]			
Underweight/normal	110 (50.00)	115 (51.80)	0.923
Overweight	69 (31.36)	68 (30.63)	
Obese	41 (18.64)	39 (17.57)	
At least once pregnant			
No	56 (25.45)	50 (22.52)	0.470
Yes	164 (74.55)	172 (77.48)	
Phase Cycle			
Luteal	102 (46.37)	95 (42.79)	0.337
Follicular	93 (42.27)	108 (48.65)	
Supressed	25 (11.36)	19 (8.56)	
STD history[e]			
No	190 (86.36)	192 (86.49)	0.970
Yes	30 (13.64)	30 (13.51)	
BV history[e]			
No	126 (57.27)	109 (49.10)	0.085
Yes	94 (42.73)	113 (50.90)	
Dyspaneuria			
No	144 (65.45)	142 (63.96)	0.743
Yes	76 (34.55)	80 (36.04)	
Cervicitis			
No	194 (88.18)	205 (92.34)	0.140
Yes	26 (11.82)	17 (7.66)	
Pruritus			
No	189 (85.91)	181 (81.53)	0.213
Yes	31 (14.09)	41 (18.47)	
Abnormal discharges			
No	135 (61.36)	125 (56.31)	0.280
Yes	85 (38.64)	97 (43.69)	
pH			
<=4.5	134 (60.91)	124 (55.86)	0.281
>4.6	86 (39.09)	98 (44.14)	
Whiff test			0.179
Negative	166 (75.45)	151 (68.02)	
Inconclusive	16 (7.27)	25 (11.26)	
Positive	38 (17.27)	46 (20.72)	
Nugent cat			

	CST I, II and V (n=220)	CST III (n=222)	P value*
Normal	192 (87.27)	160 (72.07)	0.000*
Intermediate	14 (6.36)	35 (15.77)	
BV	14 (6.36)	27 (12.16)	
PMNs (5 or more per field)			
No	211 (95.91)	198 (89.19)	0.007*
Yes	9 (4.09)	24 (10.81)	
Candidiasis			
No	209 (95.00)	200 (90.09)	0.049*
Yes	11 (5.00)	22 (9.91)	

[+]Continuous variable [++]Categorical variable [a]Grades completed; [b]12 months prior to enrollment; [c]2 months prior to enrollment; [d]Categorized according to the World Health Organization into underweight/normal (<25.0 unit), overweight (25.0-29.9) and obese (≥30.0); [e]Self reported.

Table 2: Distribution of vaginal microbiota classified by the Nugent scoring criteria, according to the community state-type (CST) determined by the vaginal microbiome in the 442 participants of the study.

	Normal (n=352)	Intermediate (n=49)	Bacterial vaginosis (n=41)
CST I	168 (47.7)	8 (16.3)	10 (24.4)
CST II	19 (5.4)	5 (10.2)	3 (7.3)
CST III	160 (45.5)	35 (71.4)	27 (65.9)
CST V	5 (1.4)	1 (2.1)	1(2.4)

Table 3. Odds ratios and 95% confidence intervals for the association between sociodemographic and behavioral factors and the vaginal microbiome dominated by *Lactobacillus iners* (CST-III).

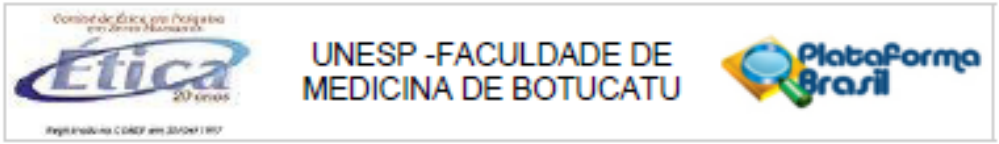
	Crude	Age- and region-adjusted	Multivariable
SOCIODEMOGRAPHIC			
Region			
South	1.00	1.00	--
Southeast	1.63 (0.92 - 2.87)	1.62 (0.92 - 2.86)	
Central	1.75 (0.97 - 3.18)	1.76 (0.97 - 3.19)	
Northeast	1.98 (1.09 - 3.60)	1.98 (1.09 - 3.60)	
North	1.22 (0.68 - 2.18)	1.22 (0.68 - 2.18)	
Age			
<35 years	1.00	1.00	--
≥35 years	1.05 (0.72 - 1.53)	1.31 (0.66 - 2.63)	
Ethnicity			
Black	1.00	1.00	--
White	0.96 (0.65 - 1.41)	1.09 (0.71 - 1.69)	
Other	0.63 (0.25 - 1.61)	0.58 (0.22 - 1.50)	
Living partner			
No	1.00	1.00	--
Yes	0.88 (0.59 - 1.30)	0.85 (0.56 - 1.29)	
Education level[a]			
≤11 years	1.00	1.00	1.00
>11 years	0.56 (0.38 - 0.82)	0.58 (0.39 - 0.87)	0.61 (0.41 - 0.91)
Has personal income?			
No	1.00	1.00	--
Yes	0.84 (0.57 - 1.25)	0.87 (0.58 - 1.31)	
BEHAVIORAL			
Milk and dairy intake			
No	1.00	1.00	1.00
Yes	0.43 (0.21 - 0.88)	0.46 (0.22 - 0.97)	0.43 (0.20 -0.90)
Smoking			
No	1.00	1.00	--
Yes	1.21 (0.64 - 2.26)	1.18 (0.62 - 2.24)	
Alcohol consumption			
No	1.00	1.00	--
Yes	1.13 (0.74 - 1.71)	1.23 (0.80 - 1.90)	
Intimate soap daily use			
No	1.00	1.00	--
Yes	1.08 (0.70 - 1.65)	1.17 (0.75 - 1.82)	
Douching			
No	1.00	1.00	--
Yes	0.84 (0.49 - 1.46)	0.89 (0.51 - 1.55)	
Sitz bathing			
No	1.00	1.00	--
Yes	1.18 (0.60 - 2.32)	1.32 (0.62 - 2.80)	
Daily pantyliner use			
No	1.00	1.00	**--**
Yes	0.68 (0.25 - 1.82)	0.92 (0.32 - 2.62)	
Number of sexual partners[b]			
0	2.37 (0.94 - 5.96)	2.51 (0.98 - 6.39)	2.34 (0.90 - 6.07)
1	1.00	1.00	1.00
2+	2.52 (1.20 - 5.27)	2.58 (1.21 - 5.49)	3.27 (1.50 - 7.11)
New sexual partner[c]			
No	1.00	1.00	--
Yes	1.26 (0.63 - 2.50)	1.42 (0.69 - 2.91)	

	Crude	Age- and region-adjusted	Multivariable
Number of sexual intercourses/week			
0	1.52 (0.89 - 2.59)	1.65 (0.95 - 2.85)	--
1-2	1.00	1.00	
3 +	1.09 (0.72 - 1.66)	1.04 (0.68 - 1.59)	
Condom use			
No	1.00	1.00	1.00
Yes	0.62 (0.41 - 0.92)	0.59 (0.39 - 0.90)	0.59 (0.38 - 0.91)
Hormonal contraceptive			
No use	1.00	1.00	--
Oral	0.74 (0.49 - 1.11)	0.74 (0.47 - 1.15)	
Injectable	1.31 (0.66 - 2.61)	1.20 (0.59 - 2.44)	
CLINICAL			
Phase cycle			
Luteal	1.24 (0.84 - 1.84)	1.31 (0.88 - 1.96)	**--**
Follicular	0.81 (0.42 - 1.57)	0.84 (0.42 - 1.64)	
STD history	0.98 (0.57 - 1.70)	1.02 (0.59 - 1.78)	
BV history	1.38 (0.95 - 2.02)	1.62 (1.08 - 2.43)	1.45 (0.98 - 2.15)
Candidiasis	2.09 (0.98 - 4.42)	1.98 (0.92 - 4.25)	2.24 (1.02 - 4.89)
Body mass index[d]			
Underweight/normal	1.00	1.00	--
Overweight	0.94 (0.61 - 1.44)	0.91 (0.59 - 1.42)	
Obese	0.90 (0.54 - 1.51)	0.82 (0.48 - 1.41)	

[a]Grades completed; [b]12 months prior to enrollment; [c]2 months prior to enrollment; [d]Categorized according to the World Health Organization into underweight/normal (<25.0 unit), overweight (25.0-29.9) and obese (≥30.0).

5. ANEXOS

1. Parecer do Comitê de Ética em Pesquisa (CEP)



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Estudo da associação entre o microbioma vaginal com variáveis sociodemográficas e de hábitos comportamentais de mulheres brasileiras em idade reprodutiva

Pesquisador: Camila Marcon
Área Temática:
Versão: 2
CAAE: 03391018.6.0000.5411
Instituição Proponente: Unidade de Pesquisa Experimental
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.094.514

Apresentação do Projeto:
Segundo os pesquisadores, a vaginose bacteriana (VB) é a principal alteração de microbiota vaginal em mulheres em idade reprodutiva. A prevalência dessa condição no Brasil é de 30% e figura entre as maiores do planeta. Embora a VB seja uma condição frequente, a composição microbiana entre os diferentes casos é bastante heterogênea. Método: Os dados do microbioma e das variáveis sóciodemográficas e comportamentais da população do projeto maior "Caracterização do microbioma vaginal de mulheres brasileiras em idade reprodutiva" (CAAE: 02381512.5.1001.5411) serão ajustados num modelo de análise multivariada em software STATA, sendo o tipo de comunidade bacteriana a variável independente e as demais variáveis serão as dependentes. O valor de significância adotado será de 5%. Tamanho amostral: 500 participantes
Trata-se de um subprojeto do projeto maior "Caracterização do microbioma vaginal de mulheres brasileiras em idade reprodutiva" (CAAE: 02381512.5.1001.5411).

Objetivo da Pesquisa:
Determinar a associação entre o microbioma vaginal com variáveis sociodemográficas e de hábitos comportamentais de mulheres brasileiras em idade reprodutiva.

Avaliação dos Riscos e Benefícios:
Riscos: O projeto de pesquisa apresenta riscos mínimos por se tratar de análises secundárias de

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dados já obtidos de amostras que compõe o biorrepositório do projeto maior.

Benefícios: Trata-se de pesquisa com benefícios indiretos as participantes. No entanto não foram esclarecidos pelos pesquisadores, contribuindo para a saúde reprodutiva da mulher, pois visa o esclarecimento dos mecanismos e presentes associações entre a composição do microbioma vaginal com aspectos da imunidade do trato genital inferior.

Comentários e Considerações sobre a Pesquisa:

Trata-se de projeto de pesquisa relevante com metodologia adequada. Os pesquisadores apresentam custos de R$ 2.400,00. O estudo será realizado utilizando recursos próprios do pesquisador. As análises serão realizadas no Laboratório de Imunopatologia da Relação Materno-Fetal, da Unidade de Pesquisa Experimental (UNIPEX) da Faculdade de Medicina de Botucatu/UNESP.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos obrigatórios foram apresentados. Os pesquisadores solicitaram dispensa do TCLE com a justificativa de tratar de análises secundárias de dados já obtidos de amostras que compõe o biorrepositório do projeto maior "Caracterização do microbioma vaginal de mulheres brasileiras em idade reprodutiva" (CAAE: 02381512.5.1001.5411), com aplicação de TCLE anteriormente.

Recomendações:

Atendidas as pendências apontadas.

Conclusões ou Pendências e Lista de Inadequações:

Após análise em reunião extraordinária, o Colegiado deliberou APROVADO o projeto de pesquisa apresentado.

Considerações Finais a critério do CEP:

Conforme deliberação do Colegiado em reunião extraordinária do Comitê de Ética em Pesquisa da FMB/UNESP, realizada em 18 de dezembro de 2018, o projeto encontra-se APROVADO, sem necessidade de envio à CONEP.

No entanto, informamos que ao final da execução da pesquisa, seja enviado o "Relatório Final de Atividades", na forma de "Notificação", via sistema Plataforma Brasil.

Atenciosamente,

Comitê de Ética em Pesquisa da Faculdade de Medicina de Botucatu - UNESP

Continuação do Parecer: 3.094.514

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1248365.pdf	11/12/2018 11:43:14		Aceito
Recurso Anexado pelo Pesquisador	Resposta_parecer_Juliano.pdf	11/12/2018 11:32:49	JULIANO NOVAK	Aceito
Declaração de Instituição e Infraestrutura	TermoDeAnuenciaInstitucionalJulianoNovak.pdf	20/11/2018 19:29:41	JULIANO NOVAK	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoDetalhado_JulianoNovak.pdf	31/10/2018 13:33:58	JULIANO NOVAK	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Dispensa_TCLE.pdf	31/10/2018 13:31:59	JULIANO NOVAK	Aceito
Cronograma	Cronograma_JulianoNovak.pdf	31/10/2018 11:51:13	JULIANO NOVAK	Aceito
Brochura Pesquisa	SubProjeto1_JulianoNovak.pdf	31/10/2018 11:35:23	JULIANO NOVAK	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	PB_PARECER_CONSUBSTANCIADO_CONEP_2929149_E2.pdf	31/10/2018 11:35:03	JULIANO NOVAK	Aceito
Folha de Rosto	FolhaDeRostoAssinada.pdf	31/10/2018 11:20:48	JULIANO NOVAK	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BOTUCATU, 19 de Dezembro de 2018

Assinado por:
SILVANA ANDREA MOLINA LIMA
(Coordenador(a))

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