UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA

Carolina Sanitá Tafner Ferreira

Avaliação proteômica do conteúdo vaginal em resposta ao tratamento da vaginose bacteriana

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

Orientadora: Profa. Dra. Camila Marconi
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Dedicatória

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Sumário

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Revisão da literatura

1. Revisão da literatura

1.1 Fluido cérvico-vaginal

O ambiente vaginal é um ecossistema complexo e dinâmico, sendo seu conteúdo constituído por água, colesterol, lipídeos, mucina, carboidratos, aminoácidos, proteínas e sais inorgânicos, que hidratam a mucosa criando uma barreira física contra a invasão de patógenos. Além disso, são encontrados nesse ambiente mediadores do sistema imune provenientes do transudato local, muco cervical, secreção das glândulas de Bartholin e Skene, células endometriais, epiteliais, inflamatórias, de descamação, e os metabólitos da microbiota local.[1,2] Esses componentes associados à comunidade bacteriana presente no trato genital inferior feminino contribuem para a manutenção da homeostase local. Dessa forma, o fluido cérvico-vaginal constitui a primeira linha de defesa contra a invasão de microrganismos patogênicos e, portanto, o equilíbrio desse ecossistema é fundamental para a saúde reprodutiva da mulher.[3]

A microbiota vaginal é considerada normal quando as espécies de *Lactobacillus* predominam em relação às outras espécies bacterianas que podem ser encontradas colonizando esse ambiente.[4] A importância da manutenção do predomínio de lactobacilos vaginais se dá pelo fato de serem encontradas inúmeras linhagens produtoras de peróxido de hidrogênio, metabólito com ação antimicrobiana contra outros microrganismos.[5] Além disso, o epitélio vaginal, sob ação estrogênica, acumula glicogênio que posteriormente será hidrolisado em glicose que, por sua vez, é metabolizada pelos lactobacilos levando à produção e liberação de ácido lático.[6] O ácido lático derivado dessa via metabólica leva à redução do pH vaginal entre 3,8 e 4,5, sendo que a manutenção desse pH ácido também contribui para a inibição do crescimento de outros microrganismos.[7] Outro fator que

confere importância aos lactobacilos vaginais é a produção de bacteriocinas que tem a função de impedir a proliferação de patógenos.[6,8-10] Um exemplo de bacteriocina produzida por lactobacilos é a gassericina A da linhagem LA39 de *Lactobacillus gasseri*[11,12] e de lactocina 160 produzida por *Lactobacillus rhamnosus*[13]. Da mesma forma, algumas linhagens de *Lactobacillus acidophilus* também são capazes de produzir bacteriocinas, cujo efeito inibitório já foi comprovado *in vitro* em linhagens de *Gardnerella vaginalis*.[14]

Já foi demonstrado que a diminuição ou mesmo depleção dos lactobacilos vaginais promovem significativas alterações no sistema imune do hospedeiro e aumentam o risco para importantes complicações ginecológicas e obstétricas como doença inflamatória pélvica,[15] infecções pós-cirúrgicas[16], rotura prematura de membranas pré-termo,[17] parto prematuro e baixo peso ao nascimento.[18] Outra séria consequência do desequilíbrio da microbiota vaginal é o aumento do risco de aquisição de infecções sexualmente transmissíveis como tricomoníase, infecção clamidiana e gonorreia, além do vírus da imunodeficiência humana (HIV).[19-21] De fato, os mecanismos relacionados ao efeito protetor dos lactobacilos contra a aquisição de infecções sexualmente transmissíveis tem sido demonstrados *in vitro*. Autores demonstraram que a produção de peróxido de hidrogênio por lactobacilos é capaz de inibir o crescimento de *Neisseria gonorroeae* e HIV.[22-24] Já estudo realizado por Graver & Wade (2011) demonstrou que a acidificação do ambiente provocada pelos lactobacilos inibe o crescimento de *Neisseria gonorroeae.*[25]

1.2 Vaginose bacteriana

Dentre as alterações de microbiota vaginal, a vaginose bacteriana é a mais comum dentre as mulheres em idade reprodutiva, com prevalência de 30%.[26,27] Tal condição é caracterizada pela substituição dos lactobacilos vaginais por outras espécies bacterianas, em

sua maioria anaeróbias.[26] Os sintomas mais reportados por mulheres com vaginose bacteriana incluem o aumento do corrimento vaginal e mal odor vaginal.[26] No entanto, já foi demonstrado que até 50% das mulheres com vaginose bacteriana não reportam tais sintomas, o que constitui um desafio para o diagnóstico e subsequente tratamento dessa alteração de microbiota.[28,29]

A alta taxa de mulheres assintomáticas representa um problema na prática clínica, visto as sérias consequências ginecológicas e obstétricas que já foram associadas à vaginose bacteriana.[15-30] Já foi demonstrado que mulheres com vaginose bacteriana apresentam maior risco para desenvolver doença inflamatória pélvica,[15] infecções pós-cirúrgicas,[16] além do aumento do risco de aquisição e transmissão de infecções sexualmente transmissíveis.[19-21] Somado a tais complicações, em gestantes a vaginose bacteriana assume ainda maior importância, visto que é associada à ocorrência de aborto espontâneo,[30] corioamnionite,[17] rotura prematura de membranas,[17] parto pré-termo e baixo peso ao nascimento.[18] Portanto, o diagnóstico da vaginose bacteriana é fundamental na prática ginecológica e obstétrica para prevenção de tais complicações associadas.

O Ministério da Saúde[31] preconiza que o diagnóstico de vaginose bacteriana seja realizado através da presença de três ou mais dos critérios estabelecidos por Amsel et al.,[32] que incluem: corrimento vaginal branco, fino e homogêneo, pH vaginal maior que 4,5, *whiff test* positivo e presença de *clue-cells* na microscopia a fresco do conteúdo vaginal. Ou ainda, utilizando esfregaços vaginais corados pelo método de Gram, pela classificação microscópica da microbiota vaginal descrita por Nugent et al.[33] que é considerada o padrão-ouro para o diagnóstico da vaginose bacteriana. Tal método baseia-se na atribuição de escores de acordo com quantidade de morfotipos bacterianos, lactobacilos ou não, presentes nas amostras, que são classificadas como normal (escores de 0 a 3), intermediária

(escores de 4 a 6) e vaginose bacteriana (escores de 7 a 10).

A vaginose bacteriana é considerada uma entidade polimicrobiana, visto que inúmeras espécies bacterianas são associadas a ela. Além disso, a composição bacteriana pode variar entre os diferentes casos.[34,35] Muitas espécies já foram identificadas como associadas à vaginose bacteriana utilizando métodos de cultura do conteúdo vaginal, dentre as quais destaca-se a *G. vaginalis*.[36] No entanto, a recente utilização de técnicas moleculares não só confirmou confirmação da presença de espécies já identificadas por métodos de cultura[37], tais como *G. vaginalis*[38], *Prevotella bivia*[39], *Mobiluncus curtisii*[39], *Mycoplasma hominis*[40], como também possibilitou a detecção de inúmeras espécies até então não identificadas como *Atopobium vaginae*, BVAB 1-3, *Leptotrichia* sp, *Megasphaera* sp., entre outros.[41,42] Além do aspecto polimicrobiano da vaginose bacteriana, trabalhos também demonstraram importantes relações sinérgica entre as espécies associadas a essa condição. Um exemplo de sinergismo bacteriano presente na vaginose bacteriana é a relação entre *P. bivia* e *G. vaginalis,* na qual a primeira produz amônia necessária para a estimulação do crescimento de *G. vaginalis* que, por sua vez, produz aminoácidos importantes para o metabolismo da *P. bivia.*[43] Ainda, outro exemplo importante de associação entre diferentes espécies na vaginose bacteriana é aquela demonstrada pela forte correlação entre as cargas de *G. vaginalis* e *A. vaginae* no conteúdo vaginal.[41] Além disso, já foi demonstrado que tais espécies são os principais componentes dos biofilmes vaginais, já que 60 a 95% da massa do biofilme é constituído por *G. vaginalis* e até 40% por *A. vaginae*.[44] Esse forte sinergismo entre espécies bacterianas na vaginose bacteriana, bem como a formação dos biofilmes vaginais, podem estar associados à maior dificuldade de tratamento dessa condição.[45]

1.3 Tratamento da vaginose bacteriana

De acordo com recomendações do Centers for Disease Control and Prevention

(CDC), o tratamento da vaginose bacteriana pode ser realizado com 500 mg de metronidazol via oral duas vez ao dia durante sete dias consecutivos.[46] Embora já tenham sido demonstrados benefícios do tratamento com metronidazol,[47,48] o tratamento da vaginose bacterina ainda é um grande desafio na prática clínica.[49] A resposta ao tratamento a curto prazo é aceitável, embora seja observada persistência ou recorrência da vaginose bacteriana em 11 a 29% das mulheres em um mês.[50-52] Apesar de poucos estudos a longo prazo terem sido realizados, alguns demonstraram taxa de recorrência acima de 70% quando as mulheres foram avaliadas em até um ano da realização do tratamento.[53-55]

Alguns fatores como a grande diversidade das bactérias presentes, a presença de linhagens com capacidade de formação de biofilmes ou então resistentes ao antibiótico tem sido propostos como a possível causa da baixa eficiência ao tratamento da vaginose bacteriana com metronidazol. Além disso, algumas espécies recentemente identificadas como bactérias associadas à vaginose bacteriana, denominadas BVAB 1, BVAB 2 e BVAB 3 já foram associadas à falha ao tratamento dessa condição.[56] Tendo em vista que tais espécies são da ordem *Clostridiales*, sugere-se que elas tenham a capacidade de produzir esporos o que resultaria numa rápida recolonização após o tratamento com o antibiótico.[56] Conjuntamente, essas espécies foram positivamente associadas com a detecção dos morfotipos sugestivos de *Mobiluncus* sp. pelo método de coloração de Gram, cujas linhagens resistentes ao metronidazol já foram demonstradas.[39,56,57] Outra espécie associada à vaginose bacteriana, o *A. vaginae*, também possui várias linhagens resistentes ao metronidazol.[58] Além da resistência já descrita ao metronidazol, o *A. vaginae* possui a capacidade de formação de biofilmes que também deve contribuir para a baixa eficiência da antibioticoterapia.[44]

Além dos aspectos microbiológicos, deve-se considerar que fatores do sistema

imune do hospedeiro também podem influenciar o padrão de resposta ao tratamento da vaginose bacteriana. De fato, estudos utilizando imunoensaios demonstraram que a vaginose bacteriana causa significativas alterações nos níveis cérvico-vaginais de vários mediadores inflamatórios, tais como Interleucina (IL)-1beta, IL-4, IL-6, IL-10, IL-8, IL-3, IL-7 e IL-12, fator estimulador de colônias de granulócitos e macrófagos, além dos níveis de peptídeos antimicrobianos, como as beta defensinas humanas produzidas pelas células epiteliais e lactoferrina.[59] Desse modo, vale ressaltar que a maioria dos estudos realizados até agora têm utilizado imunoensaios comparando mulheres com microbiota vaginal normal com aquelas com vaginose bacteriana para a pesquisa de biomarcadores.[60-65] No entanto, tais estudos permitiram a identificação de um número limitado de proteínas, ou seja, apenas daquelas para quais existem anticorpos disponíveis comercialmente para a realização dos ensaios. Além disso, muitas vezes tal metodologia não é capaz de discriminar entre variantes de algumas proteínas. Sendo assim, técnicas dependentes de anticorpos específicos não possibilitam a determinação do perfil proteômico completo e avaliação da expressão diferencial de múltiplas proteínas, portanto, estudos proteômicos com o objetivo de caracterizar o proteoma da vaginose bacteriana podem contribuir para a identificação de biomarcadores para o diagnóstico e prognóstico de infecções ginecológicas e complicações obstétricas e também predição dos casos com sucesso e falha terapêutica da vaginose bacteriana.

1.4 Proteoma do fluido cérvico-vaginal

Recentemente estudos com o objetivo de determinar a composição proteômica do conteúdo cérvico-vaginal têm sido realizados e já identificaram grande parcela das proteínas presentes nesse material.[1,3,66-73]

Mais de 800 proteínas diferentes já foram identificadas[1,3,66-73] e segundo a

classificação funcional de acordo com os processos biológicos nos quais estão envolvidas, a maioria está relacionada ao metabolismo e à imunidade e defesa contra patógenos.[66] Quando avaliadas em relação à localização, a maioria das proteínas é proveniente do citoplasma ou da região extracelular.[66] Das proteínas identificadas em maior abundância nesse material, podemos citar as proteínas S100A9 e S100A8, que formam o heterodímero calprotectina, um peptídeo que sequestra o íon zinco, inibindo o crescimento bacteriano, sendo observada expressão dessas proteínas tanto em processos fisiológicos como gravidez e parto quanto em condições patológicas, como no câncer cervical.[74] Além dessas proteínas provenientes do transudato local, também é encontrada grande quantidade de proteínas provenientes da descamação de células epiteliais e, portanto, intracelulares e derivadas dos processos metabólicos celulares e do citoesqueleto.[75]

No entanto, a composição do proteoma cérvico-vaginal difere entre os estudos já realizados e, conforme revisado por Zegels et al.,[75] varia de acordo com a população estudada e metodologia empregada. A quantidade das proteínas extracelulares e relacionadas à imunidade tende a ser relativamente menor em estudos que avaliaram um maior tamanho amostral, já que o número total de proteínas identificadas é maior. Dessa forma, tendo em vista a diminuição proporcional das proteínas relacionadas à imunidade nesses trabalhos dificulta a identificação de biomarcadores específicos.[75]

Outro aspecto que contribui negativamente para a determinação de biomarcadores fluido cérvico-vaginal em condições patológicas do trato genital inferior é a grande diferença nas abundâncias relativas das proteínas identificadas nesse material. Tendo em vista que algumas proteínas no fluido cérvico-vaginal são muito abundantes, o sequenciamento desse grande número de peptídeos em espectrômetro de massas diminui a eficiência de detecção daqueles peptídeos derivados de proteínas menos abundantes,

exigindo um constante aperfeiçoamento das técnicas proteômicas visando o aumento da sensibilidade e o poder de resolução desta metodologia. De fato, o desenvolvimento de espectrômetros de massas de alta sensibilidade e resolução permitiu a identificação de mais proteínas, possibilitando caracterizar grande parte do proteoma associado a condições patológicas e consequentemente a descoberta de biomarcadores para tais condições, como por exemplo, para o HIV.[70,76,77]

Alguns estudos de proteômica foram realizados com o objetivo de caracterizar o perfil proteômico associado a condições patológicas que comumente afetam o trato genital e possuem sérias consequências já bem conhecidas e estabelecidas, como as infecções sexualmente transmissíveis pelo Papilomavirus humano (HPV) e HIV. Tais estudos demonstraram significativas mudanças no perfil proteômico de mulheres com tais condições em relação àquelas com ausência de infecção. No caso do HIV, Buergener et. al[76] avaliaram indivíduos soronegativos que são expostos frequentemente ao HIV, como por exemplo, profissionais do sexo, hemofílicos, filhos de mães infectadas por HIV, usuários de drogas intravenosas e soronegativos que possuem parceiros soropositivos e demonstram diferenças significativas na expressão de 15 proteínas, dentre as quais algumas envolvidas na resposta imune como antiproteinases da família B de serpina e também cistatina A. A identificação dessas proteínas contribuiu para outros trabalhos que tem como objetivo a identificação de biomarcadores para prevenção do HIV.[78] Já com relação ao HPV, a maioria dos estudos de proteômica foram realizados a partir de biópsias dos tecidos de mulheres com câncer cervical para avaliação do prognóstico.[79-82] Bae et. al[79] detectaram um total de 35 proteínas no carcinoma de células escamosas, dessa 12 já eram conhecidamente associadas à presença do tumor, enquanto que as demais 21 foram descritas pela primeira vez e propiciou a caracterização do total de proteínas diferencialmente expressas nessa

condição.

Considerando as recentes evidências da associação entre o perfil proteômico cérvico-vaginal e as condições patológicas do trato genital inferior, bem como as sérias implicações da vaginose bacteriana para a saúde reprodutiva da mulher, mais estudos são necessários para a caracterização completa do proteoma associado a essa alteração de microbiota e o padrão de resposta ao seu tratamento. Tais estudos deverão contribuir com a determinação de biomarcadores para identificação de falha terapêutica da vaginose bacteriana, podendo nortear um futuro tratamento individualizado dessa condição.

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Resumo

3. RESUMO

A vaginose bacteriana é o tipo mais comum de flora vaginal anormal e pode ser definida pela diminuição, ou mesmo depleção, dos lactobacilos vaginais. Tal condição está associada ao aumento do risco de parto prematuro e aquisição de diversas infecções sexualmente transmissíveis. A eficácia a curto prazo do tratamento da vaginose bacteriana com metronidazol é baixa. Portanto, o objetivo desse trabalho foi caracterizar o proteoma do fluido cérvico-vaginal de mulheres com vaginose bacteriana e comparar o perfil proteômico entre as mulheres que foram tratadas com sucesso em relação àquelas que falharam em restabelecer a microbiota lactobacilar após 7 dias de metronidazol. A presença da vaginose bacteriana foi definida de acordo com os critérios de Nugent após coloração de Gram dos esfregaços vaginais. Os perfis proteômicos do fluido cérvico-vaginal foram determinados utilizando a metodologia de *shotgun* LC MS/MS. As análises comparativas do proteoma das 38 mulheres com vaginose bacteriana e 39 com microbiota vaginal normal identificaram e determinaram a abundância relativa de 116 proteínas. Entre elas, catepsina G e a região BRO da cadeia pesada V-III de imunoglobulina foram exclusivas de vaginose bacteriana e o inibidor de elastase de leucócitos, involucrina e a proteína associada a diferenciação de neuroblastos AHNAK exclusivas de microbiota vaginal normal. Além disso, 20 (17.2%) proteínas foram diferencialmente expressas na vaginose bacteriana, das quais 9 são envolvidas na resposta imune. Entretanto, a comparação do proteoma do fluido cérvico-vaginal das 24 mulheres com vaginose bacteriana que foram tratadas com sucesso e 11 que persistiram com esta condição após o tratamento com metronidazol não apresentou diferença. Portanto, pudemos demonstrar que a vaginose bacteriana altera significantemente o proteoma local, mas o perfil proteômico cérvico-vaginal do hospedeiro não influencia a resposta ao tratamento com metronidazol.

Artigo científico

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Proteomic aspects of bacterial vaginosis and response to metronidazole treatment

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Proteomic aspects of bacterial vaginosis and response to metronidazole treatment

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ABSTRACT

Bacterial vaginosis is the most common type of abnormal vaginal flora and defined by the depletion of vaginal lactobacilli. This condition increases the risk of premature labor and acquisition of several sexually transmitted infections. Short-term efficacy of bacterial vaginosis metronidazole treatment is low. Thus, we aimed to characterize the cervicovaginal fluid proteome of women with bacterial vaginosis and to compare the proteomic profile between women who were successfully treated with those who failed to reestablish lactobacillar flora after the 7-days course of metronidazole. Presence of bacterial vaginosis was defined according to Nugent criteria on Gram-stained vaginal smears. Proteomic profile of cervicovaginal fluids were determined using shotgun LC MS/MS. Comparative analysis of the proteome of 38 women with bacterial vaginosis and 39 with normal vaginal flora identified and determined the relative abundance of 116 proteins. Among them, cathepsin G and Ig heavy chain V-III region BRO were exclusive of bacterial vaginosis while leukocyte elastase inhibitor, involucrin and neuroblast differentiation-associated protein AHNAK of normal flora. Moreover, 20 (17.2%) proteins were differentially expressed in bacterial vaginosis, of which 9 are involved in immune response. However, the cervicovaginal proteome of 24 women with bacterial vaginosis who were successfully treated and 11 who persisted with this condition did not differ between each other. Therefore, we showed that bacterial vaginosis significantly changes the local proteome, but cervicovaginal host's proteins do not influence the response to metronidazole treatment.

Key words: bacterial vaginosis, metronidazole, LC MS/MS.

INTRODUCTION

Bacterial vaginosis is the most common type of abnormal vaginal flora in reproductive aged women.[1,2] This condition is characterized by replacement of the normal lactobacilli-dominated flora by an overgrowth of anaerobic bacteria.[3] Prevalence of bacterial vaginosis is approximately 30%, but only half of the women affected is symptomatic reporting an abnormal vaginal discharge with odor.[1] Several gynecological and obstetric complications are associated with bacterial vaginosis, such as the increased risk of pelvic inflammatory disease, post-surgical infection, acquisition of sexually transmitted infections (STI), including human immunodeficiency virus (HIV), preterm labor, preterm premature rupture of membranes and low birth weight.[4-9]

The etiology of bacterial vaginosis is defined as polymicrobial due to the high heterogeneity of bacterial species associated with this condition, among which important synergistic relations are observed.[10-12] Despite its polymicrobial feature, the conventional treatment for bacterial vaginosis recommended by Centers for Disease Control and Prevention (CDC) consists in 500 mg oral metronidazole twice a day for 7 consecutive days.[13] Although benefits of the treatment for bacterial vaginosis have been demonstrated, it is still challenging in the clinical practice.[14,15] Short-term effectiveness of metronidazole treatment is approximately 70%.[16] Only few studies assessed long term response to treatment, but they found cure rates of 30% after 6 months of end of therapy and only 42% after one year.[16,17]

Although no conclusive evidence has been demonstrated, the low efficacy of the treatment of bacterial vaginosis may be associated with the high diversity of bacterial composition among the cases.[18] Another factor that remains undetermined is whether the host immune response to bacterial vaginosis influences its treatment outcome. In fact, the

presence of bacterial vaginosis leads to changes in cervicovaginal levels of several immune mediators, such as interleukin (IL)-1beta, IL-4, IL-6, IL-10, IL-8, IL-3, IL-7, IL-12, human beta defensins, granulocyte-macrophage colony-stimulating factor and lactoferrin, when compared to women with normal vaginal flora.[19] However, none of these mediators are associated with the response to the treatment of bacterial vaginosis. Therefore, studies focusing on a broader combination of immune mediators in bacterial vaginosis may contribute to find biomarkers that could be used to identify those cases of bacterial vaginosis treatment failure.

In fact, some studies have been recently performed aiming to determine the proteome of cervicovaginal fluid.[20-22] They were able to identify a large number of proteins present in this type of sample and also revealed that a significant portion of the proteins identified is related to host's immune response and defense against pathogens.[20-22] Currently knowledge on cervicovaginal fluid proteome of bacterial vaginosis by shotgun proteomic analysis is limited and the relation between its proteome and treatment outcome has not been evaluated so far.

Thus, in this study we aimed to assess the proteome cervicovaginal fluid of women with bacterial vaginosis and to compare with those with normal vaginal flora. Secondarily, we compared the cervicovaginal proteome of those cases in which treatment of bacterial vaginosis was effective with those with treatment failure.

MATERIAL AND METHODS

Study design and subjects

From April 2013 to March 2014, non-pregnant reproductive aged women attending

one unit of Primary Health Care (Botucatu, São Paulo, Brazil) for routine cervical cancer screening were invited to participate of this prospective study that was reviewed and approved by the Ethics Committee Board of Botucatu Medical School, São Paulo State University (Protocol 478.483). Women were not included if they reported urinary loss, menstrual period, antibiotics in the previous 30 days and sexual intercourse in the last 48 hours. Considering the exclusion criteria for this study, from the total of 309 women initially recruited, those with Nugent intermediate vaginal flora (n=33, 10.7%), vaginal candidosis (n=23, 7.4%), trichomoniasis (n=6, 1.9%), chlamydia endocervicitis (n=22, 7.1%), gonorrhea (n=2, 0.6%), concurrent chlamydia and gonorrhea (n=1, 0.3%) and whose low-quality vaginal smears (n=9, 2.9%) were not considered for proteomic analyses. All subjects were informed about the study aims and signed a consent form. Sociodemographic, behavioral and clinical history data were acquired by applying individually a standardized questionnaire.

Sampling procedures and screening of co-infections

During the physical exam for collecting pap-smear, additional vaginal samples were taken to assess the pH and to perform 10% KOH test (v/v). Microscopic analyses of vaginal smears were performed to detect, by wet-mount, the presence of *Candida* sp. pseudo hyphae and *Trichomonas vaginalis* and after, Gram-staining to classify the vaginal flora using the Nugent scoring system in normal, intermediate and bacterial vaginosis.[23] Assessment of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection was performed by PCR, according to methods previously described in cervical brush samples.[24,25] Additionally, for proteomic analyses, cervicovaginal fluid samples were obtained with 3mL of sterile 0.9% NaCl solution (w/v). The solutions, after being allowed contact with the vaginal wall, were recovered using a sterile plastic pipette, centrifuged at 800 x g for 10 minutes and supernatants aliquoted and stored at -80^{o}C until analysis.

Constitution of study groups and treatment

Microscopic classification of vaginal flora of the 213 women enrolled in the study showed that 81 (38.0%) had bacterial vaginosis and 132 (62.0%) had normal lactobacilli-dominated vaginal flora. Among the 81 women with bacterial vaginosis at enrollment, 38 (46.9%) completed the 7-days regimen of metronidazole and returned at the clinics for follow-up. Thus, we included in the proteomic analyses only the cervicovaginal samples from those 38 women who had bacterial vaginosis at enrollment, performed correctly the treatment with metronidazole and returned at follow-up. As the control group, 39 out of 132 women with normal flora were randomly selected and evaluated concomitantly.

Treatment for bacterial vaginosis was performed with two daily doses of 500 mg metronidazole during 7 days, according to the preconized by CDC.[13] Medication was provided free of charge to all bacterial vaginosis-positive women who were properly instructed by the practitioner on how to perform the treatment. Follow-up visits were scheduled for 45 to 60 days of the end of metronidazole treatment according to the menstrual cycle of each subject. At follow up, before physical examination, another standardized questionnaire was applied and women were asked in different ways if they performed the treatment during the 7 days, had sexual intercourse in the progress of treatment, skipped any daily doses due to gastric discomfort or had forgotten the medication at some point, in order to assure that treatment was correctly performed in all subjects.

Additionally, at follow-up, mid-third vaginal swabs were obtained for Nugent classification of the vaginal flora in order to identify the cases in which bacterial vaginosis metronidazole treatment was successful and those women who persisted with bacterial vaginosis. Among the 38 women who completed correctly the treatment we were able to

identify 24 (63.2%) women who restored the lactobacilli-dominated flora after treatment and 11 (28.9%) persisted with bacterial vaginosis. The remaining 3 (7.9%) women who completed the treatment had vaginal candidosis and therefore were also excluded from proteomic analyses. Cervicovaginal fluid proteome between the groups with treatment success and failure were compared using the fluid samples obtained at the time of enrollment.

Total protein quantitation

Total protein concentration of cervicovaginal fluid was determined using Bradford method with the commercial kit BioRad Protein Assay (Bio-Rad Laboratories, Inc.), using a two-fold dilution standard-curve of bovine serum albumin starting at a 1mg/mL concentration.[26]

In-solution digestion

At digestion step, a solution of 8M urea was added to the sample at 1:1 and, subsequently, dithiothreitol (DTT) was added at 5mM final concentration. After incubating for 25 minutes at 56^{o}C, an alkylation step was performed with 14 mM iodoacetamide (IAA), followed by another incubation step of 30 minutes at room temperature avoiding light exposure. To quench the free IAA, samples were subjected to a new incubation step of 15 minutes with 5mM DTT. Finally, calcium chloride 1mM was added to the samples, as well as trypsin (Promega, Madison, WI) at 1:5 enzyme:substrate proportion. Digestion was completed after incubation for 16h at 37^{o}C, followed by enzyme inactivation with 0.4% (v/v) trifluoracetic acid (TFA). Digested cervicovaginal samples were centrifuged at 2500g for 10 minutes and supernatants were recovered.

Mass spectrometry analyses

Before mass spectrometry, digested proteins were desalted using Sep Pack C_{18}

columns (Waters, Mildford, MA). A liquid chromatrography-tandem mass spectrometry (LC-MS/MS) was performed using Q-Tof PREMIER API mass spectrometer (MicroMass/Waters). An aliquot of 4.5 µl of digested proteins was inject by analytic column C18 1.7 µm BEH 130 (100 µm x 100 mm) RP-UPLC (nanoAcquity UPLC, Waters) coupled with the nano-electrospray tandem mass spectrometer at a flow rate of 600 nl/min. The instrument was operated in MS positive mode, data continuum acquisition from m/z 100–2,000Da at a scan rate of 1 s and an interscan delay of 0.1 s. Search for peptide identity from LC-MS/MS experiments was performed against Human Uniprot Database (88,820 sequences; 35,204,890 residues) using Mascot Distiller v.2.3.2.0, 2009 (Matrix Science, Boston, MA) set with carbamidomethyl–cys as fixed modification (monoisotopic mass 57.0215Da), methionine oxidation as variable modification (monoisotopic mass 15.9949) and 0.1Da MS and MSMS fragment tolerances.

Statistical analysis

Comparison of discrete and continuous sociodemographics and clinical variables between the women with normal vaginal flora and bacterial vaginosis was performed, respectively, by Chi-squared and the non-parametric Mann-Whitney test using GraphPad Prism 5.0 software (GraphPad, San Diego, CA) with $P<0.05$ considered as significant. For the protein quantitation, the .dat files from Mascot were analyzed using Scaffold Q+ software (version 4.3.4, Proteome Software) and the quantitative value (normalized spectral counts) obtained was used to compare by T-test the relative abundance of peptides between the groups ($P<0.05$ as significant). Scaffold Q+ software was also used for gene ontology analysis, considering, minimum similarity of 95%.

RESULTS

Sociodemographic, behavioral and clinical data from the 213 women enrolled in the

study are displayed in **Table 1**. When variables were compared between the two groups most of them did not differ according to the type of flora. However, we found that self-reporting as white colored-skin (P=0.03) and living in a marital union (P<0.001) were protective against bacterial vaginosis. Some characteristics were close to reach statistical significance, such as previous episode of bacterial vaginosis that tend to be more frequent in women with bacterial vaginosis (P=0.05) and use of hormonal contraception in the last 12 months that was closely protective against bacterial vaginosis (P=0.05). Regarding the clinical findings at time of enrollment, vaginal pH differed between the groups (P<0.001), with highest values in bacterial vaginosis. The number of samples with positive or doubtful result at 10% KOH test and the number of women who complained of abnormal vaginal discharge and odor also differed between the groups, being more frequent in bacterial vaginosis (P<0.001).

A total of 116 proteins were identified by proteomic analysis of cervicovaginal fluid from 38 women with bacterial vaginosis at enrollment and 39 with normal vaginal flora. , (**Table S1)**. For determining the proteomic profile of bacterial vaginosis, we compared the group of women with this condition with those with normal flora. We found that among the 116 proteins identified, 2 of them, Cathepsin G and Ig heavy chain V-III region BRO were exclusively detected in bacterial vaginosis, while 3 (leukocyte elastase inhibitor, involucrin and neuroblast differentiation-associated protein AHNAK) were only found in normal vaginal flora (**Table 2**). Cathepsin G and Ig heavy chain V-III region BRO were found in, respectively, 7 (18.4%) and 4 (10.5%) women with bacterial vaginosis, while leukocyte elastase inhibitor was detected in 7 (17.9%), involucrin in 3 (7.7%) and neuroblast differentiation-associated protein AHNAK in 2 (5.1%) of the 39 women with normal vaginal flora (**Table S1**).

Quantitative analysis of normalized spectra between bacterial vaginosis and normal flora showed a total of 20 (17.2%) proteins that significantly differed between the groups (**Table 2**)**.** We could observe that among the total of proteins differently expressed, 9 (45.0%) play a role in the immune response and defense against pathogens. Among these immunity-related proteins that were differently expressed we found haptoglobin 25 times more expressed in bacterial vaginosis, kaliocin-1 and neutrophil elastase 4 times more expressed, cathepsin G, Ig lambda-2 chain, neutrophil elastase, protein S100-A8 and Ig heavy chain V-III that were also overexpressed in bacterial vaginosis, while cluster of serpin B3 and leukocyte elastase inhibitor were more abundant in normal flora. These differently-expressed proteins were also classified according to biological process, cellular component and molecular function (**Figure 1**). We observed that most of them are involved in cellular processes (16%.0), are located in the extracellular region (21.0%) or cytoplasm (20.0%) and present molecular function (35.0%) or binding activity (31.0%).

The 38 women with bacterial vaginosis that completed the treatment with metronidazole and returned for follow-up were separated into two groups according to the pattern of vaginal flora exhibited after 45 days of the end of treatment, except 3 women that were excluded as they returned with vaginal candidosis. Thus, considering the proteomic analysis of cervicovaginal fluids of 11 women who persisted with bacterial vaginosis and 24 who were successfully treated, a total of 87 proteins could be identified (**Table S2**). No statistically significant difference was detected between the relative abundance of proteins in these 2 groups. However, we found that apoliprotein A-I and cathepsin G were exclusively detected in, respectively, 3 (12.5%) and 5 (20.8%) women successfully treated with metronidazole (Data not shown).

Table 1. Sociodemographic, behavioral and clinical characteristics of 213 women whose samples were included in the study analysis, distributed according to their pattern of vaginal flora.

Variables	Normal flora (n=132)	Bacterial vaginosis (n=81)	P
Age, median (range), years[£]	34 (17-51)	31 (17-48)	0.47
Race (self-defined), n (%)[∫]			
Nonwhite (n=90)	48 (53.3)	42 (46.7)	0.03
White (n=123)	84 (68.3)	39 (31.7)	
Marital status, n (%)[∫]			
Single (n=70)	35 (50.0)	35 (50.0)	<0.001
Married (n=143)	97 (67.8)	46 (32.2)	
Years at school, median (range)[£]	9 (0 - 16)	8 (0 - 15)	0.10
Remunerated activity, n (%)[∫]			
Yes (n=121)	75 (62.0)	46 (38.0)	0.99
No (n=92)	57 (62.0)	35 (38.0)	
Smoking habit, n (%)[∫]			
Yes (n=43)	22 (51.2)	21 (48.8)	0.10
No (n=170)	110 (64.7)	60 (35.2)	
Sex partners, prior 12 months, n (%)[∫]			
0 or 1 (n=188)	120 (63.8)	68 (36.2)	0.13
2 or more (n=25)	12 (48.0)	13 (52.0)	
Number of vaginal intercourse/week, median (range) [£]	2 (0-7)	2 (0-7)	0.90
Previous BV, n (%)[∫]			
Yes (n=100)	55 (55.0)	45 (45.0)	0.05
No (n=113)	77 (68.1)	36 (31.9)	
Previous STD, n (%)[∫]			
Yes (n=17)	10 (58.8)	7 (41.2)	0.78
No (n=196)	122 (62.2)	74 (37.8)	
Consistent condom use, n (%)[∫]			
Yes (n=71)	43 (60.7)	28 (39.4)	0.76
No (n=142)	89 (62.7)	53 (37.3)	
Hormonal contraception use, prior 12 months, n (%)[∫]			
Yes (n=94)	65 (69.1)	29 (30.9)	0.05
No (n=119)	67 (56.3)	52 (43.7)	
Parity, n (%)[∫]			
0 (n=45)	30 (66.7)	15 (33.3)	0.53
≥1 (n=168)	102 (60.7)	66 (39.3)	
Vaginal pH, median (range)[£]	4.7 (4.0-5.0)	4.7 (4.0-7.0)	<0.001
KOH test, n (%)[∫]			
Positive or doubtful (n=136)	66 (48.5)	70 (51.4)	<0.001
Negative (n=77)	66 (85.7)	11 (14.3)	
Complaints, n (%)[∫]			
Discharge (n=90)	42 (46.7)	48 (53.3)	<0.001
Odor (n=54)	22 (40.7)	32 (59.2)	<0.001
Itching (n=31)	19 (61.3)	12 (38.7)	0.93

[£]Mann Whitney test, p<0.05 considered as significant;
[∫]Chi-squared test, p<0.05 considered as significant.

Table 2. Proteins differentially expressed in the cervicovaginal fluids of women with bacterial vaginosis and normal flora.

Proteins	**Normal** Mean (SD)	**Bacterial vaginosis** Mean (SD)	**P****	**Fold Change**
Leukocyte elastase inhibitor	0.22 (0.51)	-	0.01	-
Involucrin	0.42 (1.52)	-	0.09	-
Neuroblast differentiation-associated protein AHNAK	0.15 (0.85)	-	0.28	-
Cathepsin G	-	0.30 (0.72)	0.01	-
Ig heavy chain V-III region BRO	-	0.56 (1.75)	0.04	-
Small proline-rich protein 3	17.27 (11.51)	3.34 (6.42)	<0.0001	0.20
Periplakin	2.98 (2.67)	0.62 (1.31)	<0.0001	0.20
Cluster of Cornifin-B	7.14 (8.25)	1.54 (3.51)	<0.01	0.20
Cellular retinoic acid-binding protein 2 (Fragment)	0.31 (0.47)	0.02 (0.13)	<0.01	0.07
Cluster of Fatty acid-binding protein, epidermal	3.07 (1.93)	1.53 (2.18)	<0.01	0.50
Cluster of Serpin B3	3.67 (3.98)	1.31 (2.20)	<0.01	0.40
Serum albumin	26.59 (25.10)	48.24 (33.77)	<0.01	1.80
Glyceraldehyde-3-phosphate dehydrogenase	0.84 (1.22)	0.16 (0.56)	<0.01	0.20
Kaliocin-1	1.07 (1.71)	4.48 (6.90)	<0.01	4.20
Cystatin-A	1.92 (2.72)	0.39 (1.87)	<0.01	0.20
Protein S100-A11	1.80 (1.53)	1.00 (1.23)	0.01	0.60
Junction plakoglobin	2.57 (2.32)	1.41 (1.86)	0.02	0.50
Ig lambda-2 chain C regions	1.49 (1.77)	2.45 (1.88)	0.02	1.60
Neutrophil elastase	0.14 (0.41)	0.59 (1.13)	0.02	4.20
Isoform 2 of Annexin A2	3.83 (4.01)	2.17 (2.76)	0.04	0.60
Haptoglobin	0.02 (0.11)	0.46 (1.34)	0.04	25.00
Protein S100-A8	2.93 (1.83)	4.74 (5.14)	0.04	1.60

* Mean (SD: standard deviation) of normalized total spectra of peptides.
** t-test; P<0.05;

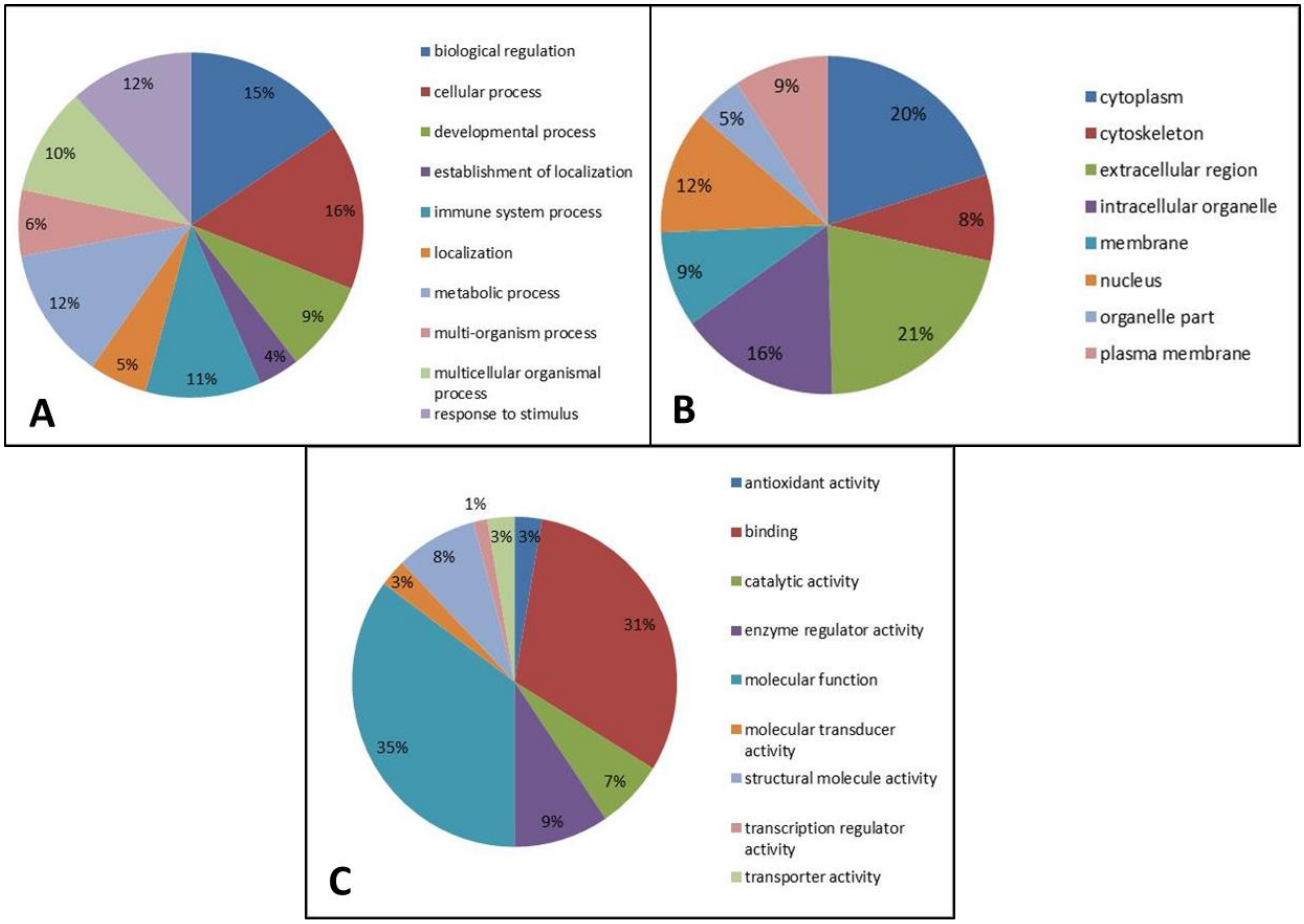

Figure 1. Pie charts representing the gene ontology categorization of the proteins differentially expressed identified by LC-MS/MS between women with normal vaginal flora and with bacterial vaginosis. Classification according to: A) biological process, B) cellular component and C) molecular function.

DISCUSSION

This study is the first to describe the full proteomic profile of cervicovaginal fluid of women with bacterial vaginosis comparing the proteome of women who were successfully treated with women who failed to reestablish lactobacillar flora after 7-days metronidazole

regimen, as recommended by CDC.[13] The inclusion criteria of women in this study were very strict to assure that none of the samples had any concurrent genital tract infection that could interfere in the profile of proteins. Although we were able to successfully identify a large number of differently expressed proteins in the cervicovaginal fluid of women with bacterial vaginosis, no peptide was associated with bacterial vaginosis treatment failure with metronidazole.

The majority of proteins identified in cervicovaginal fluids were from plasma transudate and epithelial cells secretion, which is in agreement with previous study that also evaluated cervicovaginal fluid samples by shotgun proteomic analysis.[27] In fact, our data show that a high proportion of proteins identified in the cervicovaginal fluid is located in the extracellular space and cytoplasm. Intracellular proteins, as well as those proteins involved in epidermis development and keratinization, were also frequently identified, which is explained by the constant desquamation the vaginal epithelium undergoes.[20]

When comparing bacterial vaginosis cervicovaginal fluids with normal vaginal flora, we found that a significant portion of the differently expressed proteins are involved with immune response and defense against pathogens, which is in agreement with the literature.[28] Among proteins with immune function that were differently expressed, most of them were overexpressed in bacterial vaginosis. Additionally, all proteins that were exclusively found in bacterial vaginosis also play a role in immune response. Taken together, these findings demonstrate the cervicovaginal fluid proteome is influenced by the type of flora and that dysregulated proteins are mostly immunity-related.

When evaluating the function of those proteins that were overexpressed or exclusively detected in women with normal vaginal flora, we found two proteins that act as protease inhibitors modulating the immune response: cluster of serpin B3 and leukocyte

elastase inhibitor.[29,30] In addition to these immune-related proteins, we also found that normal vaginal flora has increased abundance of isoform 2 of annexin A2 that is involved in heat-stress response.[31] The remaining proteins that were overexpressed in normal vaginal flora are responsible by epidermis development and differentiation of keratinocytes (involucrin, protein S100 A11, small proline-rich protein 3, periplakin, cornifin A, cornifin B, cellular retinoic acid-binding protein 2 and cluster of fatty acid-binding protein, epidermal).[32-36] So, these data indicate that normal vaginal flora is associated with overexpression of proteins involved in epithelium structure. This finding can be explained by the fact that women with normal lactobacilli-dominated flora have lower vaginal pH, which leads to lysis of epithelial cells and consequently increased desquamation of epithelium, which does not happen in bacterial vaginosis. We also noted that some overexpressed protein in normal flora are localized in the cell junctions, such as junction of plakoglobin, periplakin and cystatin A.[32,34,36] These proteins not only are responsible for signaling in cell to cell communication, but also are essential for epithelial cell adhesion playing an important role as epithelial barrier against the entry of microorganisms.

Of those proteins exclusively found in bacterial vaginosis or overexpressed in this condition, they were all derived from plasma transudate. Among these, serum albumin is the main protein of plasma with binding activity to several molecules.[37] Other overexpressed proteins in bacterial vaginosis include: haptoglobin, which results from hemolysis and have antimicrobial and antioxidant activity modulating many aspects of the acute phase response;[38,39] cathepsin G, a serine protease that have antibacterial activity mainly against lipopolysaccharide (LPS) of Gram-negative bacterium;[40-42] neutrophil elastase, involved in the regulation of innate immunity, inflammation and infection, can inhibits C5a-dependent neutrophil enzyme release and chemotaxis;[41,42] protein S100 A8 that

regulates inflammatory process and immune response, defense response to bacterium;[43] kaliocin-1, transferrins that prevents bacterial biofilm development and interferes with the LPS-stimulated to TLR4 signaling;[44,45] Ig lambda 2 chain C regions and Ig heavy chain V-III region BRO that are proteins with function of antigen binding.[46,47] Therefore, most proteins that were exclusive or overexpressed in bacterial vaginosis are related with immune response and defense against pathogens. Dysregulation of all these inflammatory mediators provides strong evidence that the imbalanced vaginal flora, here accessed in bacterial vaginosis, is able to interfere in several mechanisms of the immune response.

Approximately half of the women that presented bacterial vaginosis at enrollment performed correctly the 7-days course of oral metronidazole and returned for follow-up after 45 days of the end of treatment. The proteomic profile of the cervicovaginal fluid samples accessed at enrollment was compared according to the treatment outcome. We found no difference between cervicovaginal fluid proteome of women who successfully restored a lactobacilli-dominated flora after metronidazole and those who persisted with bacterial vaginosis. Based on this finding, we propose that the differences in the response to the treatment may be more associated with variations in type of bacterial community of bacterial vaginosis and in the microbiological features of the strains.

In fact, bacterial vaginosis is a polymicrobial entity in which important synergistic relations among bacterial species were already revealed. An example is the association between *Prevotella bivia* and *Gardnerella. vaginalis*.[48] *Prevotella* sp. metabolism produce ammonia, which stimulates the growth of *G. vaginalis*, which, in turn, produces amino acids, essential for *P. bivia* metabolism. Moreover, it was recently demonstrated a strong association between *Atopobium vaginae* and some biofilm-producing strains of *G. vaginalis*. These two bacterial species are the main components of vaginal biofilms recovered from

women with bacterial vaginosis. It was already shown that *G. vaginalis* contributes for 60 to 95% and *A. vaginae* up to 40% of biofilm mass.[12] Although biofilms are usually implicate in infections treatment failure, the exact role of vaginal biofilms for the response of metronidazole treatment of bacterial vaginosis remains to be addressed.[49]

Although microbiological aspects may have an important implication for the response of bacterial vaginosis treatment, we should not exclude the participation of host's mediators. We are aware that the absence of differently expressed proteins between success and treatment failure of bacterial vaginosis may be due to a limitation of the technique of LC-MS/MS mass spectrometry that allows only the identification of proteins in their primary structure. Thus, we could not compare the proteomic profile considering the post-translational modifications that the proteins identified may undergo. Another limitation of shotgun proteomic analysis to be considered is that it may detect more efficiently the more abundant peptides. As we failed to identify some proteins that are frequently detected in cervicovaginal fluids such as interleukins or defensins,[19] probably their concentration were below the detection threshold of LC-MS/MS. Studies indicate that the a pre-fractionation step prior to mass spectrometry increases the recovery of low-abundance peptides[50,51] and it should be considered for further proteomic analysis of cervicovaginal fluid for bringing new insights regarding the participation of host's proteome in the response of bacterial vaginosis treatment. Despite the limitations mentioned of the proteomic methods adopted in our study, shotgun LC-MS/MS has been widely used and accepted in world literature to characterize the human proteome.[20,26,27]

Most of the current knowledge about the protein content of cervicovaginal fluid is based on studies that investigated specific markers, mainly involved in the host immune response.[19] Thus, proteomic studies are necessary to fully characterize the proteome

associated with abnormal vaginal flora and consequently to allow the identification of biomarkers for infections and treatment outcomes. This will contribute for developing new strategies for maintenance of a healthy vaginal environment and, consequently, for reducting gynecologic and obstetric-associated complications.

CONCLUSION

Our findings show that proteomics of cervicovaginal fluid is strongly affected by the presence of bacterial vaginosis and the treatment outcome of this condition may be more influenced by its microbiological aspects rather than host´s proteomic profile. However, further studies using higher resolution proteomic techniques are still necessary to completely elucidate the role of human proteins for the response to treatment of bacterial vaginosis.

ASSOCIATED CONTENT

Supporting information

Table S1. List of identified proteins and number of positive cervicovaginal fluid samples of 38 women with bacterial vaginosis and 39 with normal vaginal flora.

Table S2. List of identified proteins and number of positive cervicovaginal fluid samples of 35 women with bacterial vaginosis who were successfully treated with metronidazole (n=24) or had persistent bacterial vaginosis (n=11).

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Notes

The authors declare no conflict of interest.

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Table S1. List of identified proteins and number of positive cervicovaginal fluid samples of 38 women with bacterial vaginosis and 39 with normal vaginal flora.

Protein Name*	Accession Number**	Molecular Weight	Number of Positive Samples	
			Normal	Bacterial vaginosis
Serum albumin	sp|P02768|ALBU_HUMAN	69 kDa	35	38
Protein S100 A8	S10A8_HUMAN	11 kDa	38	36
Protein S100-A9	S10A9_HUMAN	13 kDa	37	36
Cluster of Ig gamma-1 chain C region	IGHG1_HUMAN [3]	36 kDa	35	36
Ig gamma-1 chain C region	IGHG1_HUMAN	36 kDa	35	35
Annexin A1	ANXA1_HUMAN	39 kDa	39	34
Cluster of Keratin, type I cytoskeletal 13	sp|P13646|K1C13_HUMAN [4]	50 kDa	39	34
Cluster of Keratin, type II cytoskeletal 4	K2C4_HUMAN [11]	57 kDa	39	34
Ig kappa chain C region	IGKC_HUMAN	12 kDa	34	34
Keratin, type I cytoskeletal 13	sp|P13646|K1C13_HUMAN	50 kDa	39	33
Keratin, type II cytoskeletal 6A	K2C6A_HUMAN	60 kDa	39	33
Keratin, type II cytoskeletal 6C	K2C6C_HUMAN	60 kDa	39	33
Keratin, type II cytoskeletal 5	K2C5_HUMAN	62 kDa	37	33
Ig gamma-3 chain C region	IGHG3_HUMAN	41 kDa	32	33
Ig lambda-2 chain C region	LAC2_HUMAN (+1)	11 kDa	22	33
Keratin, type II cytoskeletal 6B	K2C6B_HUMAN	60 kDa	39	32
Keratin, type II cytoskeletal 4	K2C4_HUMAN	57 kDa	39	32
Keratin, type II cytoskeletal 2 epidermal	K22E_HUMAN	65 kDa	32	31
Ig gamma-2 chain C region	IGHG2_HUMAN	36 kDa	24	31
Keratin, type II cytoskeletal 1	K2C1_HUMAN	66 kDa	36	30
Neutrophil defensin 1	DEF1_HUMAN (+1)	10 kDa	23	28
Ig gamma-4 chain C region	IGHG4_HUMAN	36 kDa	20	27
Keratin, type I cytoskeletal 10	K1C10_HUMAN	59 kDa	33	26
Cornulin	CRNN_HUMAN	54 kDa	32	26
Heat shock protein beta-1	HSPB1_HUMAN	23 kDa	31	26
Isoform 2 of Annexin A2	sp|P07355-2|ANXA2_HUMAN (+1)	40 kDa	30	25
Small proline-rich protein 3	F5GZ12_HUMAN (+1)	17 kDa	39	23
Neutrophil gelatinase-associated lipocalin	H9KV70_HUMAN (+1)	23 kDa	23	22
Keratin, type I cytoskeletal 16	K1C16_HUMAN	51 kDa	22	22
Kaliocin-1	E7ER44_HUMAN (+2)	78 kDa	14	22
Cluster of Fatty acid-binding protein, epidermal	FABP5_HUMAN	15 kDa	37	21
Fatty acid-binding protein, epidermal	FABP5_HUMAN	15 kDa	37	21
Protein S100-A11	S10AB_HUMAN	12 kDa	30	20
Junction plakoglobin	PLAK_HUMAN	82 kDa	27	20
Cluster of Actin, cytoplasmic 1	ACTB_HUMAN [5]	42 kDa	26	20
Cluster of Alpha-actinin-4	ACTN4_HUMAN	105 kDa	23	20
Alpha-actinin-4	ACTN4_HUMAN	105 kDa	23	20
Cluster of 14-3-3 protein sigma	sp|P31947|1433S_HUMAN [3]	28 kDa	27	19
14-3-3 protein sigma	sp|P31947|1433S_HUMAN	28 kDa	27	19
Actin, cytoplasmic 1	ACTB_HUMAN (+2)	42 kDa	25	19
Cluster of Serpin B3	sp|P29508|SPB3_HUMAN [2]	45 kDa	29	18
Serpin B3	sp|P29508|SPB3_HUMAN	45 kDa	28	18
Cluster of Ig alpha-1 chain C region	IGHA1_HUMAN	38 kDa	13	18
Ig alpha-1 chain C region	IGHA1_HUMAN	38 kDa	13	18

Cystatin-B	CYTB_HUMAN	11 kDa	29	17
Glutathione S-transferase P	GSTP1_HUMAN	23 kDa	24	16
Alpha-enolase	sp|P06733|ENOA_HUMAN	47 kDa	13	16
14-3-3 protein zeta/delta	1433Z_HUMAN (+1)	28 kDa	23	15
Actin, alpha cardiac muscle 1	ACTC_HUMAN (+1)	42 kDa	22	15
Isoform H14 of Myeloperoxidase	sp|P05164-2|PERM_HUMAN (+2)	74 kDa	15	15
Protein S100 A7	S10A7_HUMAN	11 kDa	6	15
Cluster of Cornifin-B	SPR1B_HUMAN [2]	10 kDa	37	14
Desmoplakin	sp|P15924|DESP_HUMAN	332 kDa	20	14
Histone H2B	B4DR52_HUMAN (+16)	18 kDa	20	14
Cluster of Hemoglobin subunit beta	HBB_HUMAN [2]	16 kDa	17	14
Hemoglobin subunit beta	HBB_HUMAN	16 kDa	17	14
Lysozyme C	LYSC_HUMAN	17 kDa	14	14
Cornifin-A	SPR1B_HUMAN	10 kDa	36	13
Cornifin-B	SPR1A_HUMAN	10 kDa	36	13
Galectin-7	LEG7_HUMAN	15 kDa	14	13
Keratin, type I cytoskeletal 19	K1C19_HUMAN	44 kDa	12	13
Serotransferrin	TRFE_HUMAN	77 kDa	8	13
Neutrophil elastase	ELNE_HUMAN	29 kDa	5	12
Periplakin	K7EKI8_HUMAN (+1)	204 kDa	28	10
Hemoglobin subunit delta	HBD_HUMAN	16 kDa	15	10
Keratin, type II cytoskeletal 78	sp|Q8N1N4|K2C78_HUMAN	57 kDa	11	8
Alpha-2-macroglobulin-like protein 1	A2ML1_HUMAN	161 kDa	10	8
Serpin B4	SPB4_HUMAN	45 kDa	20	7
Mucin-5B	E9PBJ0_HUMAN (+1)	597 kDa	3	7
Keratin, type I cytoskeletal 14	K1C14_HUMAN	52 kDa	3	7
Cathepsin-G	CATG_HUMAN	29 kDa	0	7
Cluster of Hemoglobin subunit alpha	HBA_HUMAN [2]	15 kDa	16	6
Hemoglobin subunit alpha	HBA_HUMAN	15 kDa	15	6
Isoform 2 of Interleukin-1 receptor antagonist protein	sp|P18510-2|IL1RA_HUMAN (+3)	18 kDa	13	6
Isoform 1 of Plakophilin-1	sp|Q13835-2|PKP1_HUMAN (+1)	80 kDa	8	6
Haptoglobin	HPT_HUMAN (+1)	45 kDa	1	6
Cluster of Small proline-rich protein 2A	SPR2A_HUMAN [3]	8 kDa	18	5
Small proline-rich 2A	SPR2B_HUMAN	8 kDa	18	5
Small proline-rich 2B	SPR2A_HUMAN	8 kDa	17	5
Small proline-rich 2E	SPR2E_HUMAN	8 kDa	17	5
HCG1745306, isoform CRA_a	G3V1N2_HUMAN	12 kDa	15	5
Tubulin beta chain	Q5JP53_HUMAN (+2)	48 kDa	11	5
WAP four-disulfide core domain protein 2	sp|Q14508|WFDC2_HUMAN	13 kDa	4	5
Isoform 2 of Guanylate-binding protein 6	sp|Q6ZN66-2|GBP6_HUMAN	34 kDa	3	5
Cluster of Isoform 2 of Heat shock protein HSP 90-alpha	sp|P07900-2|HS90A_HUMAN	98 kDa	10	4
Elongation factor 1-alpha 1	EF1A1_HUMAN (+1)	50 kDa	8	4
Antileukoproteinase	SLPI_HUMAN	14 kDa	7	4
Polymeric immunoglobulin receptor	PIGR_HUMAN	83 kDa	3	4
Ig heavy chain V-III region BRO	HV305_HUMAN	13 kDa	0	4
Cystatin-A	CYTA_HUMAN	11 kDa	24	3
Glyceraldehyde-3-phosphate dehydrogenase	sp|P04406|G3P_HUMAN	36 kDa	17	3
Isoform 3 of Pyruvate kinase PKM	sp|P14618-3|KPYM_HUMAN (+1)	56 kDa	10	3

Tubulin alpha-1C chain	F5H5D3_HUMAN (+3)	58 kDa	6	3
Annexin A3	ANXA3_HUMAN (+1)	36 kDa	3	3
Alpha-1-acid glycoprotein 1	A1AG1_HUMAN	24 kDa	3	3
Cluster of Isoform 3 of Glycodelin	sp|P09466-3|PAEP_HUMAN	10 kDa	1	3
Isoform 3 of Glycodelin	sp|P09466-3|PAEP_HUMAN	10 kDa	1	3
Apolipoprotein A-I	APOA1_HUMAN	31 kDa	1	3
Peroxiredoxin-2	PRDX2_HUMAN	22 kDa	11	2
Isoform 2 of Triosephosphate isomerase	sp|P60174-1|TPIS_HUMAN (+1)	27 kDa	6	2
Ly6/PLAUR domain-containing protein 3	LYPD3_HUMAN	36 kDa	5	2
Carbonic anhydrase 1	CAH1_HUMAN	29 kDa	1	2
Keratin, type I cytoskeletal 9	K1C9_HUMAN	62 kDa	0	2
Cellular retinoic acid-binding protein 2 (fragment)	Q5SYZ4_HUMAN (+1)	9 kDa	14	1
Histone H4	H4_HUMAN	11 kDa	5	1
Peroxideroxin-6	PRDX6_HUMAN	25 kDa	4	1
Isoform long of Serine protease inhibitor Kazal-type 5	sp|Q9NQ38-3|ISK5_HUMAN	124 kDa	3	1
Myosin-9	sp|P35579|MYH9_HUMAN	227 kDa	2	1
Isoform 2 of Keratin, type II cytoskeletal 8	sp|P05787-2|K2C8_HUMAN (+1)	57 kDa	2	1
Arachidonate 12-lipoxygenase, 12S-type	LOX12_HUMAN	76 kDa	2	1
Leukocyte elastase inhibitor	ILEU_HUMAN	43 kDa	7	0
Isoform 2 of Niban-like protein 1	sp|Q96TA1-2|NIBL1_HUMAN	83 kDa	5	0
Involucrin	INVO_HUMAN	68 kDa	3	0
Neuroblast differentiation-associated protein AHNAK	sp|Q09666|AHNK_HUMAN	629 kDa	2	0
Keratin, type II cytoskeletal 2 oral	K22O_HUMAN	66 kDa	1	0
Keratin, type II cytoskeletal 74	F8W1S1_HUMAN (+1)	59 kDa	1	0

*Descending order of positive cervicovaginal samples of women with bacterial vaginosis;
**Accession number according to the Human UniProt database.

Table S2. List of identified proteins and number of positive cervicovaginal fluid samples of 35 women with bacterial vaginosis who were successfully restored a lactobacilli-dominated flora after metronidazole (n=24) or persisted with bacterial vaginosis (BV) (n=11) after treatment.

Protein Name*	Accession Number**	Molecular Weight	Number of Positive Samples	
			Restored lactobacilli-flora	Persisted with BV
Serum albumin	sp|P02768|ALBU_HUMAN	69 kDa	24	11
Cluster of Ig gamma-1 chain C region	IGHG1_HUMAN [3]	36 kDa	23	11
Protein S100-A8	S10A8_HUMAN	11 kDa	22	11
Protein S100-A9	S10A9_HUMAN	13 kDa	22	11
Ig gamma-1 chain C region	IGHG1_HUMAN	36 kDa	22	11
Ig kappa chain C region	IGKC_HUMAN	12 kDa	20	11
Ig lambda-2 chain C regions	LAC2_HUMAN (+1)	11 kDa	19	11
Annexin A1	ANXA1_HUMAN	39 kDa	21	10
Ig gamma-2 chain C region	IGHG2_HUMAN	36 kDa	18	10
Ig gamma-4 chain C region	IGHG4_HUMAN	36 kDa	14	10
Cluster of Keratin, type II cytoskeletal 4	K2C4_HUMAN [7]	57 kDa	23	9
Keratin, type II cytoskeletal 6A	K2C6A_HUMAN	60 kDa	22	9
Keratin, type II cytoskeletal 6B	K2C6B_HUMAN	60 kDa	22	9
Cluster of Keratin, type I cytoskeletal 10	K1C10_HUMAN [2]	59 kDa	19	9
Cornulin	CRNN_HUMAN	54 kDa	16	9
Keratin, type I cytoskeletal 10	K1C10_HUMAN	59 kDa	15	9
Isoform 2 of Annexin A2	sp|P07355-2|ANXA2_HUMAN (+1)	40 kDa	14	9
Keratin, type I cytoskeletal 13	sp|P13646|K1C13_HUMAN	50 kDa	23	8
Keratin, type II cytoskeletal 5	K2C5_HUMAN	62 kDa	23	8
Keratin, type II cytoskeletal 4	K2C4_HUMAN	57 kDa	22	8
Keratin, type II cytoskeletal 1	K2C1_HUMAN	66 kDa	21	8
Keratin, type II cytoskeletal 2 epidermal	K22E_HUMAN	65 kDa	20	8
Keratin, type I cytoskeletal 16	K1C16_HUMAN	51 kDa	18	8
Heat shock protein beta-1	HSPB1_HUMAN	23 kDa	17	8
Small proline-rich protein 3	F5GZ12_HUMAN (+1)	17 kDa	13	8
Cluster of Actin, cytoplasmic 1	ACTB_HUMAN [7]	42 kDa	11	8
Actin, cytoplasmic 1	ACTB_HUMAN (+2)	42 kDa	10	8
Neutrophil defensin 1	DEF1_HUMAN (+1)	10 kDa	18	7
Ig gamma-3 chain C region	IGHG3_HUMAN	41 kDa	17	7
Junction plakoglobin	PLAK_HUMAN	82 kDa	13	7
Fatty acid-binding protein, epidermal	FABP5_HUMAN	15 kDa	12	7
14-3-3 protein sigma	sp|P31947|1433S_HUMAN	28 kDa	12	7
Cluster of Ig alpha-1 chain C region	IGHA1_HUMAN	38 kDa	10	7
Ig alpha-1 chain C region	IGHA1_HUMAN	38 kDa	10	7
Protein S100-A7	S10A7_HUMAN	11 kDa	8	7
Neutrophil gelatinase-associated lipocalin	H9KV70_HUMAN (+1)	23 kDa	13	6
Alpha-actinin-4	ACTN4_HUMAN	105 kDa	13	6
Protein S100-A11	S10AB_HUMAN	12 kDa	12	6
Serpin B3	sp|P29508|SPB3_HUMAN	45 kDa	12	6
Cystatin-B	CYTB_HUMAN	11 kDa	11	6
Kaliocin-1	E7ER44_HUMAN (+2)	78 kDa	14	5
Glutathione S-transferase P	GSTP1_HUMAN	23 kDa	10	5
Desmoplakin	sp|P15924|DESP_HUMAN	332 kDa	9	5

Cluster of Cornifin-A	SPR1A_HUMAN [2]	10 kDa	8	5
Cornifin-A	SPR1A_HUMAN	10 kDa	8	5
Serotransferrin	TRFE_HUMAN	77 kDa	7	5
Keratin, type I cytoskeletal 19	K1C19_HUMAN	44 kDa	7	5
Keratin, type II cytoskeletal 78	sp|Q8N1N4|K2C78_HUMAN	57 kDa	3	5
Actin, aortic smooth muscle	ACTA_HUMAN (+3)	42 kDa	10	4
14-3-3 protein zeta/delta	1433Z_HUMAN (+2)	28 kDa	9	4
Galectin-7	LEG7_HUMAN	15 kDa	9	4
Histone H2B	B4DR52_HUMAN (+16)	18 kDa	9	4
Cornifin-B	SPR1B_HUMAN	10 kDa	8	4
Cluster of Hemoglobin subunit beta	HBB_HUMAN [2]	16 kDa	8	4
Hemoglobin subunit beta	HBB_HUMAN	16 kDa	8	4
Isoform H14 of Myeloperoxidase	sp|P05164-2|PERM_HUMAN (+2)	74 kDa	8	4
Neutrophil elastase	ELNE_HUMAN	29 kDa	8	3
Lysozyme C	LYSC_HUMAN	17 kDa	8	3
Hemoglobin subunit delta	HBD_HUMAN	16 kDa	6	3
Haptoglobin	HPT_HUMAN (+1)	45 kDa	3	3
Isoform 1 of Plakophilin-1	sp|Q13835-2|PKP1_HUMAN (+1)	80 kDa	4	2
Cluster of Hemoglobin subunit alpha	HBA_HUMAN	15 kDa	3	2
Hemoglobin subunit alpha	HBA_HUMAN	15 kDa	3	2
Guanylate-binding protein 6	F5H7G9_HUMAN (+2)	58 kDa	3	2
Tubulin beta chain	Q5JP53_HUMAN (+5)	48 kDa	3	2
Elongation factor 1-alpha 1	EF1A1_HUMAN (+1)	50 kDa	2	2
Cluster of Small proline-rich protein 2A	SPR2A_HUMAN [2]	8 kDa	2	2
Small proline-rich protein 2A	SPR2A_HUMAN	8 kDa	2	2
Small proline-rich protein 2B	SPR2B_HUMAN	8 kDa	2	2
Ig heavy chain V-III region BRO	HV305_HUMAN	13 kDa	1	2
Periplakin	K7EKI8_HUMAN (+1)	204 kDa	9	1
Polymeric immunoglobulin receptor	PIGR_HUMAN	83 kDa	3	1
Alpha-1-acid glycoprotein 1	A1AG1_HUMAN	24 kDa	2	1
Cluster of Isoform 3 of Glycodelin	sp|P09466-3|PAEP_HUMAN	10 kDa	2	1
Isoform 3 of Glycodelin	sp|P09466-3|PAEP_HUMAN	10 kDa	2	1
Isoform 3 of Pyruvate kinase PKM	sp|P14618-3|KPYM_HUMAN (+1)	56 kDa	2	1
Glyceraldehyde-3-phosphate dehydrogenase	E7EUT5_HUMAN (+2)	28 kDa	2	1
Carbonic anhydrase 1	CAH1_HUMAN	29 kDa	1	1
Ly6/PLAUR domain-containing protein 3	LYPD3_HUMAN	36 kDa	1	1
Peroxiredoxin-2	PRDX2_HUMAN	22 kDa	1	1
Isoform 2 of Keratin, type II cytoskeletal 8	sp|P05787-2|K2C8_HUMAN (+1)	57 kDa	0	1
Cathepsin G	CATG_HUMAN	29 kDa	5	0
Antileukoproteinase	SLPI_HUMAN	14 kDa	4	0
Apolipoprotein A-I	APOA1_HUMAN (+1)	31 kDa	3	0
Cystatin-A	CYTA_HUMAN	11 kDa	3	0
Keratin, type I cytoskeletal 9	K1C9_HUMAN	62 kDa	2	0
Isoform 2 of Triosephosphate isomerase	sp|P60174-1|TPIS_HUMAN (+1)	27 kDa	1	0

*Descending order of positive cervicovaginal samples of women who persisted with bacterial vaginosis after metronidazole treatment;
**Accession number according to the Human UniProt database.

Anexos

5. Anexos

Questionário – Protocolo de atendimento

Dados sócio-demográficos

Nome:..... Data da coleta:.....

Clínica:..... N° registro:..... Idade: .....

Estado civil: ( ) União-estável ( ) Solteira Cor (auto-relato):..... .....

Escolaridade (anos de aprovação escolar)..... Exerce atividade remunerada ( ) Sim ( ) Não

Reside em ( ) zona urbana/cidade ( ) zona rural

Hábitos

Fumante ( ) Sim ( ) Não Número de cigarros/dia (no ultimo ano):.....

Consumo de bebida alcoolica? ( ) Sim ( ) Não

Frequência de relações sexuais (n° relações/semana).....

N° parceiros sexuais (no ultimo ano): ( ) 1 ( ) 2 ( ) > 2

Faz uso de ducha vaginal ( ) Sim ( ) Não

Faz uso de sabonete intimo ( ) Sim ( ) Não

Antecedentes ginecológicos

História de VB: ( ) Sim ( ) Não História de DST: ( ) Sim ( ) Não Qual(is)?.....

Uso de métodos contraceptivos (4 meses anteriores): ( ) Sim ( ) Não Tipo.....

Antecedentes obstétricos

Paridade: ( ) Nulípara ( )Multípara

Intercorrências em gestações anteriores (parto prematuro, rotura prematura de membranas, aborto espontâneo, morte fetal): .....

Queixas atuais

Dor durante relação sexual ( ) Sim ( ) Não Momento:.....

Conteúdo vaginal aumentado ( ) Sim ( ) Não Odor ( ) Sim ( ) Não ( ) Prurido ( ) Sim ( ) Não

Exame físico

pH da parede vaginal :..... *Whiff test*: ( ) Positivo ( ) Negativo ( ) Duvidoso

JEC: ..... Vulvite: ( ) Sim ( ) Não Outros achados.....

Responsável pela coleta

UNIVERSIDADE ESTADUAL PAULISTA
CÂMPUS DE BOTUCATU
FACULDADE DE MEDICINA
Programa de Pós-Graduação em Patologia
BOTUCATU, SP -RUBIÃO JÚNIOR-CEP 18618-000-Fone (014) 3811-6238 ramal 236
pgpato@fmb.unesp.br

Termo de Consentimento Livre e Esclarecido

Convidamos a senhora para participar da pesquisa **"Estudo do microbioma vaginal em mulheres brasileiras em idade reprodutiva"**, que tem por objetivo conhecer os tipos de bactérias que fazem parte da vagina da mulher bem como as quantidades que elas são encontradas. Essa pesquisa será realizada pela Dra. Camila Marconi, supervisionada pela Profa. Dra. Márcia Guimarães da Silva do Departamento de Patologia da Faculdade de Medicina de Botucatu, UNESP, além de outros pesquisadores colaboradores, como Dr. Paulo César Giraldo, Dra. Ana Katherine Gonçalves, Dra. Sandra Helena Morais Leite e do Dr. Newton Sérgio de Carvalho.

Para sua participação será necessário responder as questões da entrevista e realizar um exame ginecológico com as pessoas autorizadas para isso (médicas e enfermeiras) que fazem parte do estudo. Para o exame ginecológico, será necessária a introdução de um aparelho de metal, estéril, conhecido como "bico de pato" (espéculo), que afastará as paredes vaginais, a fim de permitir a visualização das paredes vaginais e do colo do útero, bem como a coleta de amostras (secreção) para exames laboratoriais. O material da parede vaginal e do colo do útero será coletado por meio de um cotonete, para verificação da presença de microrganismos. Esse é um exame ginecológico comum em que o desconforto está relacionado à introdução do aparelho de metal (bico de pato). Esse material coletado será analisado na Universidade de Maryland nos Estados Unidos, pela responsável pelo projeto a Dra. Camila Marconi, portanto seu material será enviado para fora do país, com fins exclusivamente de pesquisa. O material coletado será o suficiente para fazer essa pesquisa, de modo que não haverá sobra de material para ser estocada, nem na Universidade de Maryland nem na UNESP.

Pelo presente instrumento, eu _____,
devidamente esclarecida, ciente dos procedimentos aos quais serei submetida, não restando quaisquer dúvidas a respeito do lido e explicado, declaro estar ciente de que as informações serão utilizadas exclusivamente pelas pesquisadoras, que manterão sigilo sobre minha identidade, inclusive em relação às amostras que serão enviadas para o exterior não serem identificadas com meu nome apenas com um número gerado para a pesquisa. Também estou ciente que os pesquisadores envolvidos nesse estudo estarão disponíveis para responder a quaisquer perguntas e de que **posso retirar este consentimento a qualquer hora sem prejuízo do meu atendimento neste Hospital,** firmo meu **CONSENTIMENTO LIVRE E ESCLARECIDO,** concordando em participar dessa pesquisa. É de meu conhecimento que não receberemos qualquer ajuda financeira para a participação no projeto, entretanto haverá o ressarcimento do transporte para a vinda ao hospital, para coleta do material, quando necessário. Está esclarecido também que esse estudo pode não trazer benefícios imediatos para mim, mas que será importante para o entendimento de como os microrganismos que vivem na vagina podem se agrupar causando alterações vaginais.

Qualquer dúvida adicional, você poderá entrar em contato com o Comitê de Ética em Pesquisa, através do telefone (14) 3811 6143. Esse documento, após a aprovação da CONEP via Plataforma Brasil, será elaborado em 2 vias, sendo uma entregue aos sujeitos da pesquisa e outra será mantida em arquivo do pesquisador.

Botucatu,_____ de _____de 201_

_____

Assinatura da paciente

_____

Dra. Camila Marconi
Rua Camilo Mazoni , nº 944
Jd. Paraíso , Botucatu, 18618-000
Fone: 14 38414272
e-mail: Marconi@fmb.unesp.br

_____

Profª. Drª. Márcia Guimarães da Silva
Rua Izidoro Bertaglia, 746
Jardim Paraíso II Botucatu, 18610-140
Fone: 14 38152417
e-mail: mgsilva@fmb.unesp.br

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Caracterização do microbioma vaginal de mulheres brasileiras em idade reprodutiva.

Pesquisador: Márcia Guimarães da Silva

Área Temática: Pesquisas com coordenação e/ou patrocínio originados fora do Brasil, excetuadas aquelas com copatrocínio do Governo Brasileiro;

Versão: 3

CAAE: 02381512.5.1001.5411

Instituição Proponente: Hospital das Clínicas da Faculdade de Medicina de Botucatu

Patrocinador Principal: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DA NOTIFICAÇÃO

Tipo de Notificação: Outros

Detalhe: Notificação de Sub-projeto

Justificativa: Solicito parecer do Comitê de Ética em Pesquisa da Faculdade de Medicina de

Data do Envio: 29/10/2013

Situação da Notificação: Parecer Consubstanciado Emitido

DADOS DO PARECER

Número do Parecer: 478.483

Data da Relatoria: 02/12/2013

Apresentação da Notificação:

Solicitação de parecer do CEP para aproveitamento de dados do projeto maior "Caracterização do microbioma vaginal de mulheres brasileiras em idade reprodutiva", já aprovado por este CEP (parecer 139.109 de 05/11/2012) e pela CONEP,em sub-estudo.

Objetivo da Notificação:

O sub-projeto "Avaliação proteômica do conteúdo vaginal em resposta ao tratamento da vaginose bacteriana" será dissertação de mestrado no Programa de Pós-Graduação em Patologia da Faculdade de Medicina de Botucatu, UNESP cujas análises já estavam previstas no projeto inicial.

Endereço: Chácara Butignolli , s/n
Bairro: Rubião Junior **CEP:** 18.618-970
UF: SP **Município:** BOTUCATU
Telefone: (14)3880-1608 **E-mail:** capellup@fmb.unesp.br

Avaliação dos Riscos e Benefícios:

Os dados já foram coletados não havendo riscos ao paciente com possiveis resultados para futuras condutas.

Comentários e Considerações sobre a Notificação:

Obrigatoria, pois dados serão utilizados para novo trabalho (previstos no projeto mãe).

Considerações sobre os Termos de apresentação obrigatória:

Não se aplica

Recomendações:

Verificação se no TCLE inicial estava previsto outros estudos

Conclusões ou Pendências e Lista de Inadequações:

O CEP aprovou a solicitação com a ressalva de que no TCLE inicial estivesse previsto outros estudos originários do projeto mãe. Não haverá agora novas coletas com os pacientes.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

O CEP em reunião de 02/12/2013, APROVOU a realização do Sub-Projeto: Avaliação proteômica do conteúdo vaginal em resposta ao tratamento da vaginose bacteriana, que será objeto de Dissertação de Mestrado de Carolina Sanitá Tafner, tendo como orientadora a Profª Drª Camila Marcone e Co-orientação da Profª Drª Márcia Guimarães Silva

OBS: O CEP aprovou a solicitação com a ressalva de que no TCLE inicial estivesse previsto outros estudos originários do projeto mãe. Não haverá agora novas coletas com os pacientes.

BOTUCATU, 04 de Dezembro de 2013

Assinador por:
Trajano Sardenberg
(Coordenador)

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