CEOG Clinical and Experimental Obstetrics & Gynecology

Vaginal microbiota in asymptomatic Brazilian women with HIV

M.K. Figueiredo Facundo[1], C.R. de Souza Bezerra Sakano[2], C.R. Nogueira de Carvalho[3], A.M. de Oliveira Machado[4], N.M. de Góis Speck[1], J. Chamorro Lascasas Ribalta[1]

[1] Gynecological Disease Prevention Nucleus (NUPREV) of the Gynecology Department of the Federal University of São Paulo, São Paulo
[2] Pathology Department, of the Federal University of São Paulo, São Paulo
[3] Gynecology Department of the Federal University of São Paulo, São Paulo
[4] São Paulo Hospital's Central Laboratory, Federal University of São Paulo, São Paulo (Brazil)

Summary

The purpose of this study was to evaluate the prevalence of different microorganisms, and the influence of menstrual cycle, CD4+ cells and viral load in vaginal flora, and compare different diagnosis methods in asymptomatic Human immunodeficiency virus HIV− and HIV+ women. Variables like contraception methods, type of sexual intercourse, and menstrual cycle phase were significant between groups. The clinical evaluation of vaginal pH and type of discharge, besides intraepithelial lesions, do not seem to have influence in microflora. Fresh wet-mount microscopy and bacterioscopy demonstrated no difference. HIV+ presented predominance of *Gardnerella*, *Candida*, *Trichomonas*, and *Mobiluncus* in cervicovaginal cytology, and vaginal culture exhibited higher prevalence of Gram+ and coagulase-negative staphylococci. Fresh wet mount microscopy showed a sensitivity of 88.9%, and the bacterioscopy sensitivity was 75%. Clinical exam specificities were 76.3% and 94.9%, respectively. Asymptomatic HIV+ women may present diversified vaginal microenvironment, possibly making them more prone to pelvic inflammatory disease, sexually transmitted infection (STI), and infertility.

Key words: HIV; HIV-seropositive; Vaginal microenvironment; Bacterial vaginosis; LGT infection; Asymptomatic women.

Introduction

Human immunodeficiency virus (HIV) has been presenting new cases of infections in Brazil. In 2014, the rates recorded 47% of all new cases counted in Latin America, and nearly 734,000 people are living with HIV. The prevalence in women aged 15-49 years old was 0.4% [1], and appear to be more easily infected with HIV than men [2]. The predominant mode of transmission is sexual intercourse, due to diversity in sexual behavior patterns, and variations in biological and behavioral co-factors [3]. Condom use, anal intercourse, male circumcision, and hormonal contraception can implicate in HIV transmission [2]; besides the stage of disease in the HIV infected partner, treatment with antiretroviral drugs, the presence of another sexually transmitted infection (STI), and bacterial vaginosis (BV) may be the most important co-factors [3].

BV is a disorder where some microbiological alterations of vaginal microflora takes place, characterized by decreased *Lactobacillus sp.* and overgrowth of *Gardnerella vaginalis*, together with anaerobes and potentially pathogenic bacteria [4, 5]. BV can be associated with HSV-2, gonorrhea, syphilis, *Trichomonas vaginalis*, and HIV infections [6, 7]. The presence of BV and absence of lactobacilli decrease the H_2O_2 concentration produced by *Lactobacillus sp*., which showed to be protective against HIV and other inflammatory conditions [1, 7]. Furthermore, this disorder can be asymptomatic in approximately half of the women who develops it [4].

Supposedly, there are three different mechanisms for increasing susceptibility to HIV infection in women with BV: disruption of the vaginal epithelium and subsequent transmission of HIV to subepithelium; number reduction of *Lactobacillus* species leading to increased pH and reduced H_2O_2 concentration [1, 8]; significant and reversible alterations in cervical immune cells and local inflammatory cytokines, influencing local HIV replication [9].

In this study, the appraisal of the vaginal microenvironment (VM) in asymptomatic HIV+ women might contribute to a better prognosis and knowledge about BV. The aims of the present study was to determine and evaluate the prevalence of different microorganisms in VM with non-molecular affordable tests, the phase of the menstrual cycle in relation to vaginal flora, the counting of $CD4^+$ cells and viral load, and the comparison of different diagnostic methods effectiveness in asymptomatic HIV+ women.

Materials and Methods

Study population

The transversal case-control observational study was conducted at Gynecological Disease Prevention Nucleus (NUPREV) of the Gynecology Department of the Universidade Federal de São Paulo - UNIFESP/EPM, within the period from October 2008

Revised manuscript accepted for publication December 22, 2015

through April 2010. Ethics and Research Committee of the Universidade Federal de São Paulo UNIFESP/EPM under protocol number 0510/08 approved the study. Written informed consent was obtained from all participants prior to enrollment.

The authors selected 98 women and the age of patients ranged from 17-40 years, that were divided into two groups identified as control or HIV− and case or HIV+. All patients presented no genital symptoms. The non-inclusion criteria consisted in women during the menstrual period, pregnancy and puerperal cycle, non-HIV immunosuppressing conditions as diabetes mellitus or corticoid and antibiotic therapy users, sexual intercourse history, and douching in the last 48 hours. Each group was submitted to a clinical exam, vaginal fresh wet mount microscopy, bacterioscopy, culture, and cytology.

Clinical exam

Briefly, the patients were submitted to an anamnesis and they were conducted to general physical exams, which included gynecological examination using a non-lubricated speculum, exposing the vaginal walls and characterizing the vaginal content related to color, odor, and appearance, following the Amsel standard methods. The Whiff test was performed using KOH 10%, following the standard method when necessary. The pH of vaginal content was measured by color-fixed indicator strips, which was evaluated and compared through the staining on the pH indicator with a measurement range from 0 to 14, previously established, according to manufacturer instructions.

The cervicovaginal swab specimens were collected according to the standard technique for fresh wet mount microscopy, bacterioscopic exam using Gram staining, aerobic bacteria culture, and cytology.

All women underwent colposcopy after initial exams. Patients with colposcopic alterations were forwarded to cervical and/or vaginal biopsy.

Fresh wet mount microscopy

Collected cells were initially prepared for fresh wet mount microscopy by directly spreading cells onto a glass slide, immediately applying a drop of sodium chloride 0.9%, and covering with a coverslip. The samples were observed through an optical microscope and the microorganisms identified.

Bacterioscopy

The samples collected by sterile swab were spread onto a glass slide and dried without fixer, and then were forwarded to the Central Laboratory of Hospital São Paulo. The slide glasses were stained by the Gram method, where initially crystal violet dye was used for one minute, followed by wash. The incubation with lugol 2% lasted one minute and the samples were washed with water. The discoloration occurred by applying acetone-alcohol 30% and washed with water, the samples were incubated in Fuchsin Ziehl, diluted in water 1:10 for 30 seconds, and finally were washed, dried, and examined in optical microscope. The assessment was conducted according to the previously established method in the Central Laboratory of Hospital São Paulo.

Culture

The samples for aerobic culture, equally collected by sterile swab and stored in tubes, were inoculated into chocolate agar PolyViteX (PVX) plate, blood agar - Columbia CNA agar + 5% sheep blood plate, and eosin methylene blue (EMB) agar plate. The samples were incubated in bacteriological incubator for five to seven days. The conditions of incubation for chocolate agar and blood agar was 35 ± 2ºC with 5-10% CO_2, and EMB agar was 35 ± 2ºC. When more than one type of bacterial growth was observed, the specimens were isolated for 24 hours, and were then identified. In aerobic culture some microaerophilic organisms were highlighted.

Some plates showed fungal growth, which were separated and forward to mycological laboratory. The sample for fungal culture was inoculated into Sabouraud's glucose agar Difco and Mycosel agar, and were incubated at 36ºC. The yeast colonies were separated from colonies of filamentous fungi. For bacteria, depending on the morphology, the colonies were selected and the species identified. As for fungi, the colonies were selected and identified by phenotype depending on the morphology.

Cytology

Cervicovaginal swab specimens were collected from vaginal fornix, ectocervix, and endocervix for cytology. The cytological exams were performed according to the standard technique. Collected cells were initially prepared for conventional cytology by directly spreading cells onto a glass slide and immediately immersed in ethyl alcohol 95% for fixation. The samples were immersed in ethanol absolute for 30 minutes, followed by alcohol 70% and alcohol 50% for one minute each. After that, the glass slides were washed with water for one minute, stained with Harris' hematoxylin solution for one minute, and washed again. The samples were immersed in alcohol solution 50% /one minute, alcohol solution 70%/one minute, ethanol absolute /one minute, and stained Orange G 6 solution. Three baths were performed with ethanol absolute for one minute each and followed by Polychrome solution EA 31 for one minute. Subsequently, the samples received three baths of ethanol absolute for one minute each one, xylene , and ethanol absolute solution (half of each) for one minute and only xylene for ten minutes. Finally, two drops of synthetic *Canada balsam* were placed on the glass slides and the samples were observed with microscopy.

Statistical analysis

Analyses were performed using SPSS version 16.0. Continuous values are expressed as mean ± standard deviation and analyzed by the Student *t*-test or Mann-Whitney U- test, when the normality was not observed. Categorical variables are presented as absolute numbers and analyzed by the chi-square test or exact test of Fisher. Two-sided p-values ≤ 0.05 indicate statistical significance.

Results

The analysis included 51 women in the control group ranging from 17 to 40 years old with a mean age of 29.69 ± 7.20 years, and 47 women in the case group ranging from 25 to 40 years old, with a mean of 33.96 ± 3.96 years. The mean of menarche age was 12.90 ± 1.72 years in the control group and 12.87 ± 1.83 years in the case group. Meanwhile, the academic level ($p = 0.04$), contraception ($p < 0.001$), and type of sexual intercourse (vaginal, oral, oral and/or vaginal, vaginal and anal) were statistically significant ($p = 0.04$).

Menstrual cycle was valued in both studied groups; in HIV− women 31.4% were in the first phase, 21.6% were in the second phase, and 47.1% were in the single phase due to hormonal contraception use. In HIV+ women, 27.7% were in the first phase, 61.7% in the second phase, and 10.6% in the single phase. The results were highly significant ($p < 0.001$).

Table 1. — *Frequency of performed essays in 98 asymptomatic women with HIV− and HIV+ and microorganisms detected.*

Groups	Control N (%)	HIV+ N (%)	Total N (%)	*p**
FRESH EXAM				0.39
Clue Cells	11 (21.6)	12 (25.5)	23 (23.5)	
Doderlein	29 (56.9)	24 (51.1)	53 (54.1)	
Trichomonas	- -	3 (6.4)	3 (3.1)	
Hyphae	6 (11.8)	6 (12.8)	12 (12.2)	
Intermediate	5 (9.8)	2 (4.3)	7 (7.1)	
Total	51 (100)	47 (100)	98 (100)	
BACTERIOSCOPY				0.90
Gram+ cocci	13 (25.5)	11 (23.4)	24 (24.5)	
Gram+ bacilli	29 (56.9)	24 (51.1)	53 (54.1)	
Yeasts	1 (2.0)	2 (4.3)	3 (3.1)	
Gram+ and - bacilli	7 (13.7)	9 (19.1)	16 (16.3)	
Gram+ bacilli	1 (2.0)	1 (2.1)	2 (2.0)	
Total	51 (100)	47 (100)	98 (100)	
CYTOLOGY				0.05
Lactobacillus sp.	38 (74.5)	27 (57.4)	65 (66.3)	
Trichomonas	- -	2 (4.3)	2 (2.0)	
Gardnerella	3 (5.9)	8 (17.0)	11 (11.2)	
Cocci	8 (15.7)	4 (8.5)	12 (12.2)	
Candida	1 (2.0)	5 (10.6)	6 (6.1)	
Other	1 (2.0)	1 (2.1)	2 (2.0)	
Total	51 (100)	47 (100)	98 (100)	
CULTURE				0.001
Staphylococcus coagulase	26 (51.0)	25 (53.2)	51 (52.0)	
Staphylococcus aureus	3 (5.9)	2 (4.3)	5 (5.1)	
Gram+ bacteria	- -	10 (21.3)	10 (10.2)	
Escherichia coli	5 (9.8)	1 (2.1)	6 (6.1)	
Doderlein	12 (23.5)	- -	12 (12.2)	
Enterococcus sp.	2 (3.9)	1 (2.1)	3 (3.1)	
Streptococcus B	- -	2 (4.3)	2 (2.0)	
Citrobacter sp.	1 (2.0)	- -	1 (1.0)	
Candida glabrata	1 (2.0)	1 (2.1)	2 (2.0)	
Candida albicans	- -	1 (2.1)	1 (1.0)	
Klebsiella pneumoniae	- -	1 (2.1)	1 (1.0)	
No growth	1 (2.0)	3 (6.4)	4 (4.1)	
Total	51 (100)	47 (100)	98 (100)	

* *p* value determined by chi-square.

Table 2. — *Vaginal flora of HIV− women and distribution according to the counting of CD4+ T cells.*

Culture	$CD4^+$ T - lymphocyte title (cells/mm3) < 200 N (%)	200-500 N (%)	> 500 N (%)	*p** Total N (%)	
Staphylococcus coagulase -	1 (4.0)	12 (48.0)	12 (48.0)	25 (100)	
Staphylococcus aureus	1 (50.0)	-	1 (50.0)	2 (100)	
Gram+ bacteria	1 (10.0)	4 (40.0)	5 (50.0)	10 (100)	
Escherichia coli	-	-	1 (100)	1 (100)	
Enterococcus sp.	-	1 (100)	-	1 (100)	0.496
Streptococcus B	-	-	2 (100)	2 (100)	
Candida glabrata	-	-	1 (100)	1 (100)	
Candida albicans	-	1 (100)	-	1 (100)	
Klebsiella pneumoniae	-	-	1 (100)	1 (100)	
No growth	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)	
Total	4 (8.5)	19 (40.4)	24 (51.1)	47 (100)	

**p* value determined by chi-square.

The analysis of clinical exam presented 86.3% of HIV− and 72.3% of HIV+ women with apparently physiological vaginal discharge. Bacterial vaginosis were detected in 13.7% and 27.7%, respectively. The vaginal pH showed that 45.1% and 34% of control and case patients were < 4.5 and 54.9%, and 66% were > 4.5, respectively.

Fresh wet mount microscopic test presented *clue cells* and *Doderlein* bacilli in more than half the women. The *T. vaginalis*, fungus shaped hyphae, intermediate flora that consists in bacilli and other bacterias (Table 1) were found in the samples; there was no statistical significance (p = 0.39). The analysis of bacterioscopy by Gram stain showed the presence of Gram+ cocci, Gram+ bacilli, yeasts, Gram+ and Gram-negative (Gram-) bacilli, and Gram- bacilli; the outcome was not significant.

In the cytology of cervicovaginal samples, a high number of women exhibited *Lactobacillus sp.*, represented by 74.5% of HIV− and 57.4% of HIV+ women. Some microorganisms, such as *T. vaginalis*, *Gardnerella vaginalis*, cocci, *Candida sp.*, *Mobiluncus*, and *Actinomyces* were found in some samples. Due to the low number found, the *Mobiluncus* and *Actinomyces* were grouped and named "other"; there was statistical significance (p = 0.05).

Culture of aerobic microorganisms from vaginal specimen is described in Table 1. When comparing the menstrual cycle phases and culture of microorganisms in HIV− and HIV+ patients, the *coagulase-negative staphylococci* highlighted the high incidence in the single phase in 58.6% of women. The first phase showed that 51.7% of women had *coagulase-negative staphylococci*, and 47.5% in the second phase, followed by 20% of Gram-positive (Gram+) bacteria in the second phase of menstrual cycle, 17% of *Doderlein* in the first, and 13.8% in the single phase (p = 0.60).

The expression of *Escherichia coli*, *Enterococcus sp.*, *Candida glabrata*, *Candida albicans*, and *Klebsiella pneumoniae* were low, but 52% of all women presented *coagulase-negative staphylococci*, 12.2% *Doderlein,* and 10.2% Gram+ bacteria (p < 0.001).

Immune status of 47 HIV+ women was evaluated by means of $CD4^+$ T cells and viral load quantification; 8.5% showed values lower than 200 cells/mm^3 featuring acquired immunodeficiency syndrome (AIDS) (Table 2). There were no significant differences in the vaginal flora between groups according to standard method (p = 0.496).

HIV+ women were divided into three subgroups classified by viral load: viral load lower than 10,000 copies/ml, viral load between 10,000-50,000 copies/ml, and viral load

more than 50,000 copies/ml. The majority exhibited viral load lower than 10,000 copies/ml, i.e. 87.3% of women; 2.1% showed more than 50,000 viral copies/ml with 515 cells/mm^3 of CD4$^+$ T cells ($p = 0.242$).

In the HIV+ group, 12.8% were not using antiretroviral drugs, but 87.2% of women were using the therapy and the vaginal flora showed more diversity, included in these samples *E. coli, Enterococcus sp., S. agalactiae, C. glabrata, C. albicans, S. aureus,* and *K. pneumoniae* with low incidence. The higher prevalence was observed for *coagulase-negative staphylococci* and Gram+ bacteria. The use of antiretroviral drugs did not influence the type of vaginal flora of HIV+ women ($p = 0.619$).

The evaluation of sensibility and specificity of diagnostic methods used, such as clinical exam, fresh wet mount microscopy test, bacterioscopy, and cytology for identification of vaginal flora of all asymptomatic women were compared to culture, which is the gold standard method. The comparison of sensibility among methods in the control group was 41.7% to clinical exam, 50% to cytology, 66.7% to fresh wet mount microscopy test, and 75% to bacterioscopy. In the case group, 44.4% to clinical exam, 55.6% to bacterioscopy, 77.8% to cytology, and 88.9% fresh wet mount microscopy test. This proportion of subject with positive outcome were properly identified by the test. Moreover, the analysis displayed that the fresh wet mount microscopy test had 64.1% specificity; the bacterioscopy had 66.7%. The specificity value was 82.1% to cytology and 94.9% to clinical exam in HIV− group. The HIV+ group showed higher specificity to clinical exam with 76.3%, followed by cytology with 65.8%.

After colposcopic examination, 54.9% of all women did not undergo biopsy. The intraepithelial lesion in cervix and/or vagina were confirmed by histopathological examination, and the HIV− and HIV+ groups showed that 11.8% and 23.4% presented low-grade intraepithelial lesions, respectively. In the high-grade intraepithelial lesions, 7.8% of HIV− and 6.4% of HIV+ groups were confirmed by biopsy. Chronic cervicitis was observed in 25.5% of HIV(-) and 10.6% of HIV(+) women ($p = 0.17$).

Discussion

The vaginal microenvironment is highly complex, due to the hormonal cycles, that results in mucosal changes, and to the multiple sexually transmitted pathogens [10, 11].

The absence of symptoms does not characterize the vaginal mucosa as a healthy microenvironment [12, 13]. Different microorganisms might be found in the lower genital tract, such as intermediate flora and BV [12]. The BV associated pathogens may activate the metabolic pathways that influence certain innate and adaptive immune responses [1, 14]. The present results revealed the presence of 11.2% of *G. vaginalis* in all samples by cytology. Gopinath and Iwasaki explained that *Gardnerella* dominance is not significantly associated with inflammatory cytokines, indicating that a single genus, even *Gardnerella*, is not a consistent marker of vaginal inflammation [15].

Levels of estrogen vary depending on the menstrual cycle phase and contraception use. Among the HIV+ women, 6.4% use hormonal contraception and almost all 61.7% were in the second phase of menstrual cycle. Estrogen increases the levels of available glycogen in epithelial cells, which facilitates lactobacilli growth and lactic acid lowering the vaginal pH [13, 15]. Moreover, the comparison between HIV+ and HIV− women and menstrual cycle seems to be highly correlated. Wijgert *et al.* affirmed that menses are the largest disturbing factor during the menstrual cycle and might contribute to lactobacilli reduction, therefore some shifts may occur that favor the appearance of BV associated bacteria, streptococci or other Gram-positive cocci [13]. The menstrual cycle phase did not influence in the composition of vaginal flora in both groups and the prevalence was the same in the first and the second phase.

The pH measured in both groups exhibited that 60.2% (59) of women were with pH more than 4.5 and 66% (31) of them were HIV+. Several studies highlighted that pH is an important characteristic and the pH less than 4.5 may prevent the transmission of pathogenic bacteria and viruses including HIV [1, 3, 7, 16].

Antiretroviral drugs use, the number of CD4$^+$ T cells, and viral load did not involve in the statistically significant difference of vaginal flora, which was emphasized by the culture or by other diagnostic methods, including the women with CD4$^+$ T cells lower than 200 cells/mm^3 and featured in stage 3 of infection by HIV.

The clinical exam of vaginal content was considered physiological in 79.6% of all women, the BV was observed in 20.4% of them, and the prevalence was in the HIV+ women. Regardless of HIV+ or HIV− women, they presented similar quantity of *clue cells*, *Dordelein bacilli*, hyphae, and intermediate flora. The features in the composition of vaginal flora in the fresh wet mount microscopy did not show large differences, but HIV+ women had *T. vaginalis*. The bacterioscopy did not exhibited expressive differences: the control group presented a little more Gram+ cocci and Gram+ bacilli than in the case group.

In the cytology and aerobic cultures, there were discrepancies statistically significant between the groups. Cervicovaginal cytology is used worldwide to observe cervical and/or vaginal precursor lesions, but the vaginal microflora were detected and showed statistical significance. A large diversity of the vaginal microorganisms was observed in the HIV+ women, besides *G. vaginalis*, *Candida sp.* and *T. vaginalis* were present in the samples. Viral infections in women with BV might be asymptomatic; once the women in both groups resulted with high-grade intraepithelial lesion in 17.3% and 7.1% with low-grade, meanwhile more studies are necessary.

The culture is considered the main method used and it was possible to observe that 18.37% of all asymptomatic women presented different pathogenic microorganisms such as *S. aureus*, *E. coli*, *Enterococcus sp.*, *Beta-hemolytic streptococci, K. pneumoniae*, *and Citrobacter sp.* The *Dorderlein bacilli* were classified in the HIV+ group like Gram+ bacteria, due to other types of Gram+ bacteria found in the samples.

In the comparison between assays, the sensibility was higher in bacterioscopy in the HIV− women, while the fresh wet mount microscopy was higher in the HIV+ women. Nevertheless, the specificity was much higher to clinical exam in control HIV− and case (HIV+) groups, as well as the positive predictive value to control group (HIV−) with 71.4%. The positive predictive value to case (HIV+) was 35% in the cytology. This outcome emphasizes the idea that the clinical exam is important in the diagnosis of asymptomatic BV infections [17, 18]. *G. vaginalis* and *clue cells* were confirmed in more than half of the women with clinical exam.

Bacterioscopy, fresh wet mount microscopy, and cytology did not differ with the cocci classification by subtypes and an accurate evaluation by the means of culture is necessary to identify the pathogenic one. The implementations of inexpensive and simple execution exams such as vaginal pH measure, fresh wet mount microscopy, and perhaps bacterioscopy by Gram stain and culture of vaginal content may select women with susceptibility to acquire infectious diseases.

Mirmonsef and Spear, in a review analysis, asserted that microbiota of the genital tract appear to play an important role in the HIV epidemic [16], and BV might be an independent risk factor for the acquisition of STIs [19-21].

In conclusion, asymptomatic HIV-seronegative and HIV+ seropositive women resulted with a higher prevalence of *Doderlein* bacilli and *coagulase-negative staphylococci* in the vaginal content. *The G. vaginalis, Trichomonas sp.* and *Candida sp.* come across as being more common in HIV+ than HIV− women. The composition of vaginal microenvironment in both groups did not seem to suffer the influence of menstrual cycle; CD4+ T cells and viral load also did not appear to be influenced in vaginal flora in this restricted group of HIV(+) women.

Clinical exam exhibited less sensibility and higher specificity in HIV+ woman and the higher sensibility was in the fresh wet mount microscopic test, while the bacterioscopy presented lower specificity. It is important to take into account that the evidences may improve the screening BV in asymptomatic women with HIV, but more studies are necessary to outline a profile of these women.

HIV+ women with BV confirmed by exams, even without gynecological symptoms, may present more diversified vaginal flora when compared to the HIV− women, and possibly this makes them more prone to pelvic inflammatory disease, STI, and infertility.

Acknowledgements

Research supported by CAPES grant.

References

[1] Ministério da Saúde: “Boletim epidemiológico HIV/AIDS”. Ministério da Saúde Secretaria de Vigilância em Saúde. Brasília 2014, *1*, 1.

[2] Petrova M. I., van den Broek M., Balzarini J., Vanderleyden J., Lebeer S.: “Vaginal microbiota and its role in HIV transmission and infection”. *FEMS Microbiol. Rev.,* 2013, *37*, 762.

[3] Buvé A., Jespers V., Crucitti T., Fichorova R. N.: “The vaginal microbiota and susceptibility to HIV”. *Aids*, 2014, *28*, 2333.

[4] Biagi E., Vitali B., Pugliese C., Candela M., Donders G. G. G., Brigidi, P.: “Quantitative variations in the vaginal bacterial population associated with asymptomatic infections: A real-time polymerase chain reaction study”. *Eur. J. Clin. Microbiol. Infect. Dis.,* 2009, *28*, 281.

[5] Baka S., Tsirmpa I., Chasiakou A., Tsouma I., Politi E., Gennimata V., Kouskouni E.: “Inflammation on the cervical papanicolaou smear: evidence for infection in asymptomatic women?”. *Infect. Dis. Obstet. Gynecol.*, 2013, *2013*, 184302.

[6] Gallegos-Arreola M. P., Figuera-Villanueva L. E., Puebla-Pérez A. M., Montoya-Fuentes H., Suarez-Rincon A E., Zúñiga-González G. M.: “Association of TP53 gene codon 72 polymorphism with endometriosis in Mexican women”. *GMR*, 2012 *11*,1401.

[7] Cone R. A.: “Vaginal Microbiota and Sexually Transmitted Infections That May Influence Transmission of Cell-Associated HIV”. *J. Infect. Dis.*, 2014, *210*, 616.

[8] Witkin S. S., Linhares I. M., Giraldo P.: “Bacterial flora of the female genital tract: function and immune regulation”. *Clin. Obstet. Gynaecol.*, 2007, *21*, 347.

[9] Rebbapragada A., Howe K., Wachihi C., Pettengell C., Sunderji S., Huibner S., *et al.*: “Bacterial vaginosis in HIV-infected women induces reversible alterations in the cervical immune environment”. *J. Acquir. Immune. Defic. Syndr.,* 2008, *49*, 520.

[10] Spear G. T., Sikaroodi M., Zariffard M. R., Landay A. L., French L., Gillevet P. M.: “Comparison of the diversity of the vaginal microbiota in HIV infected and -uninfected women with and without bacterial vaginosis”. *J. Infect. Dis*. 2008, *198*, 1131.

[11] Ameur A., Meiring T. L., Bunikis I., Häggqvist S., Lindau C., Lindberg J. H., *et al.*: “Comprehensive profiling of the vaginal microbiome in HIV positive women using massive parallel semiconductor sequencing*”. Sci. Rep*., 2014, *4*, 4398.

[12] Zozaya-Hinchliffe M., Lillis R., Martin D. H., Ferris M. J.: “Quantitative PCR assessments of bacterial species in women with and without bacterial vaginosis”. *J. Clin. Microbiol.*, 2010 *48*, 1812.

[13] Van de Wijgert J. H. H. M., Borgdorff H., Verhelst, R., Crucitti T., Francis S., Verstraelen H., Jespers, V.: “The vaginal microbiota: what have we learned after a decade of molecular characterization?”. *PLoS One*, 2014, *9*, 105998.

[14] Kyongo J. K., Crucitti T., Menten J., Hardy L., Cools P., Michiels J., *et al.*: “A cross-sectional analysis of selected genital tract immunological markers and molecular vaginal microbiota in Sub-Saharan African women with relevance to HIV risk and prevention”. *Clin. Vaccine Immunol.*, 2015, *22*, 526.

[15] Gopinath S., Iwasaki A.: “Cervicovaginal Microbiota: Simple Is Better”. *Immunity*, 2015, *42*, 790.

[16] Mirmonsef P., Spear G. T.: “The barrier to HIV transmission provided by genital tract Lactobacillus colonization”. *Am. J. Reprod. Immunol*., 2014, *71*, 531.

[17] Anderson M. R., Klink K., Cohrssen A.: “Evaluation of vaginal complaints”. *JAMA*, 2004, *291*, 1368.

[18] Yin Y., Wu Z., Lin C., Guan J., Wen, Y., Li L., *et al.*: “Syndromic and laboratory diagnosis of sexually transmitted infection: a comparative study in China”. *Int. J. STD AIDS*, 2008, *19*, 381.

[19] Saxena D., Li Y., Yang L., Pei Z., Poles M., Abrams W. R., Malamud D.: "Human microbiome and HIV/AIDS". *Curr. HIV/AIDS Rep.*, 2012, *9*, 44.
[20] Nardis C., Mosca L., Mastromarino P.: "Vaginal microbiota and viral sexually transmitted diseases". *Ann. Ig.*, 2013, *25*, 443
[21] Sharma H., Tal R., Clark N. A., Segars, J. H.: "Microbiota and pelvic inflammatory disease". *Semin. Reprod. Med.*, 2014, *32*, 43.

Corresponding Author:
M.K. FIGUEIREDO FACUNDO, M.D.
Núcleo de Prevenção de Doenças Ginecológicas
Departamento de Ginecologia
Universidade Federal de São Paulo
Rua Borges Lagoa, 380 – Vila Clementino
04038-000 São Paulo – SP (Brazil)
e-mail: mayara.kff@gmail.com