doi: 10.5216/rpt.v50i3.66032

ORIGINAL ARTICLE

RELATION BETWEEN *Candida* SPECIES ISOLATED FROM VAGINAL MUCOSA AND LESIONS CAUSED BY HIGH-RISK HUMAN PAPILLOMAVIRUS FOR CERVICAL CANCER

Ana Clara de Souza[1], Claudete Rodrigues Paula[1], Luciana da Silva Ruiz[2], Paulo Francisco Ramos Margarido[3], Marcos Ereno Auler[4], Noely Paula Cristina Lorenzi[3], Débora Moreira[5], Rennan Luiz Oliveira dos Santos[1], Maricy Tacla[3], Edgard Michel-Crosato[1] and Carina Domaneschi[1]

ABSTRACT

This study characterized and related yeasts of the genus *Candida* isolated from vaginal mucous membranes of women with lesions caused by high-risk human Papillomavirus (HPV) for cervical cancer. Forty-two women treated at the Lower Genital Tract Pathology Clinic of the University of São Paulo Medical School Hospital of Clinics were examined, with 30 high-grade (G1) uterine lesions with a mean age of 36.5 years ± 11. 1 and 12 with low grade (G2) uterine lesions with a mean age of 34.7 years ± 15.5. Clinical conditions and laboratory data on HPV were collected from patients' medical records; the socio-demographic data obtained from an appropriate questionnaire. For the study of association between the variables, Odds Ratio analysis was used from the STATA 13.1 program. Patients G1 had a higher prevalence for diabetes and the results indicated 27% prevalence of *Candida* spp. in vaginal mucosa, in G2 this was 33% in vaginal mucosa. Among the species found in vaginal mucosa of patients, *Candida albicans* was the most isolated with 88%, followed by *C. tropicalis* (8%) and *C. glabrata* (4%). The strains of *C. albicans* isolated from mucosa presented sensitivity to all antifungal agents tested, unlike the *C. tropicalis* strain isolated in G2 in vaginal mucosa, which presented a resistance profile to fluconazole. Thus, monitoring and supervision through clinical and laboratory testing of HPV patients is important, reinforcing the need for care, treatment and prevention of HPV-related infections and *Candida* spp.

KEY WORDS: vaginal mucosa; uterine cervical neoplasms; human Papillomavirus; *Candida* spp. Antifungal sensitivity.

1. Universidade de São Paulo (USP), Faculdade de Odontologia, São Paulo, SP, Brazil.
2. Instituto Adolfo Lutz, Bauru, SP, Brazil.
3. Universidade de São Paulo (USP), Faculdade de Medicina, São Paulo, SP, Brazil.
4. Universidade Estadual do Centro Oeste, Departamento de Farmácia, Guarapuava, Paraná, Brazil.
5. Instituto Educacional Irineu Evangelista de Souza, Departamento de Ciências Biológicas, Barão de Mauá, Mauá, SP, Brazil.

Ana Clara de Souza: https://orcid.org/0000-0002-6328-8449; Claudete Rodrigues Paula: https://orcid.org/0000-0003-1955-2481; Luciana da Silva Ruiz: https://orcid.org/0000-0001-7374-8640; Paulo Francisco Ramos Margarido: https://orcid.org/0000-0003-1898-520X; Marcos Ereno Auler: https://orcid.org/0000-0001-7034-1482; Noely Paula Cristina Lorenzi: https://orcid.org/0000-0001-8144-1745; Debora Moreira: https://orcid.org/0000-0001-7372-4219; Rennan Luiz Oliveira dos Santos: https://orcid.org/0000-0001-9093-3805; Maricy Tacla: http://orcid.org/0000-0002-2712-2724; Edgard Michel Crosato: http://orcid.org/0000-0001-8559-9769; Carina Domaneschi: http://orcid.org/0000-0001-8615-3283

Corresponding author: Carina Domaneschi, Av. Professor Lineu Prestes, 2227 Cidade Universitária, CEP 05508-000, São Paulo, SP, Brazil. E-mail: domaneschi@usp.br

Received for publication: 1/3/2021. Reviewed: 7/6/2021. Accepted: 14/6/2021.

INTRODUCTION

Cervical cancer is the third most commonly diagnosed cancer in the world and the fourth cause of death in women due to cancer. In 2020, there were 16,710 new cases estimated in Brazil. (Bermedo-Carrasco & Waldner, 2016; INCA, 2021; Rashid et al., 2016; Xiao et al., 2016).

Human Papillomavirus (HPV) is the main agent related to cervical cancer. Among the more than 150 different types of HPV capable of infecting the skin or mucous membranes, around 40 may infect the anogenital tract causing cellular alterations caused by persistent infections by specific types of HPV (Rashid et al., 2016).

HPV genotypes are divided into "high-risk type", known as oncogenic (genotypes 16, 18, 31, 35, 39, 45, 51, 52, 58, among others), and "low risk type", which often cause benign lesions, such as genotypes 6, 11, 40, 42, 43, 44, 55. High-risk types are involved in 99%, 40-80% and 25% of cervical, anogenital and head and neck cancers, respectively. The HPV-16 and HPV-18 genotypes are mostly associated with high-grade cervical intraepithelial neoplasia (NIC II and III) and account for 70% of all invasive cervical carcinomas (Xiao et al., 2016).

There are several risk factors attributed to HPV that still require better understanding. Some authors associate co-factors for viral action such as infections caused by *Chlamydia trachomatis*, Human Immunodeficiency Virus (HIV) and *Gardnerella vaginalis*. However, patients who acquired the human immunodeficiency virus would potentially have a higher risk of infection and development of HPV-associated lesions (Koskela et al., 2000; Shaw et al., 2016).

Recurrent inflammation facilitates cell proliferation, aids growth of malignant cells, and release of cytokines, chemokines and free radicals, which indicate that in a patient with abnormal microbiota greater cellular alterations may occur than in patients with normal microbiota (Moss & Blaser, 2005).

Thus, considering that there are few studies that aim to correlate vaginal infections and high-risk HPV infection for cervical cancer, and that the high pathogenicity of Candida species allows them to penetrate the mucosa, and induce edema and cellular exfoliation (Rodriguez-Cerdeira et al., 2012), this study aimed at monitoring the presence of Candida spp. in the mucosa of patients with high-risk HPV, even without a clinical condition of candidiasis.

METHODS

The present study was approved by the Research Ethics Committees of the following institutions: College of Dentistry of the University of São Paulo, CAAE: 52071215.8.0000.0075 and the Department of Gynecology and

Obstetrics of Hospital das Clínicas, College of Medicine, University of São Paulo (HC/FMUSP). The participation of the patients occurred through the signing of the Informed Consent Term (ICT).

This study was a cross-sectional study in which the sample consisted of 42 high-risk HPV women for cervical cancer, treated at the Lower Genital Tract Pathology Ambulatory (LGTPA), from the Gynecology Department of the HC/FMUSP.

Data on HPV and high and low-grade uterine lesions were obtained from the medical records of the patients. At the time of data indicating the sampling, presence of clinical signs of vaginal infection were recorded and the participants answered a questionnaire with sociocultural and demographic questions, personal habits and clinical data.

Participants were divided into two groups: Group 1 (G1) with 30 patients, which included high-risk HPV carriers for cervical cancer and high-grade uterine lesions and Group 2 (G2) with 12 patients, including HPV carriers of high risk for cervical cancer and low-grade uterine lesions. Pregnant women, women in the menstrual period, those who did not agree to participate in the study or who did not sign the ITC were excluded.

In order to collect material from the vaginal mucosa, a speculum without lubricant was used, and two scrapes were performed on the lateral wall of the vaginal mucosa with the aid of sterile swabs (Cefar®).

The collected material was seeded in Sabouraud Dextrose agar medium (ASD) (Difco / USA) with 2% chloramphenicol. The isolated yeasts were identified according to the methods recommended by Kurtzman et al., 2011, according to macroscopic, microscopic, physiological and reproductive aspects. Standard strains of *Candida albicans* ATCC6258 were used for the yeast identification tests. Presumptive identification of the yeasts was performed on CHROMagar™.

Descriptive statistics of the variables were performed and comparison between the two study groups was evaluated for association with the prevalence of positive culture for *Candida* spp. in the vaginal secretion. Each independent variable was evaluated separately, resulting in Odds Ratio, level of significance (95%), as a measure of association.

RESULTS

The mean age of patients with high-risk HPV for cervical cancer studied was 36 years ± 11.4 and the related chronic diseases were diabetes mellitus, erythematous systemic lupus, while HIV infection and lymphopenia status were also reported. In Group 1, three patients were diabetic, two were lupus patients and two were HIV carriers. In Group 2, there were two diabetic patients, one lupus patient, two HIV carriers and one lymphopenic.

Regarding vaginal culture, 17% (7/42) of the patients with chronic diseases presented positive cultures and 59% (25/42) of the patients without chronic diseases presented negative cultures, as shown in Table 1.

Table 1. Frequency of chronic diseases in patients with high-risk HPV for cervical cancer in relation to positive cultures for *Candida* spp. in vaginal mucosa.

	Presence of chronic disease	Absence of chronic disease
Positive culture	17%	12%
Negative culture	12%	59%

In relation to the presence of clinical signs indicative of vaginal infection, 23% (7/30) of the patients in Group 1 presented clinical signs indicative of vaginal infection and in Group 2, they presented 17% (2/12) of positivity for the clinical signs (Table 2).

Table 2. Frequency of clinical signs for vulvovaginal infection in patients with high-risk HPV for cervical cancer with high grade (Group 1) and low grade (Group 2) uterine lesions.

	Positive clinical signs	Negative clinical signs
Group 1	23%	77%
Group 2	17%	83%

The results of the yeast isolation showed that in Group 1 there was 27% (8/30) positivity for *Candida* spp. in vaginal secretion and in Group 2 there was 33% (4/12) of positivity isolation.

Among strains isolated from Group 1, seven were identified as *Candida albicans* (87.5%) and one as *C. glabrata* (12.5%); in Group 2, three were identified as *C. albicans* (75%) and one as *C. tropicalis* (25%).

Color development on CHROMagar™ revealed the same species, *Candida albicans* and *C. tropicalis*. The Triphenyl Tetrazolium Chloride medium confirmed the species *C. tropicalis*, by development of a characteristic red color.

Despite positivity in some cultures of vaginal mucosa secretion, this datum did not present a statistically significant difference between Groups 1 and 2. In the association between high and low grade uterine lesions and socio-demographic data (Table 3), cultural customs (Table 4) and personal habits (Table 5) no significant statistical difference was observed ($p > 0.05$).

Table 3. Association between high and low grade lesions with socio-demographic information of the patients.

Variable	Category	G1	G2	Odds ratio	p value
Age	Over 40 years old	11	3	1.7	0.4[b]
	Under 40 years old	19	9	1	1
BMI	Obesity	13	4	1.5	0.5[b]
	Ideal weight	17	8	1	1
Marital status	Single	13	6	0.7	0.6[b]
	Married	17	6	1	1
Education	High school	26	9	2.1	0.3[b]
	College	4	3	1	1
Monthly income	Less than 2 minimum wages	17	8	0.6	0.5[b]
	Over 2 minimum wages	13	4	1	1

[a] p value <= 0.05, with significant statistical difference; [b] value of $p > 0.05$, with no statistically significant difference. BMI: Body Mass Index. G1: patients with high-grade lesions for cervical cancer. G2: patients with low-grade cervical cancer.

Table 4. Association between high and low grade lesions and patients' cultural information.

Variable	Category	G1	G2	Odds ratio	p value
First period	At 15 years or less	25	10	1	1[b]
	Over 15 years	5	2	1	1
First sexual intercourse	At 15 years or less	9	2	2.1	0.3[b]
Number of sexual partners in last year	More than 1	7	1	3.3	0.2
	1 or none	23	11	1	1
Use of condoms	No	17	8	0.6	0.5[b]
	Yes	13	4	1	1
Other MAC	No	14	8	0.4	0.2[b]
	Yes	16	4	1	1

[a] p value < 0.05, with significant statistical difference; [b] value of $p > 0.05$, with no statistically significant difference. MAC: contraceptive method. G1: patients with high-grade lesions for cervical cancer. G2: patients with low-grade cervical cancer.

Table 5. Association between high and low grade lesions and patients' personal habits.

Variable	Category	G1	G2	Odds ratio	p value
Smoking	No	8	2	1.8	0.4[b]
	Yes	22	10	1	1
Alcohol	No	11	1	6.3	0.0[b]
	Yes	19	11	1	1
Underwear	Whatever	14	4	1.7	0.4[b]
	cotton	16	8	1	1
Intimate soap	Yes	10	5	0.7	0.6[b]
	No	20	7	1	1

[a] *p* value < 0.05, with significant statistical difference; [b] value of *p* > 0.05, with no statistically significant difference. G1: patients with high-grade lesions for cervical cancer. G2: patients with low-grade cervical cancer.

The correlation of the clinical data of patients with low grade lesions (Table 6) showed that there was a statistically significant difference between the presence of lesions in the uterine cervix and the presence of chronic diseases (p <0.05).

Table 6. Association between high and low grade lesions and clinical information of the patients.

Variable	Category	G1	G2	Odds ratio	p value
Vaginal culture	Positive	8	4	0.7	O.6[b]
	Negative	22	8	1	1
Other IST	Yes	4	2	0.7	0.7[b]
	No	26	10	1	1
Chronic disease	Yes	10	9	0.1	0.0[a]
	No	20	3	1	1
Use of antibiotics	Less than 1 month	3	2	0.5	0.5[b]
	Longer than 1 month	27	10	1	1
Symptoms of CVV	Yes	23	8	1.6	0.5[b]
	No	7	4	1	1
Signs of CVV	Yes	6	3	0.7	0.7[b]
	No	24	9	1	1

[a] p value <0.05, with significant statistical difference; [b] value of p> 0.05, with no statistically significant difference. STD: Sexually Transmitted Disease; CVV: Vulvovaginal Candidiasis. G1: patients with high-grade lesions for cervical cancer. G2: patients with low-grade cervical cancer.

DISCUSSION

The mean age of patients with high-risk HPV for cervical cancer was 36 years ± 11.4, slightly above that found in other studies (Shaw et al., 2016; Silva et al., 2014; Coser et al., 2016) ranging from 28.52 to 33.9 years of age, relating this age range to a more sexually active life, and multiplicity of partners increasing the risk of sexually transmitted infections.

Among the socio-demographic factors studied, this study did not present a statistically significant association with HPV, a fact that differs from the study by Coser et al. (2016) in which being single and earning an income below a minimum wage, indicated a statistically significant association with the presence of HPV.

Regarding the behavioral factors, in a study by Coser et al. (2016) the age of first sexual intercourse and number of sexual partners was not associated with HPV infection, a fact that corroborates our findings, since there was no statistically significant association between these factors. However, in a study conducted by Silva et al. (2014) having just one sexual partner was considered a lower risk factor for progression to a high-grade lesion caused by HPV.

In a study by Shaw et al. (2016), there was a statistically significant association between condom use and the presence of HPV, unlike this study and Coser et al. (2016). Manhart & Koutsky, in 2002 indicated that despite protection against genital warts, condom use does not prevent HPV infection. Oral-genital transmission may possibly explain this fact.

According to some studies, use of the intra uterine device (IUD has a protective association for HPV infection, which is explained by the high immune response generated by the IUD in the uterine cervix, generating a rapid elimination of infections. (Ortiz et al., 2007) On the other hand, other studies have shown that chronic infections may promote the development of HPV-related high-risk pre-malignant lesions (Fernandes et al., 2015). In the study by Silva et al. (2014), the use of hormonal contraceptives was also a protection factor regarding the progression of HPV uterine lesion. However, Roset Bahmanyar et al. (2012) indicated that prolonged use of hormonal contraceptives is associated with an increased risk of HPV infection. The biological mechanism that would explain this relationship would be the increase of the estradiol hydroxylation in cervical cells infected by oncogenic types of HPV, which in turn induced greater transcription of oncogenic agents.

Among the patients' evaluated personal habits, there was no positive association with HPV in this study. According to Coser et al. (2016) who evaluated the association between smoking and HPV, there was also no association. However, Silva et al. (2014), associated smoking as a risk factor for HPV infection.

Regarding the clinical information of the patients, our research found no association with the presence of other sexually transmitted disease, use of antibiotics or signs and symptoms of vulvovaginal candidiasis. In a study by Coser et al. (2016), there was no association between the presence of other STIs or other concomitant vaginal infections with HPV infection.

Studies report that the practice of oral sex is very common and that the use of condoms in this sexual practice is almost nil, which increases the transmission of several infections considered as sexually transmissible. (Castro-Silva et al., 2012)

Vulvovaginal candidiasis is an infection that affects about 70-75% of women of childbearing age. On estimate, of this total, 40-50% will present recurrences, and around 5 to 15% will become recurrent (Cassone, 2015). The causative agent of these infections is *Candida* spp. considered a commensal yeast that is part of the normal human microbiota and, under certain circumstances, may become pathogenic (Lott et al., 2003). An example of this situation is if a higher production level of female hormones, such as progesterone, increases the availability of glycogen in the vaginal mucosa and this becomes a source of energy, favoring growth, germination of yeasts and improving the adhesion capacity of these fungi (Lacaz, 1980; Sobel, 1990). *Lactobacillus* sp present in the vaginal mucosa is also related to a decrease in pH due to the formation of lactic acid from glycogen present in the cytoplasm of vaginal epithelial cells, whose production is also regulated by female hormones (Almeida Filho et al., 1995).

In this study, the observed proportion of *C. albicans* in both groups was 83.3%, above the world prevalence (65.3%) and European prevalence (67.9%) (Pfaller et al., 2010). *C. tropicalis* and *C. glabrata* were isolated in low percentages, therefore corroborating world literature (Pfaller et al., 2010).

The most common symptoms observed in this study reported by the participants were lumpy discharge and itching in the vulvar region. When assessing the signs and symptoms observed in these patients, there was no distinction between infection by *Candida* spp. and/or bacteria. Of the 12 patients who had a positive culture for *Candida* spp. only 17% showed clinical signs of infection and lack of these clinical signs may indicate vaginal colonization by *Candida* spp. Of the 30 patients with negative vaginal culture for *Candida* spp., 23% showed signs of infection that may also indicate infection by other microorganisms. In a study conducted by Brandolt et al. (2017), the prevalence of positive cultures for *Candida* in vaginal discharge in asymptomatic patients was 13.3%, while Bassayouni et al. (2015) found 20% positivity in healthy patients. In this study, four patients reported the use of antibiotics, one of them reported symptoms and had a positive culture, one had a complaint but the culture was negative, one had no complaint and the culture was negative and one did not report any complaints, however the culture was positive.

Recurrent inflammations facilitate cell proliferation, aid the growth of malignant cells, release cytokines, chemokines and free radicals, which indicate that in patients with abnormal microbiota, more cellular alterations can occur than in patients with normal microbiota (Moss & Blaser, 2005). An example of this would be cases of recurrent vulvovaginal candidiasis due to the high pathogenicity of *C. albicans* strains in allowing mucosal penetration, inducing edema, exfoliation of the cells (Fernandes et al, 2015) and exacerbated production of its virulence-related factors such as proteases, phospholipases and biofilms formation.

This study showed that Group 1 patients had 80% less chance of positivity for *Candida* spp. than Group 2 patients, which may lead us to think of a competition between yeast and bacteria. Culture positivity for *Candida* spp. in the vaginal mucosa did not present a statistically significant association with the presence of HPV, the same was observed in a study conducted by Rodriguez-Cerdeira et al. (2012).

In the study by Nogueres et al. (2010), the profile of women with inflammatory processes by *Candida* spp. is associated with a history of sexually transmitted disease, among which infection by HPV is highlighted. Voog et al. (1995) identified the possibility of *Candida* infection activating latent HPV. Other studies (Sobel, 1993; Melo et al., 2003) have mentioned these infections as co-factors in determining cervical neoplasias associated with HPV. In this study, the prevalence of positive cases of *Candida* cultures in the vaginal discharge, in patients with high-risk HPV for cervical cancer, was 27% in Group 1 and 33% in Group 2, slightly above that usually found in patients without HPV (Melo et al., 2003).

Thus, monitoring through clinical and laboratory tests for the presence of yeast and hyphae and not only the clinical conditions of candidiasis, in patients infected by HPV, is important, reinforcing the need for prevention and treatment.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTS

We would like to thank the Research Support Foundation of the State of São Paulo (FAPESP), Higher Education Personnel Improvement Coordination (CAPES), National Council for Scientific and Technological Development (CNPq) for financial support.

REFERENCES

1. Almeida Filho GL, Passos MRL, Gouvêa TVD. Candidíase. *In:* Passos MRL. *Doenças sexualmente transmissíveis.* 4 ed. Cultura Médica: Rio de Janeiro, 1995. 562p.
2. Bassyouni RH, Wegdan AA, Abdelmoneim A, Said W, AboElnaga F. Phospholipase and Aspartyl Proteinase Activities of *Candida* Species Causing Vulvovaginal Candidiasis in Patients with Type 2 Diabetes Mellitus. *J Microbiol Biotechnol 25*: 1734-1741, 2015.
3. Bermedo-Carrasco S, Waldner CL. The role of socio-demographic factors in premature cervical cancer mortality in Colombia. *BMC Public Health 16*: 981, 2016.
4. Brandolt TM, Klafke GB, Gonçalves CV, Bitencourt LR, Martinez AM, Mendes JF, Meireles MC, Xavier MO. Prevalence of *Candida* spp. in cervical-vaginal samples and the *in vitro* susceptibility of isolates. *Braz J Microbiol 48*: 145-150, 2017.
5. Castro-Silva II, Coutinho LA, Júnior JAS, Pires AR, Bastos OM. Percepcão de Vulnerabilidade ao HPV e Câncer de Cabeça e Pescoço: Comportamentos Sexuais e de Risco em Jovens de Niterói, RJ. *DST J Bras Doenças Sex Transm 24*: 85-92, 2012.
6. Cassone A. Vulvovaginal *Candida albicans* infections: pathogenesis, immunity and vaccine prospects. *BJOG 122*: 785-794, 2015.
7. Coser J, BoeiraTda R, Wolf JM, Cerbaro K, Simon D, Lunge VR. Cervical human papillomavirus infection and persistence: a clinic-based study in the countryside from South Brazil. *Braz J Infect Dis 20:* 61-68, 2016.
8. Fernandes JV, DE Medeiros Fernandes TAA, DE Azevedo JCV, Cobucci RNO, De Carvalho MGF, Andrade VS, DE Araújo JMG. Link between chronic inflammation and human pappilomavirus-induce carcinogenesis (review). *Oncol Lett 9*: 1015-1026, 2015.
9. INCA. Instituto Nacional do Câncer (Brasil). *Estatísticas de câncer*. [Brasília, DF]: Instituto Nacional do câncer, 2021. Available in: inca.gov.br/ numeros-de-cancer. Accessed in: 28 Jul. 2021.
10. Koskela P, Anttila T, Bjørge T, Brunsvig A, Dillner J, Hakama M, Hakulinen T, Jellum E, Lehtinen M, Lenner P, Luostarinen T, Pukkala E, Saikku P, Thoresen S, Youngman L, Paavonen J. *Chlamydia trachomatis* infection as a risk factor for invasive cervical cancer. *Int J Cancer 85*: 35-39, 2000.
11. Kurtzman CP, Fell JW, Boekhout T. *The yeasts: a taxonomic study*. 5th ed. Elsevier: Amsterdan, 2011. 2354p.
12. Lacaz CS. *Candidíases*. USP: São Paulo, 1980. 190p.
13. Lott TJ, Fundyga RE, Brandt ME, Harrison LH, Sofair AN, Hajjeh RA, Warnock DW. Stability of allelic frequencies and distributions of *Candida albicans* microsatellite loci from US population-based surveillance isolates. *J Clin Microbiology 41*: 1316-1321, 2003.
14. Manhart LE, Koutsky LA. Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia? A meta-analysis. *Sex Transm Dis 29:* 725-735, 2002.
15. Melo VH, Araújo ACL, do Rio SMP, de Castro LPF, de Azevedo AA, de Castro MM. Problemas ginecológicos mais frequentes em mulheres soropositivas para o HIV. *Rev Bras Ginecol Obstet 25*: 661-666, 2003.
16. Moss SF, Blaser MJ. Mechanisms of disease: inflammation and the origin of cancer. *Nat Clin Pract Oncol 2*: 90-97, 2005.
17. Nogueres IB, Zimmermmann JB, Gonçalves LG, Fontes L.C, Alves LF, Gontijo CC. Associação entre a infecção pelo papilomavírus humano (HPV) e outras infecções genitais femininas. *HU Rev 36*: 19-28, 2010.
18. Ortiz ME, Croxatto HB. Copper-T intrauterine device and levonorgestrel intrauterine system: biological bases of their mechanism of action. *Contraception 75*: S16-S30, 2007.

19. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, Rodloff A, Fu W, Ling TA. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* Species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J Clin Microbiol 48*: 1366-1377, 2010.

20. Rashid S, Labani S, Das BC. Knowledge, Awareness and Attitude on HPV, HPV Vaccine and Cervical Cancer among the College Students in India. *PLoS One 11*: e0166713, 2016.

21. Rodriguez-Cerdeira C, Sanchez-Blanco E, Alba A. Evaluation of Association between Vaginal Infections and High-Risk Human Papillomavirus Types in Female Sex Workers in Spain. *ISRN Obstet Gynecol 2012*: 240190, 2012.

22. Roset Bahmanyar E, Paavonen J, Naud P, Salmerón J, Chow SN, Apter D, Kitchener H, Castellsagué X, Teixeira JC, Skinner SR, Jaisamrarn U, Limson GA, Garland SM, Szarewski A, Romanowski B, Aoki F, Schwarz TF, Poppe WA, De Carvalho NS, Harper DM, Bosch FX, Raillard A, Descamps D, Struyf F, Lehtinen M, Dubin G; HPV PATRICIA Study Group. Prevalence and risk factors for cervical HPV infection and abnormalities in young adult women at enrolment in the multinational PATRICIA trial. *Gynecol Oncol 127:* 440-450, 2012.

23. Silva C, Almeida EC, Côbo E de C, Zeferino VF, Murta EF, Etchebehere RM. A retrospective study on cervical intraepithelial lesions of low-grade and undetermined significance: evolution, associated factors and cytohistological correlation. *Sao Paulo Med J 132*: 92-96, 2014.

24. Sobel JD. Vaginal infections in adult women. *Med Clin North Am 74*: 1573-602, 1990.

25. Sobel JD. *Candida* Vulvovaginitis. *Clin Obstet Gynecol 36*: 153-165, 1993.

26. Shaw E, Ramanakumar AV, El-Zein M, Silva FR, Galan L, Baggio ML, Villa LL, Franco EL, Ludwig-McGill Cohort Study. Reproductive and genital health and risk of cervical human papillomavirus infection: results from the Ludwig-McGill cohort study. *BMC Infect Dis 16:* 116, 2016.

27. Voog E, Bolmsted A, Olofsson S, Ryd W, Löwhagen GB. Human papilloma virus infection among women attending an STD clinic correlated to reason for attending, presence of clinical signs, concomitant infections and abnormal cytology. *Acta Derm Venereol 75*: 75-78, 1995.

28. Xiao M, Xu Q, Li H, Gao H, Bie Y, Zhang Z. Prevalence of Human Papillomavirus Genotypes Among Women With High-Grade Cervical Lesions in Beijing, China. *Medicine (Baltimore) 95*: e2555, 2016.