



Co-occurrence of *Gardnerellavaginalis* and *Candida sp.* in women with and without vulvovaginitis

José Eleutério Junior¹, Ana Katherinne Gonçalves de Oliveira², Paulo César Giraldo³, Ana Beatriz Gordiano¹, Fernanda Queiroz¹,*Renata Mírian Nunes Eleutério⁴ and Iara Moreno Linhares⁵

¹Motherhood and Child Health Department – Federal University of Ceará – Fortaleza - Brazil

²Obstetrics and Gynecology Department – Federal University of Rio Grande do Norte – Natal - Brazil

³Obstetrics and Gynecology Department – State University of Campinas – Campinas - Brazil

⁴Pharmacy Department – Federal University of Ceará – Fortaleza - Brazil

⁵Obstetrics and Gynecology Department – State University of São Paulo – São Paulo - Brazil

Correspondence E-mail: renatameleuterio@gmail.com

ABSTRACT

Aims: To detect the co-occurrence of *Gardnerellavaginalis* and *Candida sp.* in women with and without vaginal symptoms. **Methods:** A cross-sectional study was performed in women seeking treatment in a Brazilian city from March to November 2014. Data, such as age and symptoms, were noted from patients. Vaginal content samples were obtained with a swab of the vaginal wall and were fixed in ATTS (Ambient Temperature Transport System). The Affirm VPIII test (Becton Dickinson and Company, Sparks, MD, USA) was used to identify the pathogen. Fisher's exact test with a 95% confidence interval was used for the statistical analysis. **Results:** In total, 160 women were studied, and 13 cases were excluded. Of the 147 remaining women, fifty-two women were asymptomatic, and 95 women reported symptoms. An association between *Gardnerellavaginalis* (Gv) and *Candida sp.* (Ca) was noted in 9 cases (6.1%). Gv + Ca was observed in 1 case (1.9%) in the asymptomatic group and in 12 cases (12.6%) in the symptomatic group ($p= 0,0361$). Vaginal inflammation signs were observed in 8/8 (100%) cases of co-occurrence ($p<0.005$). **Conclusion:** The co-occurrence of *Gardnerellavaginalis* and *Candida sp.* is not rare and is frequently associated with symptoms and mucosal inflammation signs.

Key words: Bacterial vaginosis, *Candida*, Vaginitis, vaginosis, diagnostic

INTRODUCTION

Vaginal discharge, occasionally accompanied with itching, is an extremely common symptom in the gynecological services [1]. Numerous causes, such as infectious processes, reaction to a foreign body, vaginal environment imbalance, allergic and autoimmune phenomena and even physiological causes, have been noted [2]. However, bacterial vaginosis, candidiasis and trichomonas vaginitis more frequently occur [3].

The adequate diagnosis of these conditions based on the clinical characteristics of the vaginal discharge is problematic, leading to inappropriate treatment and no resolution of the symptoms [4]. Diagnosis has been based on cytological methods (Gram stain [5] and Papanicolaou [6]) and culture [5] in daily practice. The limited sensitivity of the cytological methods and the high costs of culture has led to misdiagnosis and stimulated the search for simpler and more sensitive methods, taking into account their cost-benefits [4].

It is possible that *Gardnerellavaginalis* and *Candida sp.* simultaneously occur in vaginitis. However, few studies have reported the frequency of this finding [7]. Several mechanisms can contribute to low rates of *Candida sp.* among women with bacterial vaginosis (BV), including bacterial toxin production; competition for available energy sources, micronutrients, and binding sites in mucosa; and immunological mechanisms. Cytokines also appear to be involved in this process. In women who develop candidiasis associated with the presence of *Gardnerellavaginalis*

(Gv), low IL-8 levels could attenuate the immune response that is important in the pathogenesis of symptomatic vaginal candidiasis, given that IL-8 is a potent chemoattractant for neutrophils [8].

On the other hand, in the presence of lactobacilli appears to have a stimulatory effect by increasing IL-8 in the vaginal mucosa, thus stimulating the immune response and inhibiting fungal proliferation [8].

Few articles address the issue of mixed vaginitis, assessing its prevalence among asymptomatic women with discharge. The use of biomolecular methods with high sensitivity and specificity results in more reliable findings, which is essential for a more appropriate management of this condition [9].

The aim of this study was to evaluate the co-occurrence of *Gardnerellavaginalis* and *Candida sp.* among women of reproductive age with and without genital symptoms.

MATERIALS AND METHODS

A cross-sectional study was performed from March to November 2014 with symptomatic and asymptomatic women at a university hospital gynecology clinic in a Brazilian city (Fortaleza). Data, such as age and complaints, were collected from patients undergoing research. After speculum placement, material was obtained from the vaginal wall with a swab and stored in tubes with ATTS (Ambient Temperature Transport System). The Affirm VPIII test (Becton Dickinson and Company, Sparks, MD, USA) was used to identify the pathogen. The test has three steps: denaturation of the materials to release the nucleic acids specific for each pathogen, automatic processing, and release of the test results after 32 minutes. The experiment includes negative and positive controls in each Probe Analysis Card.

To diagnosis colpitis (vaginal inflammation), aDF Vasconcelos® (Valença, Riod de Janeiro, Brazil) colposcope was used, which has a lens with five increases. Using the colposcope, the vaginal mucosa was evaluated without acetic acid and then with 3% acetic acid to identify petechiae and mucosal hyperemia. Finally, Lugol was applied to determine non-iodine uptake in outbreaks.

The prevalences of each pathogen and co-infections, as well as correlations with complaints and inflammatory mucosal signs, were evaluated.

For the statistical analysis, Fisher's exact test with a 95% confidence interval was employed (Graphpad Prism 6.0). The study was approved by the Ethics Committee of the Federal University of Ceará.

RESULTS

Initially, 160 cases of women attending the gynecology outpatient clinic were studied, and 13 cases were excluded due to lack of clinical data. Of the remaining 147 patients, fifty-two women (35.4%) were asymptomatic, and 95 women (64.6%) reported genital symptoms (Table 1).

Table 1. Frequency of diagnosis by Affirm VIII of *Gardnerellavaginalis*, *Candida sp* and co-occurrence in women with no vaginal symptoms and with vaginal symptoms

Affirm test	No symptoms		With symptoms		P (CI 95%)*
	N(%)	Discharge only N(%)	Discharge and pruritus N(%)	Discharge and odor N(%)	
Negative	28 (53.8)	29 (54.7)	13(43.3)	5(41.7)	0.6107
<i>Gardnerella</i>	19 (36.5)	19 (35.8)	4(13.3)	6(50)	0.5912
<i>Candida</i>	4 (7.7)	3 (5.7)	4(13.3)	0(0)	1
<i>Gardnerella</i> + <i>Candida</i>	1 (1.9)	2 (3.8)	9(30)	1(8.3)	0.0361
Total	52 (100)	53 (100)	30(100)	12 (100)	

* Fisher exact test

The average age of the patients was 32.1 (+9.7) years. In general, among all cases studied by Affirm VPIII, *Gardnerella vaginalis* (Gv) was detected in 51 cases (34.7%), *Candida sp.* (Ca) was noted in 13 cases (8.9%) and *Trichomonas* was observed in no cases. An association of *Gardnerella* and *Candida* pathogens was observed in 9/147 cases (6.1%).

When Gv was identified, 19/48 patients (39.6%) had no symptoms, 19/48 (39.6%) reported discharge, 4/48 (8.33%) reported discharge with itching and 6/48 (12.5%) reported discharge with odor (p = 0.5912). In cases of *Candida sp.*, 4/11 patients (36.4%) were asymptomatic, 3/11 (27.3%) reported discharge and 4/11 (36.4%) reported pruritus

($p = ns$). When the two pathogens were simultaneously identified (Gv + Ca), only one of 13 patients (7.7%) was asymptomatic, and 12 of 13 patients were symptomatic ($p = 0.0361$). Of these patients, 2/13 (15.4%) complained of discharge, 9/13 (69.2%) reported itching and 1/13 (7.7%) reported odor. In asymptomatic patients, 24/52 (46.2%) had Gv and/or Candida at the following frequencies: Gv 19 (36.5%), Ca 4 (7.7%) and Gv + Ca 1 (1.9%). However, pathogens were identified in 48/95 symptomatic patients (50.52%) [Gv 29(30.5%); Ca 11(11.6%) and Gv+Ca 12(12.6%)]. Gv+Ca was found significantly more frequent in symptomatic patients ($p = 0.0361$) (Table 1).

Evaluating the presence of colpitis, 33/48 (68.8%) of the negative Affirm test cases had no signs of mucosal inflammation, and 15/48 (31.2%) had colpitis ($p=0.0043$). In the cases with Gv, 19/34 patients (55.9%) had no inflammatory mucosal signs, and 15 (44.1) exhibited mucosal inflammation ($p=0.8321$). On the other hand, colpitis signs were observed in 7/7 (100%) and 8/8 (100%) cases of Candida ($p=0.0035$) and Candida co-occurrence with Gv ($p=0.0015$), respectively (Table 2).

Table 2. Frequency of diagnosis by Affirm VIII of *Gardnerellavaginalis*, *Candida sp* and co-occurrence among women with inflammatory mucosal signs or not

	No inflammatory mucosal signs N(%)	Inflammatory mucosal signs N(%)	Total	p(CI 95%)
Negativo	33 (68,8)	15 (31,2)	48 (100)	0.0043
Gardnerella	19 (55,9)	15 (44,1)	34(100)	0.8321
Candida	0	7 (100)	7(100)	0.0035
Gardnerella + Candida	0	8 (100)	8(100)	0.0015

* Fisher exact test

DISCUSSION

A healthy vagina is not easy to define, especially considering that the microbiome is extremely dynamic, with a substantial difference in its composition under endogenous and exogenous influences [10,11]. Vaginal content results from secretions from the endocervix and the vestibular and periurethral glands, vaginal transudate and desquamation, and can thus produce a daily volume of approximately 4 ml [11]. Therefore, discharge can result from normal conditions. Discharge can also be associated with a microbial imbalance and inflammation [3,9].

Vaginosis and vaginitis are frequent noted in medical practice, and their diagnoses are primarily based on biochemical reactions and morphological findings. However, sensitivity for the identification of etiologic agents is an important limiting factor, even for evaluating the presence of more than one pathogen associated with vaginitis and vaginosis [1,3].

More recently, some researchers [1,4,6] have used molecular biology techniques to evaluate the vaginal microbiome. These techniques exhibit an increased sensitivity that can identify the actual frequency of each agent between asymptomatic and symptomatic women, correlate the information and identify associations.

In this report, Gv was the most frequently identified pathogen in asymptomatic (19/52, 36.5%) and symptomatic (51/95, 57.7%) women. Among those with GV, co-occurrence with Candida was noted in 6.1% of the patients studied, independent of symptoms. Co-occurrence was observed in 1.9% of asymptomatic patients and 12.6% of symptomatic patients. Other studies have reported an association between Gv and Ca in 6.7 to 22.1% of cases, depending on the technique used [12]. Using Affirm, Sobel et al. [13] reported the presence of co-infection (Ca + Gv) in 10 to 16% of symptomatic women. Among women from a STD clinic in the USA, co-occurrence was noted in 12.4% of women with vaginal symptoms using microscopy and culture [9]. In a study evaluating more than 600 patients using Gram status, culture, and Affirm, the authors observed that Gv was the most prevalent, followed by Candida and *Streptococcus agalactia*. The most frequent association was that of Gv and Candida (11.7%). [14]. Using the same methodology, other groups reported a 7% co-infection with two or more microorganisms [6].

The association between Gv and Ca was infrequent among asymptomatic cases (1/52 [1.9%]) and significantly more frequent among symptomatic women (12/95 [12.6%]). In the study by Rivers et al. [9], the presence of an odor was more typical in cases of Gv alone compared with other conditions. Subjectivism can be a limiting factor in the assessment of this variable. On the other hand, patients with mixed vaginitis significantly referred to vaginal soreness in this study.

Colpitis signs in the colposcopy were noted in 31% of negative patients, 44% of patients with Gv alone, and 100% of cases with Ca alone and Gv + Ca. Mucosal inflammation was also more frequent in another study using Gram stain and culture for cases of Candida (63.2%) and Gv + Candida (66.7%) [14].

Symptoms and signs modestly benefit from the identification of mixed vaginitis [13]. However, in this study, the mixed cases were significantly more symptomatic, and pruritus was a major complaint, suggesting that the diagnosis of BV with itching and evidence of colpitis should be investigated regarding cases of possibly mixed vaginitis.

The presence of mucosal inflammation signs along with the complaint of itching and vaginal odor could be evidence of the presence of *Candida sp* in patients with VB. The co-occurrence of *Gardnerella vaginalis* and *Candida sp*. concomitantly is not rare and is frequently associated with symptoms and mucosal inflammation signs.

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