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# Prevalence of *Gardnerella vaginalis* in cervical Papanicolaou smears of patients attended at a laboratory of pathology in Bento Gonçalves, RS

## *Prevalência de Gardnerella vaginalis em exames citopatológicos de pacientes atendidas em um laboratório de patologia de Bento Gonçalves, RS*

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## ABSTRACT

This study aimed to verify the prevalence of *Gardnerella vaginalis* in cervical Papanicolaou smears of patients attended at a laboratory of pathology in the city of Bento Gonçalves, RS. A retrospective cross-sectional and descriptive study was performed by analyzing 49617 cytopathological reports issued from January 1, 2016 to December 31, 2020. A total of 21.86% (10839/49617) positive reports for *G. vaginalis* were found, and the years 2020 and 2017 presented the highest and lowest prevalences, corresponding to 25.28% and 19.40%, respectively. The mean age of patients was 45.59 years, and the highest prevalence was found in patients aged 51 to 60 years corresponding to 22.74%. The fungus *Candida* sp. was observed in 3.60% of the reports positive for *G. vaginalis*. Among the cytopathological alterations associated with *G. vaginalis*, the atypical cells of undetermined significance (ASC-US) result was the most predominant, in 3.97% of the reports. Therefore, the results demonstrated a considerable prevalence of the bacteria in pap smears reports. Thus, it is necessary to inform the female population about the diagnosis and treatment of the bacteria, to avoid possible complications.

**Keywords:** Bacterial vaginosis. *Clue Cells. Gardnerella vaginalis*. Papanicolaou. Vaginal microbiota.

## RESUMO

Este estudo teve por objetivo verificar a prevalência da *Gardnerella vaginalis* em exames citopatológicos do colo do útero em pacientes atendidas em um Laboratório de Patologia na cidade de Bento Gonçalves, RS. Foi realizado um estudo transversal, retrospectivo e descritivo por meio da análise de 49617 laudos citopatológicos emitidos no período de 01 de janeiro de 2016 a 31 de dezembro de 2020. Foram encontrados 21,86% (10849/49617) de laudos positivos para a *G. vaginalis*, sendo que os anos de 2020 e 2017 apresentaram a maior e menor prevalência, correspondendo a 25,28% e 19,40%, respectivamente. A média de idade das pacientes foi de 45,59 anos, e a maior prevalência foi verificada em pacientes com 51 a 60 anos, correspondendo a 22,74%. O fungo *Candida* sp. foi observado em 3,60% dos laudos juntamente com a *G. vaginalis.* Dentre as alterações citopatológicas associadas à *G. vaginalis*, o resultado de células atípicas de significado indeterminado (ASC-US) foi o mais predominante, em 3,97% dos laudos. Portanto, os resultados obtidos evidenciaram uma prevalência considerável da bactéria nos laudos de citologia cervicovaginal. Desta forma, é preciso alertar a população feminina sobre o diagnóstico e tratamento da bactéria, a fim de evitar possíveis complicações.

**Palavras-chave**: *Clue Cells. Gardnerella vaginalis*. Microbiota vaginal. Papanicolau. Vaginose bacteriana.

## 1. INTRODUCTION

During the reproductive age, healthy women present a polymicrobial vaginal microbiota, represented mainly by lactobacilli, denominated as Döderlein bacillus. These lactobacilli play an important role in body defense, balance the vaginal microbiota, and are responsible for maintaining the vaginal pH (FADER et al., 2021). Some species of bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae*, *Mobiluncus* sp., can also be found and are commensal, thus non-pathogenic (GAMBONI & MIZIARA, 2013).

Bacterial vaginosis is caused by the imbalance of the vaginal microbiota, when there is a reduction or absence of lactobacilli leading to an increase of the pH above 4.5, which triggers the excessive growth of anaerobic bacteria in less acidic media, mainly *G. vaginalis* and *Mobiluncus* sp. (TORTORA et al., 2017). Some women are asymptomatic, which makes the diagnosis difficult, whereas others have symptoms such as a white and pasty discharge, also presenting a yellow or gray color, with a bullous appearance, and with a foul odor (MURRAY et al., 2017).

*G. vaginalis* is a facultative anaerobic, gram-variable coccobacillus, immobile, non-spore-forming bacterium and does not present a capsule. Its cell surface is covered by fimbriae, responsible for its attachment to the vaginal epithelial cells (ONDERDONK et al., 2016). This bacterium produces organic acids (e.g. acetic acid), for its proliferation, and aromatic amines (e.g. putrescine, cadaverine and trimethylamine) which, in the presence of high pH, cause a bad odor. In addition, amines and acids are cytotoxic and cause exfoliation of epithelial cells, causing vaginal discharge (CONSOLARO & MARIA-ENGLER, 2016).

Several factors are associated with the imbalance and pH variation, such as vaginal showers, immune system disorders, chemotherapy, and/or poor hygiene habits. Although vaginosis is not considered a sexually transmitted infection, women during the reproductive period with multiple sexual partners are more susceptible to vaginosis (ONDERDONK et al., 2016). Despite being considered more an irritation than a serious infection, vaginosis is associated with adverse health conditions. In pregnant women, it represents a risk factor for premature births, premature rupture of membranes, and postpartum or post-abortion endometritis, consequently, mortality in preterm births is increased as a result of vaginosis during pregnancy. However, the investigation of this pathology is not routine in prenatal care (DENNEY & CULHANE, 2009).

Metronidazole is the most used drug for the treatment of bacterial vaginosis. The effectiveness rate is up to 80% after 7-10 days of treatment. Another alternative that has

been studied is the use of probiotic lactobacilli to restore the balance and pH of the vaginal microbiota (BROLAZO et al., 2009).

The cervical-vaginal cytology or Pap smear test is efficient in identifying cervical-vaginal infections such as *G. vaginalis*, *Candida* spp., *Trichomonas vaginalis* and *Actinomyces* sp. The report of the presence of these microorganisms in the samples is useful and alerts physicians, especially in asymptomatic patients, as it is routinely performed as a prevention for cervical cancer (NAYAR & WILBUR, 2015; SMITH et al., 2018). The main microscopic finding of bacterial vaginosis is the presence of clue cells, also called target cells, which are squamous cells with cellular surfaces and borders covered by coccobacilli, making the cytoplasmic limits imprecise, associated with a reduction or absence of lactobacilli (CONSOLARO & MARIA-ENGLR, 2016).

Due to the risk of major complications, it is important to obtain and disseminate information on the prevalence of the microorganism to contribute to the update of epidemiological data. Therefore, the present study aimed to assess the prevalence of *G. vaginalis* in cytopathological tests, relate its occurrence to other cytopathological alterations and microorganisms, and verify the age range of the affected patients attended at a laboratory of pathology located in Bento Gonçalves, RS.

## 2. MATERIAL AND METHODS

This is a cross-sectional, retrospective, descriptive and qualiquantitative study, carried out by the analysis of 49617 cervical cytopathological reports in patients treated by the *Sistema Único de Saúde* (SUS) in a laboratory of pathology in the municipality of Bento Gonçalves, RS, in the period between January 1st, 2016 and December 31st, 2020. This study was approved by the Research Ethics Committee of *Centro Universitário CNEC de Bento Gonçalves* (protocol n. 4.717.221 - CAAE: 46337721.7.0000.5571).

The research was conducted through the database of the *Sistema de Informação do Câncer* (SISCAN/SISCOLO). The sampling was carried out considering the reports issued in the period proposed by the research, whereas reports issued in other periods and/or with an unsatisfactory sample were excluded. The variables analyzed were age, year of exam, positive result for *G. vaginalis* and whether, of these positive results, other microorganisms such as *Candida* spp., *Trichomonas vaginalis* and *Actinomyces* spp. were found. The occurrence of cytopathological alterations was also observed, such as atypical cells of undetermined significance (ASC-US), atypical squamous cells of undetermined significance

which cannot exclude high-grade lesions (ASC-H), atypia of undetermined character in glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL). The cervicovaginal slides are stained using the Papanicolaou method and the nomenclature of the reports followed the Bethesda System.

For the analysis of results regarding age, women were categorized into six age groups (under 21, 21-30, 31-40, 41-50, 51-60 and over 60 years). Data were tabulated using the Microsoft Office Excel® 2016 program for basic descriptive analysis and are expressed in absolute values (n) and relative frequencies (%).

## 3. RESULTS

Among the 49617 reports of cytopathological examinations issued during the research period, 22 were excluded due to an unsatisfactory sample, thus, a total of 49595 satisfactory reports were analyzed. The prevalence of *G. vaginalis* was observed in 10839 reports, corresponding to 21.86%.

Table 1 shows a temporal analysis of the prevalence of positive and negative cases for *G. vaginalis*, where a higher prevalence is observed in 2020 and a lower prevalence in 2017 (25.28 and 19.40%, respectively).

**Table 1.** Prevalence of *Gardnerella vaginalis* in patients attended in a Laboratory of Pathology in Bento Gonçalves, RS, from January 1st, 2016 to December 31st, 2020, according to the year of examinations.

| YEAR | Negative | | Positive | | Total |
|---|---|---|---|---|---|
| | n | % | n | % | |
| 2016 | 7315 | 80.02 | 1827 | 19.98 | 9142 |
| 2017 | 9700 | 80.60 | 2334 | 19.40 | 12034 |
| 2018 | 8412 | 79.05 | 2229 | 20.95 | 10641 |
| 2019 | 7828 | 75.15 | 2588 | 24.85 | 10416 |
| 2020 | 5501 | 74.72 | 1861 | 25.28 | 7362 |
| *Total* | 38756 | 78.14 | 10839 | 21.86 | 49595 |

Regarding the age group, there was a higher prevalence of *G. vaginalis* in patients aged 51 to 60 years (22.74%), followed by 41 to 50 years (21.56%), with a lower prevalence being observed in patients younger than 21 years (3.52%), with a mean age of 42.34 years and a median of 46 years (Table 2). Among the microbial agents observed concerning the microbiota associated with *G. vaginalis*, the fungus *Candida* sp. was the most prevalent,

corresponding to 3.60% (390/10,849) of the data (Figure 1). Other associations included the microorganisms *T. vaginalis* and *Actinomyces* sp.

**Table 2.** Prevalence of *Gardnerella vaginalis* in patients attended in a Laboratory of Pathology in Bento Gonçalves, RS, from January 1st, 2016 to December 31st, 2020, according to the age group.

| Age group | Positive | % |
|---|---|---|
| <20 years | 382 | 3.52 |
| 21-30 years | 2327 | 21.47 |
| 31-40 years | 2302 | 21.24 |
| 41-50 years | 2337 | 21.56 |
| 51-60 years | 2465 | 22.74 |
| >61 years | 1026 | 9.46 |
| *Total* | 10839 | 100 |

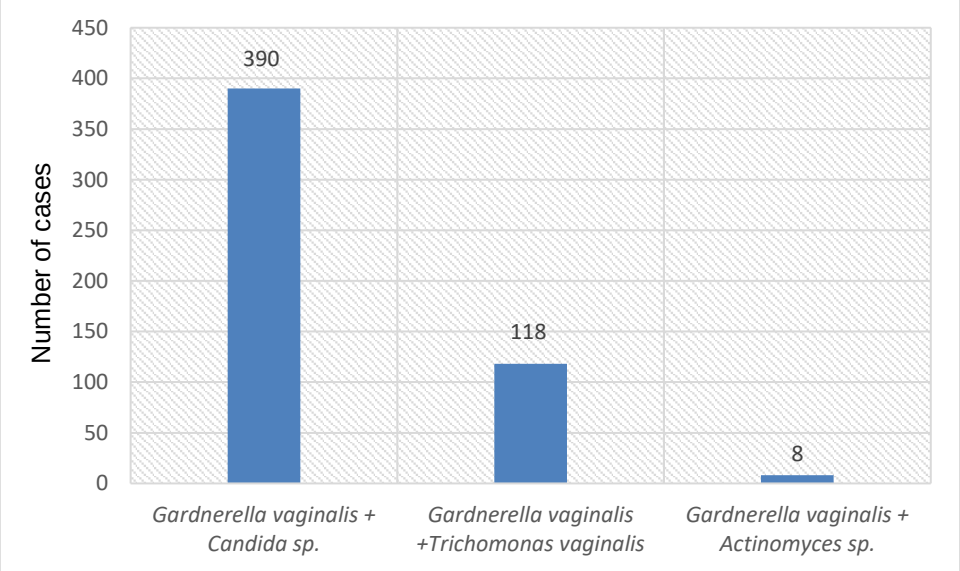


**Figure 1**. Associated microbiota with *Gardnerella vaginalis* in patients attended at a laboratory of pathology in Bento Gonçalves, RS, from January 1st, 2016 to December 31st, 2020.

As for the cytopathological alterations found in the period of the present study, the negative reports for malignancy (NILM) were the most frequent (92.46%). However, a prevalence of 7.54% of cytopathological alterations was observed in patients with *G.*

*vaginalis*, and the ASC-US result was the most predominant finding, with a prevalence of 3.90% (Table 3).

**Table 3.** Prevalence of cytopathological changes in patients with *Gardnerella vaginalis* attended in a Laboratory of Pathology in Bento Gonçalves, RS, from January 1st, 2016 to December 31st, 2020.

| **Cytopathological findings** | **n** | **%** |
|---|---|---|
| Negative for Intraepithelial Lesion or Malignancy (NILM) | 10022 | 92.46 |
| Atypical squamous cells of undetermined significance (ASC-US) | 423 | 3.90 |
| Atypical squamous cells of undetermined significance cannot exclude high-grade lesions (ASC-H) | 157 | 1.45 |
| Low-grade Squamous Intraepithelial Lesion (LSIL) | 167 | 1.54 |
| Atypical squamous cells of undetermined significance cannot exclude high-grade lesion (HSIL) | 64 | 0.59 |
| Atypia of undetermined character in glandular cells (AGC) | 6 | 0.06 |
| *Total* | 10839 | 100 |

## 4. DISCUSSÃO

Bacterial vaginosis is caused by an imbalance in the vaginal microenvironment where there is an exacerbated proliferation of bacterial species and a decrease in lactobacilli, with *G. vaginalis* being the most predominant microorganism. The results of the present research indicated a prevalence of 21.86% for *G. vaginalis* in the cytopathological exams of patients who underwent the exam in the proposed period in the municipality of Bento Gonçalves, RS, which corroborates the prevalence found in other studies carried out in different Brazilian regions. Rocha et al. (2016) observed a prevalence of 23.48% of *G. vaginalis* in 2202 cytopathological exams of patients in Belém, PA. Similarly, Coelho et al. (2014) observed 21.67% of *G. vaginalis* in 563 cytopathological exams in the city of Floriano, PI. In another study, the authors found 12.7% of *G. vaginalis* in 363 cervical smears from women in Fortaleza, CE (TAVARES et al., 2020). Similar results were observed by Diniz et al. (2020), since among the 356 cytopathological exams of patients seen at a health unit in the city of Caruaru, PE, *G. vaginalis* was the most predominant microbiological finding, in 15.17% of the reports.

Regarding similar studies conducted in the state of Rio Grande do Sul, the same region as this study, Santos et al. (2006) and Becker et al. (2011) observed in the city of Porto

Alegre, RS, prevalences of *G. vaginalis* in 17.11% and 15.6% of the reports, respectively. Yet, Ródio et al. (2010) found a prevalence of 28.8% for the microorganism in Erechim, RS. These findings are in accordance with those obtained in the present study. On the other hand, Jantsch et al. (2019) found a lower prevalence in Santiago, RS, corresponding to 13.28%, and Dall'Alba and Jaskulski (2014) found a higher prevalence in patients from Santo Expedito, RS, with 51% of positive reports. Thus, in general, there is little variation in the prevalence of *G. vaginallis* among different municipalities in the state and in the country.

Considering the age group, the mean age of patients with *G. vaginalis* verified in the present study was 42.34 years and a higher prevalence was observed in patients aged 51 to 60 years. Other studies carried out in Brazil reported higher prevalences of the microorganism in younger women, aged between 21 and 39 years (OLIVEIRA et al., 2007; BRINGEL et al., 2012; BARBOSA et al., 2021). Only one study, carried out in Maringá, PR, reported a higher prevalence of *G. vaginalis* in women older than 40 years, in which the mean age of the patients was 40.91 years (TONINATO et al., 2016).

In this context, the present research presented data in disagreement with previous studies. This can be explained by the fact that menopausal women present an absence of lactobacilli in the vaginal microbiota due to the decrease in the production of hormones, such as estrogen and progesterone. As lactobacilli absorb these hormones present in squamous cells and produce lactic acid and hydrogen peroxide that helps to maintain vaginal balance, their absence or decrease can lead to the proliferation of commensal microorganisms, causing vaginitis (KIM et al., 2017). The divergence of the data found regarding the most affected age group in the present study can be attributed to the differences between the characteristics of the populations studied, such as the use of an intrauterine contraceptive device (IUD), poor hygiene habits, use of vaginal showers, among others, as well as sociodemographic factors, which include ethnicity and level of education (RIBEIRO et al., 2007). Due to the lack of this information from the patients analyzed in the present, it was not possible to compare these conditions.

Among the microorganisms associated with *G. vaginalis*, the fungus *Candida* sp. was the most frequent, corresponding to 3.60%. Similarly, Santos et al. (2006), Dall'Alba and Jaskulski (2014) and Junior et al. (2016) found prevalences corresponding to 2.06, 3.0 and 6.1%, respectively, of *G. vaginalis* and *Candida* sp. Vulvovaginitis is caused by fungi, bacteria and protozoa and can often be associated with a mixed infection. According to

Fader et al. (2021), approximately 25% of women have *Candida* species living commensally in the vaginal microbiota, which can proliferate when an imbalance in the microbiota occurs.

The cervical-vaginal examination is the method of screening for cervical cancer in Brazil. According to the Bethesda System, the cytopathological alterations that can be observed in the cytology include: ASC-US, which presents cellular alterations but are insufficient to define the diagnosis of the intraepithelial lesion; ASC-H, which may be suggestive of HSIL; AGC, which are nuclear alterations related to glandular cells but which do not necessarily represent anaplasia; and LSIL. Furthermore, this test proved to be also effective in detecting microorganisms with pathogenic potential, since some women are asymptomatic.

A prevalence corresponding to 7.54% of cytopathological alterations in patients with *G. vaginalis* was found in the present study, which differs from the study of Zattoni et al. (2013), which reported a 34% prevalence of bacterial vaginosis with cellular atypia diagnosed by cervical cytopathological examination. The authors suggest that there is a relationship between the imbalance of the vaginal microbiota and lesions caused by HPV. In this scenario, a meta-analysis found an association between bacterial vaginosis and cervical infection caused by the HPV virus in 6372 women, corresponding to a prevalence of 32% (GILLET et al., 2011). It is postulated that the presence of the virus can alter the vaginal environment, making it more susceptible to the proliferation of bacteria, which can trigger bacterial vaginosis (CHEN et al., 2020). According to Platz-Christensen et al. (1994), there is a possibility that bacterial vaginosis is somehow associated with the development of cervical intraepithelial neoplasia, thus acting as a cofactor of HPV. In addition, there is also evidence that the pathogenic bacteria present in vaginosis can alter immunological signals, reducing the immune system's ability to protect the host, leaving the mucosa more susceptible to HPV infection and cytological abnormalities (MARCONI et al., 2013; PASSOS et al., 2020).

Therefore, the evaluation of molecular mechanisms in the presence of bacterial vaginosis that corroborates the persistence of HPV would be essential to define whether there is a causal association between bacterial vaginosis and precursor lesions of cervical cancer (ZATTONI et al., 2013). It should be noted that in the present research, only the cytopathological reports were analyzed, in which the alterations might or not be associated with HPV. In this scenario, other more specific tests are necessary to verify the possible association between vaginosis and HPV.

## 5. FINAL CONSIDERATIONS

This is the first study with epidemiological data on *G. vaginalis* carried out in the municipality of Bento Gonçalves, which found a considerable prevalence of the microorganism in the population studied. In this way, it is important to highlight that the cytopathological examination is easily accessible in health units and is extremely essential for the prevention of cervical cancer. Furthermore, this test proved to be effective in detecting microorganisms with pathogenic potential in asymptomatic women.

Finally, it should be noted that *G. vaginalis* inhabits the normal microbiota of most women, but when an imbalance occurs, there is an exacerbated proliferation of the microorganism that becomes pathogenic. Therefore, its identification and treatment are extremely important to avoid possible gynecological and obstetrics complications.

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