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Prevalence and susceptibility of microorganisms in vaginal isolates

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Abstract

Microbiological analysis of genital secretion allows isolate and identify the microorganisms responsible for infection in the vaginal canal. The objective of this research was to isolate and identify the microorganisms that cause vaginal infection and analyze antimicrobial susceptibility. 210 samples from women with a clinical diagnosis of vaginal infection who attended the microbiology service were analyzed. Microbial analysis, Gram staining was performed, and they were seeded in specific culture media, as well as in Mueller-Hinton for sensitivity tests. 115 microorganisms were identified, 57% of the isolated correspond to bacterial species, the most frequent was *Gardnerella vaginalis* with 38.5%. 43% of the isolated were yeasts, the predominant species was *Candida albicans* with 78%. On the other hand, testing for susceptibility to various antibiotics, *Gardnerella vaginalis* showed 100% resistance to gentamicin and netilmicin. Ampicillin, norfloxacin and sulfamethoxazole/trimethoprim showed sensitivity greater than 80%. In antifungal susceptibility tests, *Candida albicans* showed 100% resistance to nystatin and 90 to 100% sensitivity to fluconazole and ketoconazole, respectively. Of the isolated species, *Gardnerella vaginalis* and *Candida albicans* were the most frequent in vaginal infections; in addition, both microorganisms presented a variable susceptibility to antimicrobials.

Keywords: Vaginal infection; *Gardnerella vaginalis*; *Candida albicans*; Antibiotics; Antifungals

1. Introduction

It is important to know the typical composition of the vaginal microbiota, which refers to the set of microorganisms that normally inhabit the female reproductive system. In women of childbearing age, the vagina normally contains two types of bacteria, Doderlein-type lactobacilli and some acidophilic bacteria. These bacteria help maintain a healthy balance in the vagina by producing lactic acid from sugars, which maintains an acidic pH and prevents other harmful bacteria from overgrowing. Vaginal acidity therefore constitutes a protective mechanism resulting from the multiplication of the flora that itself promotes [1].

Vaginal infection is defined as a condition in which the female genital area is affected by the abnormal proliferation of microorganisms, such as bacteria, yeasts, viruses or parasites. These infections can cause a series of uncomfortable symptoms, such as itching, burning, abnormal vaginal discharge, unpleasant odor and discomfort in the genital area [2]. The three most important types of vaginal infections are bacterial vaginosis (40-50%), candidal vaginitis (20-25%) and trichomoniasis (15-20%) [2-4].

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Bacterial vaginosis (BV) is the most common cause of vaginal infections. It is an imbalance in the composition and functions of the microorganisms that normally live in the vagina. This occurs when the healthy vaginal flora is replaced by a set of different bacteria, mostly anaerobic. This change includes the formation of a dense layer of multiple types of bacteria, the main one being *Gardnerella vaginalis*, which adheres strongly to the vaginal walls, in addition to *Mobiluncus sp* or *Mycoplasma* [5]. BV is asymptomatic in 11-48% of cases and is the most common cause of vaginal problems, accounting for one third of vulvovaginal infections [6] and most frequently occurring in women aged 15-44 years [7]. The prevalence varies depending on factors such as race, age, and socioeconomic status, but generally occurs in more than 29.2% of cases, being more common in women of childbearing age. Furthermore, the frequency varies from 4.9% to 35% in high-income countries, while in low- and middle-income countries, it ranges from 20% to 51% [8-9]. BV is diagnosed using Amsel criteria, which include four features. To confirm the diagnosis, at least three of these criteria must be met: a) the vaginal discharge has a milky, grayish or yellowish appearance, b) the vaginal pH is higher than 4.5, c) when an alkaline solution (10% KOH) is added to the vaginal discharge, emits an unpleasant fish-like odor (positive amine test), d) clusters of shed cells, known as clue cells, are observed in the sample [2, 5].

Candidal vaginitis (CV) is a condition characterized by fungal colonization, most commonly *Candida albicans*. In the United States, CV represents the second most common cause of vaginal infections, affecting 70–75% of women during their lifetime and resulting in approximately 1.4 million outpatient visits each year [10-11]. Although most women with vaginal fungal colonization are asymptomatic, many have varying degrees of vaginal itching, the most specific symptom of CV. Some patients may also experience vaginal pain, swelling, dyspareunia, dysuria, or increased discharge [11]. VC can be diagnosed clinically by microscopy or by yeast culture, and most cases are treated with azole antifungals [12]. CV is caused by different species of the *Candida* genus, the most common being *Candida albicans*, which accounts for 60–80% of the total disease-causing species; *C. glabrata* is the second most common species (between 5 and 15%). Other species detected less frequently in gynecological infections are *C. tropicalis*, *C. pseudotropicalis* and *C. krusei*; a significant increase in the detection of non-*C. albicans* species and a higher rate of recurrence of vulvovaginitis episodes has been observed [13].

Trichomoniasis is one of the most common sexually transmitted diseases worldwide, caused by the parasite *Trichomonas vaginalis*. Women with this disease present itching and profuse, whitish, greenish or yellowish vaginal discharge with a foul odor [14]. Direct observation by microscopy with saline solution of mobile *Trichomonas* with their characteristic spasmodic movements is 100% specific but only 50% sensitive. Sensitivity is reduced by delaying microscopic observation of the sample for just 10 minutes [2].

Accurate diagnosis of a vaginal infection requires an appropriate medical evaluation, which may include a physical examination, laboratory tests (smear and vaginal culture), and additional tests depending on the person's symptoms and medical history. Treatment of vaginal infections usually includes antifungal, antibiotic, or antiparasitic medications depending on the type of infection. The aim of this study was to determine the prevalence of microorganisms isolated from vaginal infections and to analyze antimicrobial susceptibility.

2. Material and methods

This study was an observational, prospective, and descriptive; it was conducted in the Research Laboratory of the Academic Unit of Chemical Biological and Pharmaceutical Sciences of the Autonomous University of Nayarit and in a clinical institution in Tepic, Nayarit, during the period September-November 2018.

2.1. Sample collection

210 samples of clinical cases of patients with vaginal discharge who attended the microbiology service were included. Clinical characteristics observed include homogeneous discharge, vaginal odor, itching, and increased amine production. A sample of vaginal discharge was obtained by inserting two swabs into the cervix and vaginal wall; one of the swabs was placed in Stuart medium, the other was used for Gram staining and subsequently placed in sterile saline solution to perform the microbial examination. In addition, the amine test was performed, three drops of 10% KOH were added, the detection of a fish-like odor was considered positive [15-16].

2.2. Microbiological analysis

Samples were plated on MacConkey agar, blood agar, mannitol salt and Biggy agar (Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 24 hours; The same swab was plated on Thayer-Martin agar (bio-Mérieux, Lyon, France), the plates were incubated in the presence of 5% CO_2 at 36°C for 48 hours. Bacterial identification was performed with the help of colonial morphology, Gram staining and by the MicroScan autoSCAN-4 System (Siemens Healthcare Diagnostics). *Candida* species were identified using CHROMagar (MCD Lab, Mexico), which indicates the species based on the color produced by the colonies. Only pathogens isolated and identified were reported in the results. Normal vaginal flora was not reported.

2.3. Susceptibility tests

The agar diffusion test described by Kirby and Bauer [17-18] was used to determine antimicrobial susceptibility. The bacterial suspension was prepared by transferring 3 isolated colonies in tubes with 3.0 mL of sterile saline solution, shaken and turbidity in the tubes was measured by comparing it with the 0.5 standard MacFarland tube, which is constant turbidity equal to 1.5×10^{8} cells/ml. Subsequently, 100 µL of the suspension was taken and placed on a plate with Mueller-Hinton agar, and a sterile swab was used to spread the bacterial inoculum by rotating it until it became a grass-like shape. The plate was left to dry for two minutes. Next, disks impregnated with the following antimicrobials for Gram-positive bacteria were placed: ampicillin, cephalothin, cefotaxime, ciprofloxacin, clindamycin, dicloxacillin, erythromycin, gentamicin, penicillin, tetracycline, sulfamethoxazole/trimethoprim and vancomycin (Multibac I.D.). For Gram-negative strains, amikacin, ampicillin, carbenicillin, cephalothin, cefotaxime, ciprofloxacin, chloramphenicol, gentamicin, netilmicin, nitrofurantoin, norfloxacin, and sulfamethoxazole/trimethoprim were used. For *Candida* strains, the discs impregnated with the antifungals were nystatin, econazole, miconazole, ketoconazole, clotrimazole, and fluconazole (Bio-Rad). The plates were then incubated at 37°C for 24 hours. For interpretation, the breakpoints and the classification scheme in categories (susceptible, intermediate resistance, and resistant) recommended by the National Committee for Clinical Laboratory Standards were applied [18].

2.4. Statistical analysis

The Microsoft Office Excel program was used to process the information collected in this research. The data was analyzed by applying descriptive statistics, presenting comparisons of the results obtained through frequency tables and/or equivalent graphs.

3. Results and discussion

3.1. Microbiological identification

Between September and November 2018, 210 swabs were collected for the study from women with vaginal infections. Of the total number of vaginal exudates analyzed, only 102 showed bacterial growth, of which 115 microorganisms were isolated and identified. Not all samples revealed pathogenic organisms, as approximately 49% did not show pathogen growth. Several reasons could explain this; but it is important to show that not all vaginal discharges reported by women during clinic visits are diseases or caused by pathogenic organisms [19-20].

The results obtained show that infections of bacterial origin correspond to 56.52% of the isolates and *Candida spp* to 43.48%. These results coincide with other research, where bacterial isolation has been reported in 15-50% and the presence of *Candida spp* in 15-30% [3-4, 21-22]. Another study conducted in pregnant women reported that the most frequently isolated microorganism was *Candida spp*, followed by *Gardnerella*, *Trichomonas vaginalis* and Gram-negative diplococci [23]. For identification, the growth of microorganisms was analyzed. On Sabouraud dextrose agar *Candida albicans* grows forming white, soft, creamy and smooth colonies; on chromogenic agar the colonies are blue or light green. On blood agar, *Gardnerella vaginalis* forms small colonies of 0.25 to 0.44 mm in diameter, transparent white and presenting β-hemolysis. Escherichia coli grows on MacConkey agar forming pink-reddish colonies, surrounded by a zone of precipitated bile due to lactose fermentation. Microscopic observation of Gram stain, *Candida albicans* was observed to contain yeasts, *Gardnerella vaginalis* was observed to contain short Gram-negative bacilli and *E. coli* as Gram-negative bacilli (Figure 1).

Gardnerella vaginalis was the predominant pathogen with 38.47%, followed by *Escherichia coli* 23.08%, *Enterococcus faecalis* 15.38%, *Staphylococcus aureus* 15.38% and with lower prevalence *Streptococcus agalactiae* and *Enterobacter cloacae* with 6.15 and 1.54%, respectively (Table 1). No growth of *N. gonorrhoeae* was obtained. Similar results were found by Salas et al. [24], where *Gardnerella vaginalis* was present in 39% of cases, being the most prevalent microorganism in bacterial vaginosis. Similarly, in the study conducted by Padilla et al. [25], it was observed that *Gardnerella vaginalis* is the microorganism most frequently isolated from samples of women with a clinical diagnosis of vaginal infection (33.2%), followed by *E. coli* (23.0%). Other frequent isolates were *Candida albicans* and *Trichomonas vaginalis* with 15.5 and 9.1%. The latter contrasts with our results because the presence of *Trichomonas vaginalis* was not reported.

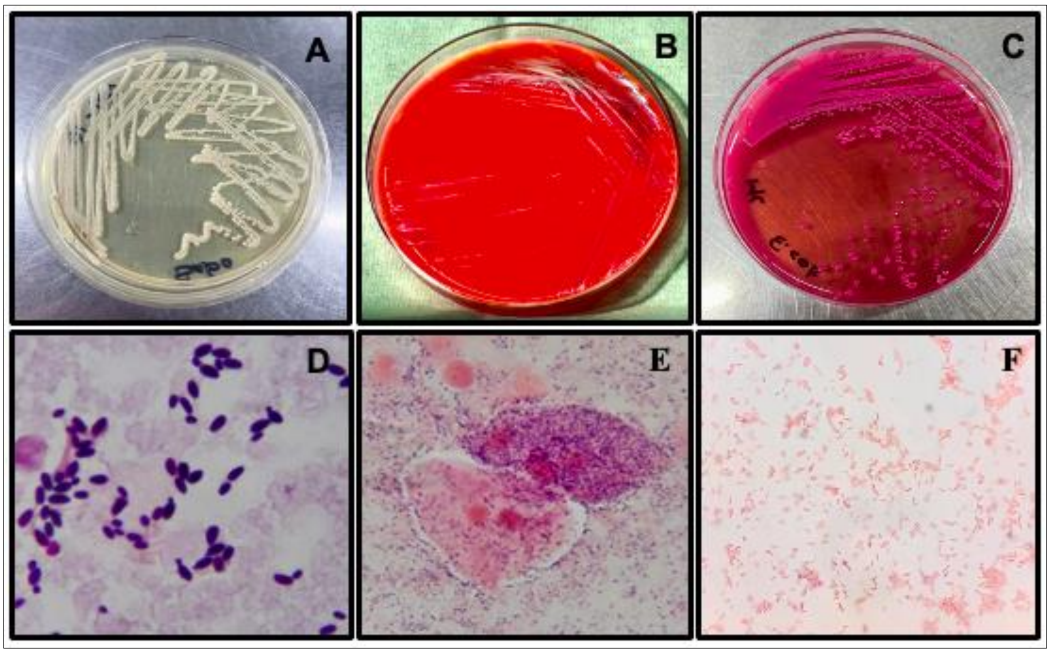

Figure 1 Morphological characteristics of microorganism isolated. *Candida albicans* growth on Sabouraud dextrose agar and microscopic morphology (A and D). *Gardnerella vaginalis* growth on blood agar and microscopic morphology (B and E). *Escherichia coli* growth on MacConkey agar and microscopic morphology (C and F).

Candida isolated, the species *albicans*, *glabrata*, *tropicalis* and *krusei* were identified with a prevalence of 78, 16, 4 and 2%, respectively. It is important to highlight that the predominant specie was *C. albicans* (Table 1). These results agree with those reported by Frías-De León et al. [26] who indicate that the predominant causal agent in candidiasis infections is *Candida albicans*. Betancourt & Carrera [27] studied 201 women, where *Candida albicans* was also the most frequent species. Orellana & Pacheco [28] show that *Candida albicans* was the most prevalent in vaginal secretion samples with 92.6%, followed by *Candida glabrata* 6.6% and *Candida parapsilosis* with 0.7%.

Table 1 Microorganisms identified in vaginal isolates.

Microorganism	**Number of isolates**	**%**
Bacteria		
Gardnerella vaginalis	25	21.74
Escherichia coli	15	13.04
Enterococcus faecalis	10	8.70
Staphylococcus aureus	10	8.70
Streptococcus agalactiae	4	3.48
Enterobacter cloacae	1	0.87
Yeast		
Candida albicans	39	33.91
Candida glabrata	8	6.96
Candida tropicalis	2	1.74
Candida krusei	1	0.87
TOTAL	115	100

3.2. Antimicrobial susceptibility testing

The susceptibility test was carried out using the Kirby-Bauer method [17], both for bacteria and for *Candida* species. This test consists of spreading disks impregnated with the antimicrobial on a Mueller-Hilton agar base. *Gardnerella vaginalis* isolates show 100% resistance to gentamicin and netilmicin; 96% resistance to nitrofurantoin. In contrast, 92% are sensitive to norfloxacin, 84% to sulfamethoxazole/trimethoprim, 80% to ampicillin, and 48% to carbenicillin (Table 2). Knupp de Souza et al. [29] reported that out of 42 vaginal isolates, high resistance to ampicillin was obtained (54.4%), metronidazole (59.8%), tinidazole (60.3%) and secnidazole (71.6%), which slightly disagrees with the results obtained. In addition, susceptibility tests to metronidazole were not performed.

Escherichia coli presented 100% resistance to amikacin, 80% to ampicillin and 100% sensitivity to carbenicillin, cephalothin, netilmicin and norfloxacin (Table 2). Ruiz [30] reported that *E. coli* in vaginal isolates shows a resistance rate to sulfamethoxazole/trimethoprim of 36% and ciprofloxacin of 17%, as well as a 100% sensitivity to chloramphenicol. On the other hand, Guzman et al. [31] points out that *E. coli* in vaginal infections presents resistance of 96.1% to ampicillin, 59.4% to cephalothin, 39.6% to sulfamethoxazole/trimethoprim and 29.7% to ciprofloxacin. In Mexico, Martínez et al. [1] report that in vaginal isolates *E. coli* has a sensitivity of 66% to gentamicin and 40% to sulfamethoxazole/trimethoprim.

Table 2 Antibiotic susceptibility-resistance in *Gardnerella vaginalis* y *E. coli.*

Microorganism	***Gardnerella vaginalis***		***Escherichia coli***	
Antiobiotic	**Resistance**	**Sensitivity**	**Resistance**	**Sensitivity**
Amikacin	10 (40)	15 (60)	15 (100)	0 (0)
Ampicillin	5 (20)	20 (80)	12 (80)	3 (20)
Carbenicillin	13 (52)	12 (48)	0 (0)	15 (100)
Cephalothin	20 (80)	5 (20)	0 (0)	15 (100)
Cefotaxime	18 (72)	7 (28)	5 (33)	10 (67)
Ciprofloxacin	18 (72)	7 (28)	4 (27)	11 (73)
Chloramphenicol	10 (40)	15 (60)	3 (20)	12 (80)
Gentamicin	25 (100)	0 (0)	2 (13)	13 (87)
Netilmicin	25 (100)	0 (0)	0 (0)	15 (100)
Nitrofurantoin	24 (96)	1 (4)	2 (13)	13 (87)
Norfloxacin	2 (8)	23 (92)	0 (0)	15 (100)
Sulfamethoxazole Trimethoprim	4 (16)	21 (84)	4 (27)	11 (73)

The resistance and susceptibility data for Gram-positive bacteria such as *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus agalactiae* against antibacterial agents showed variable susceptibility. *Enterococcus faecalis*, 100% of the isolates were resistant to dicloxacillin and 80% to ampicillin and erythromycin. In contrast, 100% were sensitive to cefotaxime and penicillin and 90% to gentamicin and ciprofloxacin. Martínez et al. [1] reported that *Enterococcus faecalis* had a sensitivity of 11% to tetracycline and 100% to vancomycin, results that differ since a sensitivity of 30% was obtained for tetracycline and 50% for vancomycin. *Staphylococcus aureus*, 100% of the isolates were resistant to ampicillin, cephalothin, erythromycin and vancomycin. In addition, 90% sensitivity to clindamycin and 80% to dicloxacillin, tetracycline and sulfamethoxazole/trimethoprim was observed. Sánchez & Flores [32] report *Staphylococcus aureus* presented a resistance rate of 72% to clindamycin and tetracycline; likewise, gentamicin and tetracycline show a resistance rate of 18.2%. Martínez et al. [1] reported a 100% sensitivity to gentamicin, sulfamethoxazole/trimethoprim and vancomycin, while tetracycline presented a sensitivity of 75%, results that differ from those obtained in this work since for *Staphylococcus aureus* a resistance rate of 10% to clindamycin, 100% to erythromycin and vancomycin and 40% to gentamicin was obtained.

The analysis of the resistance and sensitivity tests for the species *Candida albicans*, *C. tropicalis*, *C. krusei* and *C. glabrata* against antifungal agents showed variable susceptibility. *Candida albicans* showed 100% resistance against the agent nystatin, and 92 and 100% sensitivity to fluconazole and ketoconazole, respectively. In the case of miconazole, it shows 54% sensitivity (Table 3). In Mexico, there are clinical practice guidelines on which health personnel can use as a basis for diagnosis as well as the treatment of diseases. According to the clinical practice guide issued in 2019 by the Mexican Social Security Institute (IMSS), for the management of vulvovaginal candidiasis the use of nystatin and fluconazole is recommended [33], in the present work *Candida* demonstrates a resistance rate of 100% and 7.70% respectively. Hassan et al. [34] analyzed the antifungal sensitivity of 95 *Candida* strains from vulvovaginal isolates that included women aged 19 to 40 years. They found that 56% of the strains studied showed resistance to ketoconazole, unlike the results obtained in this work where 100% sensitivity to this antifungal was obtained. In relation to clotrimazole, in 2016 evidence of 100% sensitivity was found in *C. albicans* strains, however, in this work a sensitivity of almost 72% was observed, a lower percentage than that already reported [35].

Table 3 Antifungal susceptibility-resistance in *Candida albicans*

Microorganism	***Candida albicans***	
Antifungal	**Resistance**	**Sensitivity**
Nystatin	39 (100)	0 (0)
Econazole	10 (26)	29 (74)
Miconazole	18 (46)	21 (54)
Ketoconazole	0 (0)	39 (100)
Clotrimazole	11 (28)	28 (72)
Fluconazole	3 (8)	36 (92)

The antifungal that was least effective against the isolated strains was nystatin, which differs from has been reported by different study where it is indicated that it has an effectiveness of 70-90% [36-37]. In addition to the above, the World Health Organization in the "Model List of Essential Medicines" considers it as an effective antifungal against yeast infections and recommends the vaginal administration of 1-2 suppositories at night for at least 2 weeks [38]. In different investigations, it has been proposed that strains resistant to nystatin are those that naturally generate modified sterols that have a lower affinity for nystatin and bind to it less effectively [39-40].

4. Conclusion

The main microorganisms found in vaginal exudates were bacteria such as *Gardnerella vaginalis* (21.74%) and yeasts such as *Candida albicans* (33.91%). According to antimicrobial susceptibility tests, *Gardnerella vaginalis* showed greater resistance to gentamicin (100%), netilmicin (100%) and nitrofurantoin 896%) and high sensitivity to norfloxacin (96%), sulfamethoxazole/trimethoprim (84%) and ampicillin (80%). On the other hand, *Candida albicans* is highly sensitive to ketoconazole (100%) and fluconazole (92%) and resistant to miconazole (46%) and nystatin (100%). *Candida spp*. showed total resistance to nystatin, the antifungal drug administered in the first instance for candidiasis according to the hierarchy of pharmacological treatment by the National Health System of Mexico.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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