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Single-pathogen and Mixed Vulvovaginal Infections Among Women of Reproductive Age Consulting Gynecologists: A Cross-Sectional Study

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Abstract: Vulvovaginal infections are a public health problem that accounts for 20% of gynecological consultations. However, there are no clear data on the prevalence of the different types of vulvovaginal infections, including vaginal mixed infections, that make women seek care from gynecologists. We aimed to determine the prevalence of vulvovaginal infections and to evaluate the therapeutic approach of symptomatic women of reproductive age consulting gynecologists. A cross-sectional study was conducted among 103 women with vulvovaginitis of probable infectious etiology at 25 gynecology outpatient clinics. Women underwent clinical diagnosis following standard clinical practice. Signs and symptoms were registered as well as the therapeutic strategy prescribed by the gynecologists and self-treatment habits of the women before consultation. A microbiological culture of the vaginal discharge was performed in all women to confirm the diagnosis. According to the microbiological studies, 68% of women had an infection, being candidiasis the most prevalent (54.3%), followed by bacterial vaginosis (25.7%), mixed infection (14.3%), and nonspecific vulvovaginitis (5.7%). Most of the candidiasis involved a single pathogen, however 19.4% were mixed infections also involving other pathogens. Leukorrhea and pruritus were the most prevalent symptoms (82.5% and 81.6% respectively). Mixed infections produced vaginal odor changes more often than single candidiasis (40% vs. 24%). Among the 103 women, 71.8% received pharmacological treatments, mostly antifungals, being fenticonazole the most frequently prescribed; 48.5% received nonpharmacological treatments, either alone or as adjuvant therapy. Candidiasis is the most prevalent vulvovaginal infection in symptomatic women of reproductive age consulting gynecologists. Candidiasis usually presents as a single-pathogen infection, but additional pathogens may coexist in many women. Clinical diagnosis of mixed infections is

challenging because the most prevalent symptoms are the same as for candidiasis, so prescription of antifungals with a broad spectrum of antimycotic and antimicrobial activity is an adequate alternative for women.

Keywords: Antifungals, Bacterial Vaginosis, Gynecology, Mixed Infection, Pruritus, Vulvovaginal Candidiasis, Vulvovaginitis

1. Introduction

Vulvovaginitis is characterized as an inflammatory or infectious condition of the vulva and the vaginal mucosa. Vulvovaginal infections are a frequent reason for consultation in primary and secondary care and hospital emergency departments, accounting for 20% of gynecological consultations. About 75% of women develop symptomatic vulvovaginitis at least once in their lifetime [1]. The highest incidence is noted among 20–40 years-old [1, 2]. Bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis are the three most prevalent vulvovaginal infections [3], being VVC the first among them in Europe and the second in the USA, only surpassed by BV. Occasionally, vulvar and vaginal inflammation does not have an infectious etiology, but an irritant or allergic origin [1]. Risk factors that may predispose patients to develop vulvovaginal infections include prior vulvovaginal infection, prior pregnancy, contraceptive use, recent antibiotic use, and diabetes [1, 4, 5]. Most frequent symptoms of vulvovaginitis include vulvar pruritus and pain, vaginal discharge, or dyspareunia [6]. Due to the discomfort and the negative interference in women's quality of life, vulvovaginitis is considered a public health problem [7].

The scientific community highlights certain issues as a matter of concern. One of them is the increasing prevalence of recurrent vulvovaginal infections. Recurrent VVC and recurrent BV affect 138 million and 100 million women every year respectively [8, 9]. Recurrent vulvovaginal infections can severely affect the quality of life of affected women, causing great discomfort and changes in their routines, especially in sexual life [8, 10]. Unlike most episodic vulvovaginal infections, recurrent cases require maintenance regimens with long-term treatments. So, recurrences pose a problem for women and clinicians, apart from the economic burden from lost productivity and treatment costs [8, 11]. Recurrences may result from ineffective empiric treatments [1, 12]. Since it is not always possible to determine the etiologic agent through history and physical examination, empiric treatment of women having symptoms of vulvovaginitis is common and is perceived to cause no harm, although it is not recommended [13, 14].

Several women self-diagnose and self-treat vulvovaginal infections inaccurately based on their previous experience, leading to recurrences and increased drug resistance [1, 15, 16]. Vulvovaginal infections caused by resistant strains constitute a major challenge to reach a successful treatment [17].

Finally, mixed vulvovaginal infections constitute another important issue. They are defined as the simultaneous infection by at least two different pathogens [18]. They are rather common and their incidence is increasing [19], posing serious challenges in their management because their clinical diagnosis is further complicated due to coexistence of symptoms and because both pathogens require specific treatment for complete eradication [18, 20].

To reduce the impact caused by inappropriate treatment of vulvovaginal infections, it is advisable to standardize clinical management and provide information to patients. Despite the relevance and health implications of vulvovaginal infections, there is no clear data about the prevalence and management of this condition in Spain. To our knowledge, other countries have evaluated the prevalence, diagnostic approach, and treatment appropriateness of women with symptoms of vulvovaginitis who are seeking care, but there are no such studies in our country [13]. A few studies have addressed the prevalence of vulvovaginal infections in Spain, but focusing on specific pathogens, regions, or populations other than symptomatic women of reproductive age [21–23]. The aims of this study were (1) to determine the prevalence of vulvovaginal infections and associated signs and symptoms in women of reproductive age attending gynecology outpatient clinics in Spain and (2) to evaluate the therapeutic approach by gynecologists and the self-treatment habits of women.

2. Materials and Methods

2.1. Study Design

A multicenter, observational, descriptive cross-sectional study was conducted between November 2019 and March 2020 at 25 gynecology outpatient clinics from hospitals and medical centers in Spain, distributed across the entire country. The study included a single visit in which the inclusion and exclusion criteria were verified, the informed consent was signed, the participant was evaluated, the data were collected, and the samples were taken.

2.2. Participants

The study involved consecutive women attending gynecology outpatient clinics with signs or symptoms suggestive of vulvovaginal infection. Potential participants were enrolled in the study based on the following criteria: (1) women aged 18 years and over, (2) women of reproductive age, (3) women with signs and symptoms suggestive of vulvovaginal infection according to the investigator, (4) women considered suitable to participate in the study

according to the investigator, and (5) women who provided written informed consent. The exclusion criteria included the following: (1) pregnant women, (2) women with comprehension, reading, or writing difficulties, and (3) women who were already involved in another clinical study. The study protocol was reviewed and approved by a reference Ethics Committee for Clinical Investigation. In addition, the study was notified and classified by the Spanish Agency of Medicines and Medical Devices.

2.3. Clinical Data

The women selected for the study were managed in accordance with the standard clinical practice, consisting of a thorough history and a gynecological physical examination. Women were asked about antibiotic and/or antifungal use, prior episodes of vulvovaginal infections, contraceptive use, sexual partners, prior pregnancy, diabetes, and self-management practices of the current vulvovaginitis episode. Clinical assessment was based on office tests including vaginal pH determination and on the presence of signs and/or symptoms suggestive of vulvovaginitis. Considered symptoms included pruritus, dysuria, dyspareunia, burning, vulvar pain, leukorrhea, changes in vaginal discharge color, consistency, and odor and increased urinary frequency. Considered signs included erythema, edema and bleeding. The intensity of the symptoms and signs was assessed by classifying each feature into three categories: mild, moderate, or severe. According to the findings, the gynecologist established the suspected clinical diagnosis, which should fit in one of these six differentiated categories: VVC, BV, trichomoniasis, mixed infection, nonspecific vulvovaginitis (suspicion of the absence of infection or the presence of an uncommon or nonspecific pathogen), and others (sexually transmitted infections, etc.).

All these data were recorded by the investigators to determine the prevalence of each sign and symptom, their intensity, and the suspected diagnosis according to the clinical assessment. Information about the therapeutic approach was also collected, with paid attention to the pharmacologic and non-pharmacologic treatments prescribed by clinicians as well as the self-treatment habits referred by patients.

2.4. Microbiological Diagnosis

Microbiological analysis was conducted to confirm the suspected diagnosis assessed by the clinician. This analysis responded only to investigational purposes since the gynecologists selected the treatment exclusively based on the clinical assessment and prior to the reception of the microbiological results.

Vaginal discharge samples were collected from all women to conduct microbiological analysis. The sample was collected from the posterior fornix of the vagina using a swab. On the same day that were collected all samples were sent to a central laboratory to be tested. According to the results obtained, women were classified according to the same six categories previously defined for the clinical diagnosis. From the microbiological perspective, nonspecific vulvovaginitis was defined when the culture was positive for saprophytic and nonspecific vaginal flora, for instance *Streptococcus agalactiae, Saccharomyces cerevisiae,* or *Streptococcus pyogenes.*

These data were used to assess the main variable of the study: the prevalence of the different vulvovaginal infections, according to the microbiological study results.

2.5. Sample Size and Statistical Analysis

To calculate the sample size, the expected prevalence of vulvovaginal infections considered in our study was 67% based on published evidence [24]. At a 95% confidence interval with a precision of ± 9.5, the calculated minimum sample size was 101 patients.

Statistical analyses were performed using SAS® (Version 9.4; SAS Institute Inc., Cary, NC, USA). A descriptive statistical analysis of each of the variables was carried out. Qualitative variables were described as absolute and relative frequencies. Quantitative variables were described as mean values, median values, standard deviations, and ranges according to their distribution. Associations between qualitative variables were analyzed with the chi-squared test. *P*-values<.05 were considered statistically significant.

3. Results

3.1. Population Characteristics

During recruitment, 113 women were considered to participate in the study. Among those, ten were not eligible for participation because of the following reasons (a woman could have more than one reason for exclusion): seven women were not evaluated for the main variable of the study, three women were not of reproductive age, three women were not suitable to participate in the study according to the investigator, and one woman was pregnant. Finally, a total of 103 women were enrolled. Their baseline characteristics are shown in Table 1. Sixty-one women (59.2%) had a previous history of VVC and 18 (17.5%) of BV.

Table 1. *Baseline characteristics of women of reproductive age consulting gynecologists (n=103).*

Characteristic	Frequency (%)
Median age (range)	35 (18–55) years
Diabetes	2 (1.9)
Prior pregnancy	41 (39.8)
On contraceptive therapy	35 (34.0)
Antibiotic or antifungal use in the last 2 weeks	33 (32.0)
Unprotected sex in the last moth	78 (75.7)

3.2. Clinical Features and Diagnosis

Signs and symptoms are classified by intensity, except in the case of “discharge color change” and “discharge odor change” where the overall number of women presenting with them is shown.

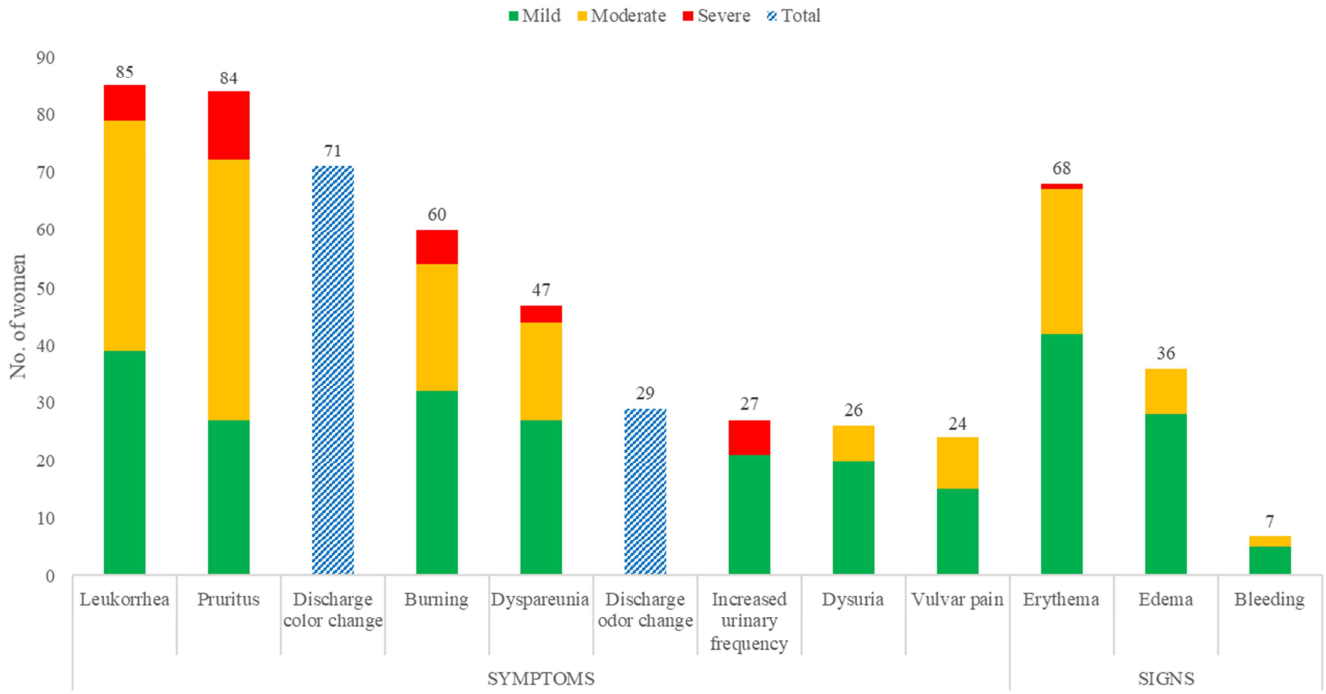

Figure 1. *Prevalence and intensity of symptoms and signs among women of reproductive age consulting gynecologists (n=103).*

Figure 1 shows the prevalence of symptoms and signs revealed in the study, as well as their intensity. Leukorrhea and pruritus were the most prevalent symptoms, reported by 82.5% and 81.6% of women, respectively. Symptoms mostly affected both the vagina and the vulva. The intensity of the symptoms was mostly mild except for leukorrhea and pruritus —the most prevalent ones—, where the intensity was moderate or severe for more than a half of the women. The most prevalent sign was erythema, which affected 66.0% of women. The intensity of the signs was generally mild. Signs usually affected both the vagina and the vulva.

The results of the vaginal cultures showed 33 (32.0%) negative cultures and 70 (68.0%) positive cultures for at least one pathogen. All the pathogens found are shown in Table 2. Among the 70 women with positive results, a single pathogen was identified in 60 women whereas a mixed infection was found in the other ten women (Figure 2). VVC was the most prevalent infection (54.3%), followed by BV (25.7%), mixed infections (14.3%), and nonspecific vulvovaginitis (5.7%). No trichomoniasis was found in any woman. Of the 47 women with *Candida* species identified in the vaginal discharge, nine had a mixed infection (19.1%) and 26 (55.3%) had a previous history of VVC. Of the 28 women with a microbiological diagnosis of BV, ten had a mixed infection (35.7%) and five (17.9%) had a previous history of BV.

Table 2. *Pathogens isolated from the positive vaginal discharge cultures.*

Pathogen	**Number**
Candida albicans	44
Candida glabrata	3
Gardnerella vaginalis	26
Bifidobacterium breve	1
Staphylococcus aureus	1
Streptococcus agalactiae	3
Streptococcus pyogenes	2
Saccharomyces cerevisiae	1
Total	81

All mixed infections included *Gardnerella vaginalis, Candida* species were present in nine cases, one case associated *Streptococcus agalactiae*, and one case with *Saccharomyces cerevisiae* (Figure 2).

The prevalence of the symptoms and signs found in women with mixed infections, VVC, BV, and negative cultures is shown in Table 3 and Table 4. The comparison of the clinical features of each infection type showed that VVC and mixed infection profiles of symptoms were similar. For instance, 97% of women with VVC and 90% with mixed infections presented with pruritus, the most frequent symptom in both subgroups. In contrast, only 57% of women with BV presented with pruritus.

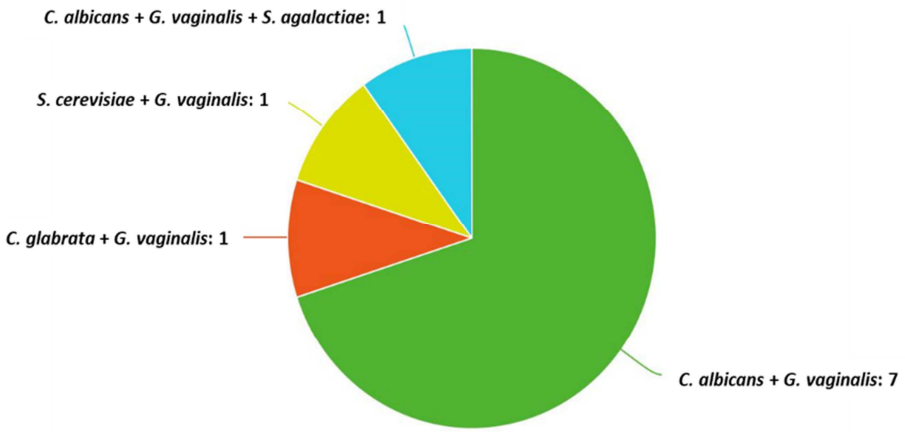

Figure 2. *Pathogens isolated from the positive vaginal discharge cultures in women with vulvovaginal mixed infections (n=10).*

Table 3. *Prevalence of symptoms among women of reproductive age consulting gynecologists classified by type of infection.*

	VVC	BV	Mixed infection	No infection
	N=38 (%)	*N*=28 (%)	*N*=10 (%)	*N*=33 (%)
Symptoms				
Pruritus	97	57	90	82
Dysuria	31	21	30	21
Dyspareunia	50	39	60	42
Burning	63	36	60	67
Vulvar pain	24	28	50	15
Leukorrhea	84	82	80	79
Discharge color change	68	71	80	67
Discharge odor change	24	57	40	12
Increased urinary frequency	32	36	40	15

BV, bacterial vaginosis; VVC, vulvovaginal candidiasis.

Table 4. *Prevalence of signs among women of reproductive age consulting gynecologists classified by type of infection.*

	VVC *N*=38 (%)	BV *N*=28 (%)	Mixed infection *N*=10 (%)	No infection *N*=33 (%)
Signs				
Erythema	77	57	80	64
Edema	45	35	60	30
Bleeding	4	11	10	6

BV, bacterial vaginosis; VVC, vulvovaginal candidiasis.

3.3. Therapeutic Approach

Among the 103 women, 77 (74.8%) received pharmacological treatment, either alone (*n*=42) or in combination with nonpharmacological adjuvant measures (*n*=35) (see Table 5). Pharmacological treatment included topical imidazole antifungals (prescribed in 38 women), topical polyene antifungals (*n*=2), systemic triazole antifungals (*n*=17), systemic imidazole antifungals (*n*=1), macrolides (*n*=10), and antiseptics (*n*=19). Among the women who took antifungals, only 13 received additional prescriptions for their sexual partners. Gynecologists prescribed nonpharmacological treatments in 45 women (43.7%), representing the exclusive therapy in 10 cases. These measures included 58 products (23 probiotics, 20 feminine hygiene products, and others) and three recommendations for a low-sugar diet. Sixteen women did not receive any pharmacological nor nonpharmacological treatment.

Among the women with a clinical diagnosis of VVC, fenticonazole was the most prescribed antifungal (56.3%), followed by clotrimazole (25.4%), ketoconazole (9.9%), and miconazole (8.5%). Vaginal suppositories and cream were prescribed combined in 48.8% of cases, especially in those women where nonpharmacological adjuvant treatment was prescribed. Among nonpharmacological treatments, probiotics were the most used (44.8%), followed by feminine hygiene products (34.5%). Surprisingly, 9.6% of women with a clinical diagnosis of VVC did not receive pharmacological nor nonpharmacological treatment. The most prescribed pharmacological treatment for BV was dequalinium chloride (54.5% of cases), combined or not with nonpharmacological treatment. Up to 17.8% of women diagnosed with BV did not receive any treatment. Among the 16 women clinically diagnosed with non-specific vulvovaginitis, there was less consensus on treatment choice. The most common approach was no treatment or treatment with dequalinium chloride (five

women in both cases).

Thirty-two women (31.1%) had self-treated the current episode of vulvovaginitis before consulting the gynecologist. Twenty-seven of them decided to take a pharmacological treatment. However, 15 out of 20 women who took antifungals had a positive culture despite having undergone treatment. In addition, 1 out of 2 women who took dequalinium chloride had also a positive culture afterward. Among the 32 women, the most used nonpharmacological measures were feminine hygiene products (n=12) and probiotics (n=6). A significant association was found between women presenting with pruritus, burning, vulvar pain or edema and self-treatment practices (p=.032; p=.006; p=.022; and p=.031 respectively).

Table 5. *Treatment modalities prescribed according to the suspected clinical diagnosis.*

	VVC *N*=52 *n* (%)	**BV *N*=28 *n* (%)**	**Non-specific vulvovaginitis *N*=16 *n* (%)**	**Mixed infection *N*=6 *n* (%)**	**Chlamydia infection *N*=1 *n* (%)**	**TOTAL *N*=103 *n* (%)**
Treatment modality						
Only pharmacological treatment	23 (44.2)	10 (35.7)	5 (31.2)	4 (66.6)	0 (0.0)	42 (40.8)
Only non-pharmacological treatment	4 (7.6)	1 (3.6)	4 (25.0)	1 (16.6)	0 (0.0)	10 (9.7)
Pharmacological and non-pharmacological treatment	10 (38.4)	12 (42.8)	2 (12.5)	1 (16.6)	0 (0.0)	35 (34.0)
No treatment	5 (9.6)	5 (17.8)	5 (31.2)	0 (0.0)	1 (100)	16 (15.5)

BV, bacterial vaginosis; VVC, vulvovaginal candidiasis.

4. Discussion

The present study evaluates the epidemiology of vulvovaginal infections among women of reproductive age attending gynecology outpatient clinics in Spain, together with the therapeutic approach followed by clinicians and the self-treatment habits of women.

Vulvovaginitis accounts for a high proportion of gynecology consultations and usually presents with troublesome symptoms such as pruritus, burning, vulvar pain, odor, dysuria, and dyspareunia [1, 6]. In our study, pruritus (categorized moderate-severe in the majority of women) was the most prevalent symptom for both VVC and mixed infections. These data are consistent with the literature, that points to pruritus as the predominant symptom of VVC, although not all women who report pruritus suffer from VVC [25]. As shown in Table 3, VVC and mixed infections usually presented with similar symptoms. However, there are clinical features such as discharge color and odor changes that seem more prevalent in mixed infections.

Women with signs and symptoms of vulvovaginitis should be advised to seek medical care since they cause great discomfort and interfere negatively with women's self-esteem and quality of life [7, 10]. The aim of treating VVC is to relieve symptoms, so every symptomatic woman should be treated [1].

In our sample, most women (68%) attending gynecology outpatient clinics with symptoms of vulvovaginitis had indeed an infection. Differential diagnosis between vulvovaginal infections might be particularly difficult since all of them cause similar signs and symptoms. In fact, misdiagnosis of this condition approaches 50% and lack of awareness of the etiological pathogen is the main cause of therapeutic failure in vulvovaginitis [4, 20]. Thus, once the gynecologist has assessed the patient's clinical features, it is important to identify in which cases a culture is needed to complete the diagnosis [4].

In our study, VVC represented the most common infection, followed by BV. In particular, the microbiological study of the vaginal discharge samples revealed that approximately half of the women of reproductive age with symptoms of vulvovaginal infections who consult gynecologists have VVC, and a quarter have BV, which is consistent with the literature in terms of frequency order [1]. In our study, 44 out of 47 women with VVC (isolated or combined with other pathogens) had *Candida albicans* in their culture, which has been previously described as the predominant species in women with acute VVC [25]. The other three VVC were caused by *Candida glabrata*, which is, by definition, a complicated VVC. Furthermore, its incidence has increased in recent years [1].

Mixed infections represented a small fraction of all infections in our study population (14.3%). However, it is noteworthy that they were present in 1 out of 5 VVC and 1 out of 3 BV. In our study, most of the mixed infections revealed two pathogens in the culture, mainly *Candida albicans* and *Gardnerella vaginalis*. Other studies reported that approximately 20–30% of women with BV are coinfected by the *Candida* species [18], which is quite consistent with the results of our study (32.1%). Mixed infections constitute a matter of special concern. As mentioned before, they have similar symptoms to single VVC, so their clinical diagnosis is quite challenging and complicates the choice of the most appropriate treatment [20]. Moreover, their incidence is increasing and their recurrence is frequent [19, 26]. Therefore, the characterization of this kind of infection might be helpful to guide treatment options. Mixed infections may require treatment with multiple drugs to eradicate all the microorganisms involved. In this sense, the prescription of antifungals with a broad spectrum of antimycotic and antimicrobial activity may be an appropriate choice in the therapeutic approach of women with signs and symptoms of VVC. In fact, the study of Tumietto *et al.* concluded that the use of topical fenticonazole can be recommended for the first-line empiric treatment of vulvovaginal mixed infections, minimizing the risk of selecting drug-resistant microbial strains [18]. Regarding the therapeutic approach, fenticonazole was the most prescribed antifungal for VVC,

followed by clotrimazole. In VVC, oral and topical azole drugs achieve cures in 80–95% of acute cases without pregnancy. Antifungal therapy with azole drugs applied topically for 3–7 days leads to an improvement of symptoms and negative fungal cultures in 80–90% of patients who complete the therapy [27]. As confirmed in our study, symptoms of VVC mostly affect both the vagina and the vulva. When VVC symptoms involve the vulva outside of the introitus vaginae or inguinal region, an antifungal cream is recommended. Treatment of the vulva alone, without simultaneous eradication of microorganisms in the vaginal reservoir, may provide temporary symptomatic relief but may not lead to definitive treatment success. According to some studies, combined treatment using intravaginal ovules and topical cream for the external genital region and vulva seems to achieve more favorable healing results than intravaginal treatment alone [25]. When VVC is diagnosed, it is important to ask the patient about the sexual partner because antifungal treatment should be indicated if they show signs and symptoms of infection [1].

BV was mostly treated with dequalinium chloride, combined or not with non-pharmacological treatment. Dequalinium chloride is an antiseptic and disinfectant agent recommended as a first-line treatment for patients with BV [1].

Among the women included in the study, 15.5% did not receive pharmacological nor nonpharmacological treatment. Surprisingly, 9.6% of women diagnosed with VVC and 17.8% of women diagnosed with BV received no treatment. These data highlight the need to insist on following clinical guidelines and standardizing practice to improve women's healthcare, in order to avoid recurrences and the appearance of resistant strains.

Non-pharmacological treatment was prescribed in almost half of the women, either alone or in combination with other drugs. These alternatives might be of special interest to relieve the most prevalent and troublesome symptoms such as pruritus and burning in those women with a negative microbiological culture, which represented a third of the whole population in this study. However, this approach might not be limited to this subpopulation. Demand is increasing for new alternative strategies to replace or to be combined with standard therapies to prevent and treat vulvovaginal infections more efficiently [10, 27], aimed at achieving better tolerability and fewer side effects while offering improved quality of life in terms of disease prevention [27]. Alternative strategies may include substances that reestablish the physiologic vaginal environment (probiotics, prebiotics, acidifying agents, etc.) while improving the local immunity response [10]. Even so, albeit limited, adjuvant therapies to treat vulvovaginal infections do have support in the scientific literature [25, 27]. There is still some controversy regarding the use of probiotics as main or adjuvant therapy for the treatment of vaginal infections [10]. To date, several studies support a beneficial role for probiotics in the treatment of several vulvovaginal infections, although in some cases the evidence is weak and requires further investigation [28–32]. Two systematic reviews on the use of probiotics indicated that they might be effective to treat common vaginal infections in women [28, 29]. Regarding BV, probiotics, either alone or combined with antibiotics, may have also a positive effect as demonstrated by two additional systematic reviews [30, 31]. Another systematic review about the use of probiotics for VVC showed that, compared with conventional antifungal drugs used alone, probiotics as adjuvant therapy could enhance their effectiveness in improving the rate of short-term clinical and mycological cure and relapse at one month [32]. Finally, in the study by Cancelo-Hidalgo *et al.,* 123 Spanish gynecologists reached a high consensus on the benefits of probiotics associated with antibiotics to treat vaginitis and prevent recurrences and complications [33]. Information about the effect of other alternatives such as feminine hygiene products is scarce. Zelesse® was the most prescribed non-pharmacological intimate solution in our study (45.0%). It has demonstrated to be effective, alone or as an adjuvant along with antimicrobial therapy, for improving the signs and symptoms of acute vulvovaginitis, especially pruritus. It could be an alternative to relieve symptoms when an infectious etiology is not suspected, or when the prescription of the specific treatment must be delayed until the microbiological diagnosis is made [34]. Dietary changes such as a low-sugar diet — recommended to some women in our study — might also be helpful. Diets with high sugar content directly affect the vaginal microenvironment, which can lead to alterations in the vaginal microbiota, eliminating the beneficial bacteria and stimulating the overgrowth of pathogenic microorganisms instead [27].

Our study finally investigated the women's habits regarding self-diagnosis based on symptoms and self-treatment with over-the-counter antifungals. These are common practices since the sale of antifungal drugs is not subject to prescription control by pharmacies [16, 25]. Almost a third of women included in the study had already treated the current episode of vulvovaginitis on their own before consulting a gynecologist, most of them with drugs. Nevertheless, they all had to seek specialist gynecology care eventually because the symptoms persisted. Most of them had a positive culture despite having undergone treatment, which suggests that the treatment options chosen by women might not be the most appropriate for their particular condition, so they should be advised to consult a physician to be properly diagnosed and treated. In fact, it has been demonstrated that symptom relief is higher in physician-treated cases compared to those who self-medicate [35]. As previously discussed, symptoms alone do not allow reliable differentiation of the causes of vulvovaginitis [25]. Inaccurate treatment of infections may affect the ecological characteristics of pathogenic agents and worsen the outcomes of infections, resulting in recurrences, mixed infections, and even the appearance of resistant strains [16, 18]. Only recurrent VVC affects approximately 138 million women worldwide annually and 492 million women over their lifetimes [8, 36]. According to Denning *et al.,* the population of women with recurrent VVC is going to increase [8]. BV recurrence rates are high, up to approximately 80% three

months after effective treatment [5]. Treatment should only be administered after a correct, medically confirmed diagnosis to avoid further resistance and unjustified side effects of treatment [25]. Interestingly, we found a statistical association between women with pruritus, burning, vulvar pain or edema and self-treatment practices. However, this association should be confirmed with studies of greater methodological rigor.

This study has some limitations. Despite the suitability of the present cross-sectional design to determine the prevalence of vulvovaginal infections and their signs and symptoms, its value in identifying risk factors is limited. On the other hand, the sample was small to generalize the results obtained for the different subgroups, especially when it comes to mixed infections. The strength of this study is that it is a pioneering initiative in Spain since, to our knowledge, there is no clear data about the prevalence of vulvovaginal infections in women of reproductive age attending gynecology outpatient clinics. This work could serve as base for future research in the topic, both in Primary Care and specialized gynecological setting.

5. Conclusions

Most women of reproductive age attending gynecology outpatient clinics in Spain with symptoms and signs of vulvovaginitis have an infection (68.0%). Leukorrhea and pruritus are the most prevalent symptoms, followed by changes in color discharge and burning. Infections are mainly caused by a single pathogen, being *Candida albicans* the most prevalent. Mixed infections are a major diagnostic challenge and are present in 14.3% of women with symptomatic vulvovaginal infections, being *Candida albicans* and *Gardnerella vaginalis* the most frequent combination. Several women self-diagnose and self-treat based on earlier experience without a proven diagnosis, leading to recurrence and resistance. Health education interventions are recommended to raise women's awareness of the importance of maintaining good hygiene and self-care habits and consulting a physician when necessary. Finally, demand is increasing for new alternative strategies to replace or to be combined with standard therapies to treat infectious and noninfectious vulvovaginitis more effectively. Nonpharmacological treatments seem to have a relevant role, but more studies are needed to support their use since the evidence so far is promising.

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