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EPIDEMIOLOGY OF FUNGAL INFECTIONS (TM CHILLER AND JW BADDLEY, SECTION EDITORS)

Vulvovaginal Candidiasis: Epidemiology and Risk Factors, Pathogenesis, Resistance, and New Therapeutic Options

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Abstract

Purpose of Review Vulvovaginal candidiasis (VVC) is an infection of the vaginal mucosa caused by fungi of the genus *Candida* and can become pathogenic under special conditions. It affects about 75% of women at least once in life and is characterized by leukorrhea, intense pruritus, vulvar hyperemia, dysuria, and dyspareunia. The imbalance between the microbiota and these yeasts causes candidiasis. In addition, recurrent vulvovaginal candidiasis (RVVC) can affect a very significant number, approximately 138 million women worldwide. Pregnancy, diabetes mellitus, use of hormonal contraceptives, hormone replacement therapy, antibiotics and steroids, immunosuppressive diseases, and hygienic habits are contributing factors. The evaluation of VVC and RVVC requires clinical criteria associated with laboratory findings, the latter being essential for an accurate diagnosis and a satisfactory therapeutic result.

Recent Findings New drug formulations already known or carriers promise to increase the effectiveness of the antifungal activity of drugs against VVC and RVVC.

Summary This review aims to describe the epidemiology, pathogenesis, clinical and laboratory diagnosis, resistance, and new therapeutic options in primary and recurrent VVC and RVVC infections.

Keywords Diagnosis · Pathogenesis · Resistance · Therapeutic · Vulvovaginal candidiasis

Introduction

Vulvovaginal candidiasis (VVC) is caused by abnormal yeast growth in the mucosa of the female genital tract and is one of the most common conditions diagnosed in women seeking gynecological care [1] and affects approximately 75% of women at least once in their lifetime [2••, 3]. As VVC is easily treated, often with medicines available over the counter, it is perceived as a common or nuisance condition [4–8]. Environmental changes increase the chance of opportunistic pathogens as a genus *Candida* [9].

Based on the previous statement, the presence of *Candida* spp. does not necessarily indicate a vaginal infection; however, its presence combined with inflammation and vulvovaginal symptoms such as itching, burning, and discharge indicates a clinical diagnosis of VVC. VVC is defined as signs and symptoms of inflammation in the presence of *Candida* species and in the absence of other infectious agents [10]. A more complicated form of VVC is referred to as recurrent VVC

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(RVVC), which is defined as at least four confirmed episodes in a year, with the following clinical symptoms: vulvar erythema, vaginal discharge, edema, and burning/soreness. Culture of *Candida* spp. and/or wet mount microscopy is mandatory, and these tests should be positive [3, 11].

The clinical symptoms of VVC are nonspecific and can be associated with many other vaginal diseases, such as bacterial vaginitis, trichomoniasis, and gonorrhea [12]. The most common clinical manifestations are vulvar pruritus and burning accompanied by vaginal soreness and irritation leading to dyspareunia and dysuria. Vulvar and vaginal erythema, edema, and fissures are also commonly found [13]. Its laboratory diagnosis consists of media culture, PCR, DNA probe, monoclonal antibody staining, antigen test, chromogenic culture media, and direct agglutination test [11].

Nevertheless, *C. albicans*, a well-characterized biofilm forming organism, remains a prominent pathogen in this disease. Resistance to antifungal therapy because of biofilm formation is a likely contributor to failed treatment. Although biofilms are widely accepted to contribute to the pathogenesis of bacterial vaginosis and evidence point to it [14, 15], their role in VVC remains contested [16••, 17, 18]. Thus, this review purposes to describe the epidemiology, risk factors, pathogenesis, resistance, and new therapeutic options of the vulvovaginal candidiasis.

Epidemiology and Risk Factors

VVC is not a reportable disease, and therefore, the information on its incidence is incomplete and based on epidemiological studies often hampered by inaccuracies of diagnosis and/or by the use of non-representative populations [19].

Vulvovaginal candidiasis can be diagnosed by visualization of yeast hyphae on potassium hydroxide preparation in a woman with typical symptoms. It can also be diagnosed using antigen or DNA probe testing, with sensitivities from 77 to 97% and specificities from 77 to 99%, compared with culture as the diagnostic standard. Women with vulvovaginal candidiasis have a normal acidic vaginal Ph [20, 21].

The distribution of the different *Candida* species identified in women suffering from VVC varies widely depending on the locations as well as the populations studied [10]. *Candida albicans* has been reported as the cause of symptomatic VVC in 85–95% of cases. In many parts of the world, non-*albicans* isolates—notably *C. glabrata*—affect 10–20% of women [20]. In North America and in many European and Asian countries, several studies have shown a general shift from predominance of *C. albicans* in VVC to non-*Candida albicans*, notably *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* [21–23].

The highest incidence of *C. albicans* in VVC was reported by epidemiological studies conducted in Brazil, with 92.3% [24]; Argentina, with 85.95% [25]; and Pakistan, with 47.7% [26••]. A study with 898 sexually active nonpregnant women aged between 16 and 30 years from communities around Mysore, India, found that positive values for diagnosis of vulvovaginal candidiasis with signs or symptoms were low (< 19%) [27].

Nurat et al. [28] observed a higher prevalence of candidiasis (33.8%) in women in the age range of 20–29 years, followed by those (24.3%) in the age range of 30–39 years. The overall prevalence of VVC was 25%.

Candida africana has been reported as a cause of VVC in African, German, Spanish, and Italian patients [29–32]. *Candida dubliniensis* is a yeast closely related to *Candida albicans* and has been found in vaginal samples. *Candida africana* and *C. dubliniensis* strains are often misidentified as *C. albicans* [33, 34]. *Candida dubliniensis* shares many phenotypic similarities with *C. albicans*, and thus, it may easily be misidentified as such [35, 36]. This *Candida* specie appears to be most commonly associated with recurrent episodes of oral candidiasis in HIV and non-HIV-infected individuals [37].

Healthy women may develop VVC sporadically; however, this infection is often attributed to the presence of host-related and behavioral factors that disrupt the vaginal environment including pregnancy, hormone replacement, uncontrolled diabetes mellitus, immunosuppression, use of antibiotics and glucocorticoids, stress, and genetic predispositions. Behavioral factors for VVC include the use of contraceptives, intrauterine device, spermicides, and condoms, as well as some sexual, hygienic, and dressing habits (Fig. 1) [9].

Pregnancy is an important factor for triggering vaginal candidiasis due to high hormonal levels, mainly progesterone, and to an increased vaginal secretion of glycogen with important acidification of the medium, reducing bacterial population, and favoring fungal proliferation. Estrogens in pregnancy increase the avidity of vaginal epithelial cells for adherence of *Candida* and formation of yeast mycelia. As most oral contraceptives contain estrogen and progesterone for the same reasons, they also promote fungal growth [38]. Using intrauterine devices (IUDs) also increases the risk of VVC due to their ability to adhere to medical devices and to form biofilm of *Candida* species on their surface, contributing to colonization, reduced susceptibility to antifungal, and consequent occurrence of VVC, mainly in the recurrent form [3, 39].

Another important factor that can increase susceptibility to fungal infection is diabetes mellitus [22, 39]. When decompensated or poorly controlled, high blood glucose levels increase glucose and vaginal glycogen concentration, with medium acidification and yeast proliferation. Vaginal colonization by *Candida* is higher in women taking insulin or oral hypoglycemic agents [40].

The use of antibiotics is an important predisposing factor for vaginal candidiasis. The use of broad-spectrum systemic

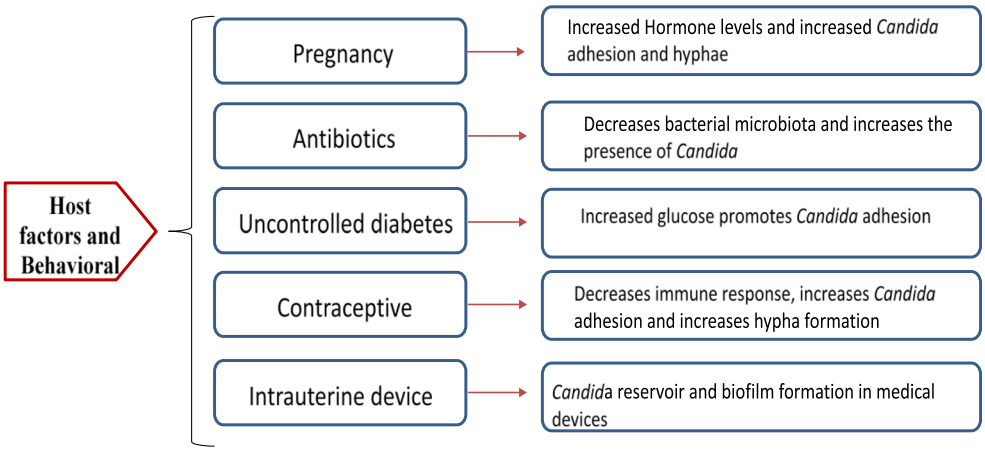

Fig. 1 Host and behavioral factors for vulvovaginal candidiasis and its respective effects (adapted from Gonçalves et al. (2016)) [9]

antibiotics reduces the normal vaginal bacterial microbiota, particularly Döderlein bacilli, decreasing competition for nutrients and facilitating *Candida* proliferation. Using local antibiotics can also lead to the same sequence of events [27].

All *Candida* infections depend on the host's response. Changes in the vaginal environment are usually necessary for switching from commensal to opportunistic. Despite scientific advances, many mechanisms involved in the development of this infection still need to be clarified [9]. Genetic polymorphisms in innate immunity genes have been also associated with increased susceptibility to VVC, including polymorphisms in mannose-binding lectin (MBL) gene, dectin-1 stop-codon, and interleukin-4 (IL-4) gene [41]. Corticosteroids, immunosuppressants, and cytostatic agents also predispose to *Candida* infection.

In HIV-infected patients, while oroesophageal candidiasis is known to appear at any time during the course of HIV infection progression, symptomatic VVC develops significantly later [41]. Ohmit et al. [42] reported that although the prevalence of symptomatic VVC is higher among HIV-infected women, it did not significantly differ from HIV serostatus of the participants at baseline. However, the authors found that during follow-up visits, the rate of acquisition of symptomatic VVC was significantly higher among HIV-infected women compared with HIV-uninfected women. Cross-sectional and cohort studies have shown only a moderate increase in VVC in HIV-positive women not receiving antiretroviral therapy compared with HIV-negative women, and a modest increase in the incidence of VVC in HIV-positive women compared with HIV-negative women in relation to increased occurrence of oropharyngeal candidiasis [19].

Pathogenesis of VVC

The pathogenicity of microorganisms results from their need to defend themselves against environmental attacks. *Candida* species produce metabolites derived from their natural metabolism and induced by stimuli in the host microenvironments. These substances that act on host tissues, an adaptation to parasitism and interaction with the individual's immune defenses, are called virulence factors and may also be part of the inherent makeup of cells. Each virulence factor contributes to tissue invasion, multiplication, and survival of the yeast, so it can evade the host's immune defenses [43].

Generally, VVC occurs concomitantly with an imbalance between the factors of vaginal protection and the virulence of the fungus, caused by physiological or non-physiological changes, favoring the colonization for the development of yeasts. Thus, the infection results from a change in the commensalism relation between this fungus and the host [9]. *Candida albicans* are very well adapted to the human body and can colonize it without producing signs of disease. Maintaining the integrity of mucosal tissue barriers, the harmonic relationship of the autochthonous microbiota, and the proper functioning of the immune system, among others, protect the organism and avoid an infectious process triggered by the fungus that colonizes the vaginal environment. In turn, the fungus expresses a series of specific strategies to establish, colonize, cause disease, and overcome the susceptible host defenses in a balanced way [44].

Several virulence factors and adaptive attributes support the infection capacity. Virulence factors are considered to be the morphological transition between yeast and hyphae forms, resulting in adhesin and invasion expression on the cell surface, the thigmotropism, the secretion of hydrolytic enzymes, and the biofilm formation [9, 45] (Fig. 2). A reversible morphological alternation between unicellular yeast cells and filamentous phase (hyphae and pseudohyphae) is an important virulence factor for some *Candida* species. *Candida albicans* can form hyphae and/or pseudohyphae, *C. parapsilosis* can generate pseudohyphae, *C. tropicalis* pseudohyphae and possibly true hyphae, and *C. glabrata* grows only as blastoconidia [46]. Filamentous forms give more mechanical strength, enhancing colonization and invasion of host tissues, and

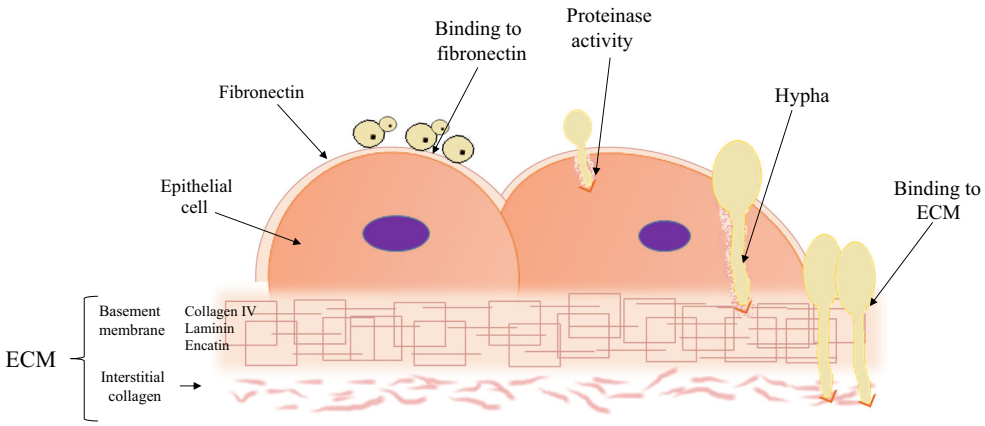

Fig. 2 Schematic representation of adhesion, invasion, and infection by *Candida* sp.

demonstrate increased resistance to phagocytosis [46, 47]. Hyphae are believed to play an important role in tissue invasion, and in vitro investigation has shown that *C. albicans* lacking hyphal formation exhibited lower ability to invade tissue compared with the wild-type strains [48]. Phospholipase D is necessary for yeast-to-hyphal transition in *C. albicans* [49], and the expression of some secreted aspartyl proteinase (SAP) genes (SAP4-6) occurs specifically during hyphal development [50, 51].

Candida species secrete several hydrolytic enzymes, which play an important role in adhesion, invasion, and destruction of host tissues [52, 53]. The enzymes most frequently implicated in *Candida* pathogenicity are secreted aspartyl proteinases (Saps), but phospholipases, lipases, and hemolysins are also involved in *Candida* virulence [54, 55]. Mohandas and Ballal [56] detected higher proteinase and phospholipase activity in vaginal isolates than in urinary and respiratory isolates of candidiasis-infected patients, relating Sap production to site of strain isolation. Studies have reported higher expression of SAPs and higher proteinase activity by *Candida* species isolated from women with VVC than from asymptomatic vaginal *Candida* carriers [57]. It has been also demonstrated that the expression of *C. albicans* SAP1 and SAP3 has a strong and specific correlation with VVC [58]. Phospholipases hydrolyze one or more ester bonds in glycerophospholipids, contributing to host-cell membrane damage and to the adhesion of yeasts to host tissues. Several *Candida* species can produce extracellular phospholipases, but this ability is highly strain dependent [46].

Adherence to various surfaces has been shown to play a central role in the pathogenesis of many microbial infections, representing the first step in pathogenesis mechanisms, and suggests ways to control infection at an early stage [59]. Adherence is mediated by *C. albicans* cell wall glycoproteins and by specific molecules (called adhesins) on the fungal surface, which interact with specific ligands of the host cell as components of the extracellular matrix (ECM). This interaction can be influenced by temperature, pH, nutrients, secretory IgA, and cellular surface hydrophobicity [53, 60]. One of the main characteristics that establish infections by *Candida* species as a successful pathogen is their adaptability to successfully thrive in different conditions present in various host niches, such as nutrient availability, pH, hypoxia, and CO_2 levels. *C. albicans* has metabolic flexibility, being able to use alternative carbon sources simultaneously for its survival and virulence [53]. This adaptation resulted from the absence of inactivation of catabolites due to the recovery of ubiquitination sites in metabolic enzymes [61].

Current Reality in Vulvovaginal Candidiasis Therapy and Resistance to Antifungals

Azoles, particularly fluconazole, miconazole, and ketoconazole, are considered the first choice for the treatment of *Candida albicans* infections. These antifungal agents act by inhibiting lanosterol 14α-demethylase (CYP51), encoded by ERG11, which is a key enzyme involved in the biosynthesis of ergosterol (the main component of the fungal cell membranes). Other effective therapeutic strategies employed against *Candida* spp. include polyene antimycotics such as nystatin, natamycin, and amphotericin B, which bind with sterols in fungal membranes and form pores and echinocandins such as anidulafungin, caspofungin, and micafungin, which inhibit the activity of the enzyme 1,3-β-D-glucan synthase and, consequently, inhibit the synthesis of glucan of fungal cell wall [62].

The first-line treatment for VVC uses antifungals of the azole group, which can be administered orally or topically. Polyene antifungals, mainly nystatin, are generally used as a topical treatment, and azoles, such as fluconazole, are used orally. Nowadays, fluconazole and topical imidazole drugs are preferred as first-line agents; however, when infective species are resistant, such as *Candida krusei* and some strains of *C. glabrata*, alternatives to this treatment must be considered [63].

The vast majority of cases of vulvovaginal candidiasis are caused by *C. albicans*. In common cases, *C. albicans* does not have significant high resistance to azole antifungals. Thus, these drugs are the agents chosen for the treatment of this infection and can be taken orally or intravaginally (Table 1).

When the patient does not respond to conventional therapy, identification analysis of other species of *Candida* or resistant *C. albicans* may be necessary. Immunosuppressed patients generally require more intensive therapy with intravaginal azole administration for at least 1 week or oral treatment once every 3 days for three doses [54].

For recurrent vulvovaginal candidiasis (RVVC), the therapy chosen is usually systemic and oral. Individual episodes of RVVC caused by *C. albicans* respond well to azole therapy. Nevertheless, to maintain clinical and mycological control, specialists recommend a longer duration of initial therapy. A therapeutic option is administration for 7 to 14 days of topical therapy or an oral dose of fluconazole 100 mg, 150 mg, or 200 mg every 3 days for 3 doses. Oral fluconazole weekly for 6 months is the first-line maintenance regimen [64].

If severe or recurrent vulvovaginal candidiasis does not respond to initial treatment, culture may guide therapy when non-*albicans* species are identified. Infections with non-*albicans* species are less responsive to fluconazole. Topical imidazoles (i.e., econazole, clotrimazole, miconazole, and ketoconazole) are more effective in eradication [20].

In general, all oral or topical azole drugs cure 80–95% of acute cases, in the absence of pregnancy; polyenes such as nystatin solve VVC in 70–90% of cases. Over 3 days, higher concentrations and doses of topical drugs are effective, with lower doses of the same formulations requiring prolonged therapy. The treatment of asymptomatic women is not recommended as about 40–50% has commensal fungi in the vagina [63].

Complicated VVC therapy is more complex as the infections involve several factors, some of which are not yet understood. Studies have shown that most cases of recurrence result from diagnostic errors. Patients who have RVVC, with four or more episodes of infection, should be referred for clinical and laboratory evaluation to confirm the presence of the fungus and the species, and to rule out other causes. If the diagnosis of RVVC is confirmed, an effective control measure is a suppressive therapy with triazoles for 6 months. Naturally, the success of RVVC treatment requires follow-up and adherence to treatment by the patient [65, 66].

New Therapeutic Options and Future Perspectives

The development of alternative therapies for the treatment of vulvovaginal candidiasis has been increasing, associated with the search for less side effects, better tolerability, and lower cost. Some substances with antifungal activity are indicated as alternative treatments, because they restore the balance of the vaginal microbiota and demonstrate the inhibition capacity on the factors of microbial virulence [63].

Although adequate use of the therapeutic alternatives contributes to the treatment of RVVC, some chemicals and even natural products may have undesirable side effects. Without drawing the merit and importance of experimental studies (in vitro and in vivo), well-designed and reliable clinical trials are required to confirm the safety and efficacy of a new substance or new substance formulation with activity already known for the treatment of VVC [63].

Some clinical trials have shown the efficacy of some natural products in the treatment of RVVC: *Ageratina pichinchensis* extract [67], *Calendula officinalis* [68••], Garcin® [garlic tablet] [55], and propolis [69]. The use of natural products, such as plant extracts, presents some efficacy problems in in vivo model despite their potent in vitro biological activity. Most of the biologically active constituents of the extracts, such as flavonoids, tannins, and terpenes, are highly water soluble but have low absorption, either because they are unable to cross the lipid membrane of the cells or because of their high molecular size, hindering their absorption and losing part of their effectiveness [70]. Zida et al. [71] reviewed 142 natural substances (extracts, essential oils, and isolated natural products) with potential anti-*Candida* activity. The action mechanisms of all these substances include inhibition of yeast-hyphal transition, inhibition of biofilm formation, membrane depolarization induction, and cell membrane integrity disruption by pore formation.

The use of pharmaceutical nanotechnology in medicinal extracts has opened new horizons for a better activity and promotion of the release of nanoparticles [72]. Thus, further

Table 1 First-line of topical treatment for VVC

Drug	Pharmaceutical form—concentration	Form of use
Clotrimazole	Cream—1%	Intravaginally for 7–14 days
Clotrimazole	Cream—2%	Intravaginally for 3 days
Miconazole	Cream—2%	Intravaginally for 7 days
Miconazole	Cream—4%	Intravaginally for 3 days
Miconazole	Vaginal suppository—100 mg	One suppository/day for 7 days
Miconazole	Vaginal suppository—200 mg	One suppository per day
Miconazole	Vaginal suppository—1.200 mg	One suppository per day
Thioconazole	Ointment—6.5%	Intravaginally in a single dose

Adapted from CDC 2018

studies with carriers are needed to enhance, potentiate the action of natural products, and even provide a substantivity of their action.

New drug formulations already known or carriers promise to increase the effectiveness of the antifungal activity of these drugs. One should remember that the development of new formulations may improve the antifungal activity of existing drugs; however, it does not allow treating infections resistant to a particular drug but it may decrease the dose required for the treatment of infections of susceptible species and, consequently, the side effects such as dose-related toxicity. One study evaluated the in vitro and in vivo efficacy of amphotericin B-loaded poly (lactic acid-co-glycolic acid) nanofibers and concluded that nanofibers can be applied as an alternative strategy for the local treatment of vulvovaginal candidiasis, without induced resistance fungus, guaranteeing the patient's compliance [73].

A vaginal mucoadhesive gel was developed and evaluated for delivery of fluconazole, using nanolipid carriers to increase tissue deposition in the treatment of vulvovaginal candidiasis. The release of fluconazole was maintained showing 30.69 ± 1.02% deposition on the porcine vaginal mucosa after 8 h, with improved antifungal activity against *C. albicans* during well diffusion studies. The optimized gel was non-irritating to the vaginal mucosa of Wistar rats, with no signs of erythema or edema [74••].

Although photodynamic therapy (PDT) is not a clinically widespread technique in antimicrobial treatment, it has been reported as promising for RVVC treatment. One study evaluated the efficacy of PDT using two photosensitizers, methylene blue, and protoporphyrin IX, to treat treatment VVC induced in rats. Two LEDs with 660 nm and 630 nm wavelength at 800 mW were used for irradiation. Using PDT as a therapeutic option to decrease fungal infection resulted in a significant reduction in the *C. albicans* population. Both photosensitizers were effective in preventing reinfection within 7 days. In addition to having a fungicidal effect, swelling and abscess formation reduced, as well as micro abscesses in the coating of the cornified epithelial layer and neutrophil accumulation in the submucosa [75].

The treatment available for RVVC is represented by antifungal drug therapy, but without a definitive cure. However, limitations of these drugs show that other approaches are urgently needed. An obvious prevention measure for candidemia or relapse of RVVC would be to immunize at-risk patients with an effective vaccine against *Candida* infections [76]. Although there is no clinically available vaccine, immunotherapy is already a reality in clinical trials in humans. A randomized, double-blind, placebo-controlled exploratory study evaluated an immunotherapeutic vaccine (NDV-3A) containing a recombinant adhesin/invasive *C. albicans* protein to prevent RVVC. The study in 188 women with RVVC showed that 1 intramuscular dose of NDV-3A was safe and generated fast and robust B cell and T cell immune responses. The study showed statistically significant increase in the percentage of symptom-free patients at 12 months post-vaccination (42% vaccinated vs. 22% placebo) and a median doubling time for the first symptomatic episode (210 vaccinated days vs. 105 placebo days) subgroups of patients aged < 40 years (n = 137). The results support the further development of the NDV-3A vaccine and provide guidance for significant clinical parameters for the immunotherapeutic management of RVVC [77].

Final Considerations

Despite the effectiveness of antifungal agents currently available on the market, the prevalence of RVVC is still high. This fact is due to the increase resistance of *Candida* species by different virulence factors. Prospecting new molecules with antifungal activity should be encouraged to obtain new drugs with action mechanisms different from those of known antifungal agents. With the advances of biotechnology, nanotechnology, and bioinformatics, pharmacological planning for the development of new drugs can be optimized and the future prospects are optimistic. Regarding the alternative approaches to antifungal treatment, immunization proves to be the most promising one, but more clinical studies and more investments are needed, so that this alternative will no longer be a future perspective, becoming a reality.

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Compliance with Ethical Standards

Conflict of Interest None.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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