

REVIEW ARTICLE

Vulvovaginal candidiasis: Epidemiology, microbiology and risk factors

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Abstract

Vulvovaginal candidiasis (VVC) is an infection caused by *Candida* species that affects millions of women every year. Although *Candida albicans* is the main cause of VVC, the identification of non-*Candida albicans Candida* (NCAC) species, especially *Candida glabrata*, as the cause of this infection, appears to be increasing. The development of VVC is usually attributed to the disturbance of the balance between *Candida* vaginal colonization and host environment by physiological or nonphysiological changes. Several host-related and behavioral risk factors have been proposed as predisposing factors for VVC. Host-related factors include pregnancy, hormone replacement, uncontrolled diabetes, immunosuppression, antibiotics, glucocorticoids use and genetic predispositions. Behavioral risk factors include use of oral contraceptives, intrauterine device, spermicides and condoms and some habits of hygiene, clothing and sexual practices. Despite a growing list of recognized risk factors, much remains to be elucidated as the role of host versus microorganisms, in inducing VVC and its recurrence. Thus, this review provides information about the current state of knowledge on the risk factors that predispose to VVC, also including a revision of the epidemiology and microbiology of VVC, as well as of *Candida* virulence factors associated with vaginal pathogenicity.

Keywords

Behavioral factors, *Candida* species, host factors, vaginal infection, virulence factors

History

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Introduction

Species from genus *Candida* have a wide distribution in nature and can be found in humans, domestic and wild animals as well as in diverse environments including hospitals (Sidrim & Rocha, 2004). These yeasts belong to the normal flora of humans and can colonize the mucosal surfaces of the genital, urinary, respiratory and gastrointestinal tracts, as well as oral cavity, nails, scalp and skin (Dignani et al., 2009). However, *Candida* species can be commensal organisms or transform a symptomless colonization into an infection. Thus, these species are characterized as opportunistic and can change from harmless to pathogenic upon variation of the host conditions. *Candida* infections are mainly superficial, but in severely immunocompromised patients serious systemic infections can occur (Ascioglu et al., 2002). Most, if not all women carry *Candida* in vagina at some point of their lives, yet without symptoms of infection (Beigi et al., 2004). *Candida* organisms gain access to the lower genital tract mainly from the adjacent perianal area (Bertholf & Stafford, 1983). There is a balance between *Candida* organisms and vaginal defence mechanisms against *Candida*, such as lactobacilli and immune responses that allow the persistence of *Candida* species as vaginal commensals (Ferrer, 2000), thereby changes in the host vaginal/vulvar environment

can lead to a *Candida* infection, named vulvovaginal candidiasis (VVC). VVC is defined as signs and symptoms of inflammation in the presence of *Candida* species and in the absence of other infectious agents (Achkar & Fries, 2010). VVC can be classified into uncomplicated and complicated cases, a classification that has been accepted by several authors (Achkar & Fries, 2010; Pappas et al., 2009; Sobel et al., 1998). Uncomplicated VVC is characterized by fewer than four episodes per year with mild to moderate severity caused by *Candida albicans* in apparently healthy women. Complicated VVC include episodes due to non-*Candida albicans Candida* (NCAC) species or severe cases caused by any *Candida* species. Moreover, recurrent VVC (RVVC), which is characterized by four or more episodes per year, and VVC in the presence of recognized risk factors (e.g. pregnancy, diabetes and immunosuppression) are also classified as complicated VVC (Achkar & Fries, 2010; Pappas et al., 2009; Sobel et al., 1998).

The clinical symptoms of VVC are nonspecific and can be associated with a variety of other vaginal diseases, such as bacterial vaginosis, trichomoniasis and gonorrhoea (Anderson, 2004). 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 (Sobel, 2005). Topical antimycotic agents (e.g. creams, lotions and vaginal suppositories) and oral therapy are available for VVC. Topical polyenes (e.g. nystatin) achieve a mycological cure rate of about 75–80% and topical azoles of about 85–90% (Sobel et al., 1995), in uncomplicated cases.

Oral azole agents (e.g. fluconazole and itraconazole) achieve comparable cure rates than do topical agents (Watson et al., 2002) and most patients prefer the convenience of oral administration (Tooley, 1985). However, oral azoles have a potential side effect of toxicity (Fidel et al., 1999b). In addition, topical and oral azoles are poorly effective in VVC caused by *Candida glabrata* in comparison to *C. albicans* (Fidel et al., 1999b). In fact, complicated infections such as those caused by NCAC species or in the presence of risks factors are much more difficult to treat requiring intensive regimens (Sobel et al., 2001). Prevention treatments, such as topical vaginal use of recombinant mannose-binding lectin (Petersen et al., 2006) and anti-*Candida* vaccines (Spellberg et al., 2006), have been investigated as possible preventions for VVC.

VVC affects millions of women every year and has been considered an important public health problem. Although not associated with mortality, the morbidity associated with VVC makes it a major cause of mental distress, causing pain, great discomfort, altered self-esteem, anxiety, impairing work performance and interfering with sexual and affective relations (Sobel, 2007). VVC is also associated with considerable direct and indirect economic costs (Foxman et al., 2000) and enhanced susceptibility to human immunodeficiency virus (HIV) infection (Røttingen et al., 2001). Especially when VVC is left untreated, many complications has been appointed as its consequence, such as pelvic inflammatory disease, infertility, ectopic pregnancy, pelvic abscess, spontaneous abortion and menstrual disorders. Therefore, prevention, early diagnosis and prompt treatment of VVC, especially among risk groups, are essential to avert the complications (Nwadioha et al., 2010).

Despite therapeutic advances VVC remains a common disease, for which triggering factors and their underlying mechanisms are not fully understood. Thus, this review provides information about the current state of knowledge on the risks factors predisposing to VVC, also including a revision of the epidemiology and microbiology of VVC, as well as of *Candida* virulence factors associated with vaginal pathogenicity.

Epidemiology of VVC

VVC is not a reportable disease, and therefore, the information on its incidence is incomplete and based on epidemiology studies that are often hampered by inaccuracies of diagnosis and/or the use of non-representative populations (Sobel, 2007). VVC is considered the second most common cause of vaginitis after bacterial vaginosis (Anderson, 2004). It is estimated that approximately 10–15% of asymptomatic women are colonized with *Candida*, 70–75% of women will experience an episode of VVC in their lifetimes, 50% of initially infected women will suffer a second VVC event and 5–10% of all women will develop RVVC (Sobel, 2007).

Symptoms and signs of VVC are not specific to the disease (Anderson, 2004) and the presence of *Candida* in the vagina is not necessarily indicative of VVC since asymptomatic women can be colonized. Therefore, the diagnosis of VVC requires correlation of clinical findings and laboratory confirmation of *Candida*. Table 1 highlights the more relevant epidemiologic studies published during the last years concerning the incidence of VVC diagnosed in symptomatic women with laboratory confirmation. It also shows the incidence of vaginal colonization by *Candida* species whenever asymptomatic women were also included in the study. The incidence of VVC in symptomatic women varies depending on the locations as well as the populations studied. The studies published during the last years reported incidences of the disease in symptomatic women that range from 12.1% to 57.3% (Table 1). The highest incidences were reported by epidemiologic studies made in African countries (Nigeria (Okungbowa et al., 2003) and Tunisia (Amouri et al., 2011), with 57.3% and 48.0%, respectively), followed by Brazil (Andrioli et al., 2009; Rosa & Rumel, 2004) and Australia (Bradshaw et al., 2005). The lowest incidences were reported in European countries (Greece (Grigoriou et al., 2006) and Italy (Tibaldi et al., 2009), with 12.1 and 19.5%, respectively) and India (17.7 to 20.4%) (Ahmad & Khan, 2009; Mohanty et al., 2007; Vijaya et al., 2014). All these epidemiologic studies were consensual in reporting higher incidence of VVC in women at reproductive age (20–40 years) than those at menopause. Regarding asymptomatic colonization, Brazilian (Andrioli et al., 2009;

Table 1. Epidemiologic studies published from 2003 to 2014, concerning the VVC incidence in symptomatic women and the incidence of vaginal colonization by *Candida* in asymptomatic women.

Country	Year(s) of study	Symptomatic women		Asymptomatic women		References
		Number	With VVC (%)	Number	Colonized (%)	
Brazil	2002	23	43.5	112	14.3	(Rosa & Rumel, 2004)
Brazil	2005–2007	121	47.9	165	17.0	(Andrioli et al., 2009)
Jamaica	–	422	29.6	–	–	(Jackson et al., 2005)
Italy	1996–2005	13014	19.5	11 551	11.6	(Tibaldi et al., 2009)
Austria	2000–2004	10463	30.5	–	–	(Paulitsch et al., 2006)
Greece	2002–2004	4743	12.1	–	–	(Grigoriou et al., 2006)
Turkey	2004–2005	569	42.2	–	–	(Cetin et al., 2007)
India	2003–2004	601	18.5	–	–	(Mohanty et al., 2007)
India	–	1050	20.4	–	–	(Ahmad & Khan, 2009)
India	2011–2012	300	17.7	–	–	(Vijaya et al., 2014)
Israel	–	208	35.5	100	15.0	(Dan et al., 2003)
Tunisia	2006–2008	481	48.0	–	–	(Amouri et al., 2011)
Nigeria	–	902	57.3	–	–	(Okungbowa et al., 2003)
Australia	2003–2004	342	42.7	–	–	(Bradshaw et al., 2005)

Rosa & Rumel, 2004), Italian (Tibaldi et al., 2009) and Israeli (Dan et al., 2003) studies reported vaginal colonization by *Candida* species in women without any symptoms of VVC with a prevalence between 11.6% and 17.0% (Table 1). VVC affects women globally, and therefore, further large studies, from diverse countries, are needed to a better knowledge of the incidence of VVC and vaginal asymptomatic colonization by *Candida* species.

Microbiology of VVC

The most common *Candida* species associated with VVC are *C. albicans*, *C. glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei*. Typically, a single species is identified, but two or more species have been found in some women with VVC (1–10%) (Amouri et al., 2011; Cetin et al., 2007; Fan et al., 2008b; Mahmoudi et al., 2011; Paulitsch et al., 2006; Richter et al., 2005). Most of these mixed infections are caused by an association between *C. albicans* and *C. glabrata* (Amouri et al., 2011; Mahmoudi et al., 2011; Paulitsch et al., 2006; Richter et al., 2005). In fact, *C. albicans* is the most common species identified in women with VVC followed by *C. glabrata* (Table 2). A recent *ex vivo* study demonstrated higher colonization and invasion of vaginal tissue by *C. albicans* than by *C. glabrata*, but an enhanced invasion by *C. glabrata* in co-infection with *C. albicans* (Figure 1) (Alves et al., 2014b).

Table 2 highlights the more relevant epidemiologic studies published during the last years concerning the distribution of the most common *Candida* species identified in women with VVC. Most of these studies reported higher association of *C. albicans* with VVC than NCAC species. In American (Richter et al., 2005), European (Corsello et al., 2003; Grigoriou et al., 2006; Paulitsch et al., 2006; Sojakova et al., 2004) and Australian (Holland et al., 2003) studies *C. albicans* was the most common specie identified in women with the disease (70.0–89.0%), followed by *C. glabrata* (3.4–20.0%). Also, Chinese (Fan et al., 2008b), Tunisian (Amouri et al., 2011) and Iranian (Mahmoudi et al., 2011) studies show a predominance of *C. albicans* (65.1–90.4%), however in some Asian and African studies (Turkey (Cetin et al., 2007),

India (Ahmad & Khan, 2009; Mohanty et al., 2007) and Nigeria (Okungbowa et al., 2003)), NCAC species appear to be more commonly associated with VVC, especially *C. glabrata* (29.5–50.4%) (Table 2). *Candida tropicalis* is the second most common NCAC species, with the higher percentages identified in Nigeria (17.9%) (Okungbowa et al., 2003), Jamaica (11.2%) (Jackson et al., 2005) and India (10.8%) (Mohanty et al., 2007).

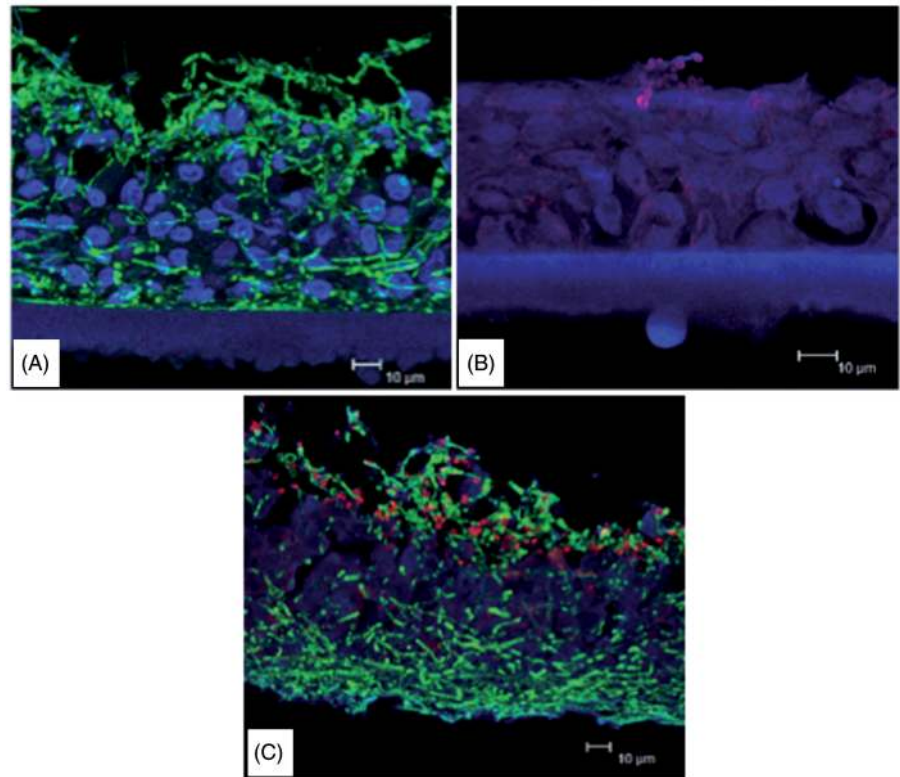
Historically, 85–95% of *Candida* species identified in women with VVC were *C. albicans* (Linhares et al., 2001; Lynch & Sobel, 1994; Otero et al., 1998; Saporiti et al., 2001; Spinillo et al., 1997b); however, most studies, published during the last years, reported incidence of *C. albicans* below 85% and in some countries even below 50% (Table 2). It has been suggested that the widespread and inappropriate use of antifungal treatments (self-medication and prolonged antifungal therapy) may lead to the selection of NCAC species (such as *C. glabrata*), which are more resistant to the commonly used antifungal agents than *C. albicans* (Sobel et al., 1998; Spinillo et al., 1994). In fact, NCAC species have been more commonly isolated among patients with RVVC than in women with sporadic VVC (Amouri et al., 2011; Grigoriou et al., 2006; Holland et al., 2003; Richter et al., 2005), possibly due to a higher antifungal exposure and widespread use of over the counter antimycotics among patients with RVVC (Nyirjesy et al., 1997). High percentages of NCAC species causing VVC, mainly *C. glabrata*, have been also associated with increasing age (Dan et al., 2006; Fan et al., 2008b; Holland et al., 2003), patients with uncontrolled diabetes (Goswami et al., 2000, 2006) and HIV-infected women (Corsello et al., 2003; Spinillo et al., 1997b). These associations are possible due to changes in patient physiology, hormone balance and decrease in immune functions.

Compared to *C. albicans*, NCAC species are generally associated with higher resistance to the azoles (Ribeiro et al., 2001; Richter et al., 2005; Sojakova et al., 2004), the most commonly prescribed class of antifungal agents. The use of nonazole antifungals, such as boric acid and flucytosine, has been shown to be effective in treating VVC caused by NCAC species, especially *C. glabrata* (Sobel et al., 2003), which

Table 2. Epidemiologic studies published from 2003 to 2014, concerning the distribution of *Candida* species in women with VVC.

Country	Year(s) of study	No. of subjects	<i>C. albicans</i> (%)	<i>C. glabrata</i> (%)	<i>C. tropicalis</i> (%)	<i>C. parapsilosis</i> (%)	<i>C. krusei</i> (%)	References
USA	1998–2001	429	75.7	15.6	1.1	3.8	3.8	(Richter et al., 2005)
Jamaica	–	125	83.2	4.8	11.2	–	–	(Jackson et al., 2005)
Brazil	2001–2003	40	70.0	20.0	7.5	–	–	(Boatto et al., 2007)
Italy	1999–2001	909	77.1	14.6	2.3	0.6	4.0	(Corsello et al., 2003)
Slovakia	2000–2001	227	87.7	6.2	0.9	0.9	2.2	(Sojakova et al., 2004)
Austria	2000–2004	3243	87.9	3.4	0.2	0.5	0.3	(Paulitsch et al., 2006)
Greece	2002–2004	576	80.2	7.3	1.6	2.1	1.0	(Grigoriou et al., 2006)
Turkey	2004–2005	240	44.2	29.6	–	–	5.8	(Cetin et al., 2007)
India	2003–2004	111	35.1	50.4	10.8	0.9	2.7	(Mohanty et al., 2007)
India	–	215	46.9	36.7	2.8	10.2	1.4	(Ahmad & Khan, 2009)
China	2003–2006	1070	90.4	7.9	0.9	0.6	0.1	(Fan et al., 2008b)
Iran	2006–2008	175	65.1	13.1	6.2	0.6	4.0	(Mahmoudi et al., 2011)
Tunisia	2006–2008	295	76.3	19.3	1.4	–	–	(Amouri et al., 2011)
Nigeria	–	517	20.3	33.8	17.9	4.3	–	(Okungbowa et al., 2003)
Australia	2000	1221	89.0	7.3	0.7	1.0	1.0	(Holland et al., 2003)

Figure 1. Reconstituted human vaginal epithelium (RHVE) infected with *C. albicans* (A), with *C. glabrata* (B) and co-infected with *C. albicans* and *C. glabrata* (C) (Alves et al., 2014a).



demonstrates intrinsically low susceptibility to the azoles and the ability to develop high resistance to them (Redding et al., 2003; Richter et al., 2005; Safdar et al., 2002). The high resistance levels of NCAC species to the commonly used treatments associated with an increasing identification of these species in women with VVC highlights the importance of identifying *Candida* species within vaginal samples, in order to provide physicians with information concerning the proper treatment for their patients.

Candida virulence factors

Until a few decades ago, it was believed that *Candida* microorganisms passively participated in the establishment of an opportunistic fungal infection, caused only by an organic weakness or an immunocompromised host. Today, there is consensus that these yeasts actively participate in the pathogenesis of the disease process, using mechanisms of aggression called virulence factors (Tamura et al., 2007). Thus, the pathogenicity of *Candida* species is mediated by a number of virulence factors that include adhesion, biofilm formation, extracellular hydrolytic enzyme production, hyphal formation and phenotypic switching.

Adhesion

Adhesion of *Candida* to host surfaces is required for initial colonization of human tissues, contributes to persistence of the microorganism within the host and is essential in the establishment of infection (Silva et al., 2012). Therefore, the primary event in VVC is the adhesion of *Candida* species to vaginal epithelial cells. Some studies have confirmed that *Candida* species have the ability to adhere to this type of cells (King et al., 1980; Vidotto et al., 2003). In addition, *Candida*

species can adhere to the surface of medical devices, often promoting device-related infections, like VVC in women using intrauterine device (IUD) as contraceptive method (Demirezen et al., 2005). The initial attachment of *Candida* cells to biotic and abiotic surfaces is mediated by cell surface physicochemical properties and promoted by specific cell surface proteins, called adhesins (Verstrepen & Klis, 2006). Adhesins recognize host ligands, such as serum proteins and components, of the extracellular matrix of host tissues (e.g. laminin, fibronectin, collagen, vitronectin and entactin), or promote the binding to abiotic surfaces through hydrophobic interactions (Chaffin, 2008). In *C. albicans*, a major group of adhesins is encoded by the agglutinin-like sequence (*ALS*) gene family that comprises eight members (*ALS1-7, 9*) (Hoyer et al., 2008). Cheng et al. (2005) detected expression of all *ALS* genes in vaginal specimens of women with VVC, but expressions of *ALS1*, *ALS2*, *ALS3* and *ALS9* were detected more frequently than of *ALS4* to *ALS7*. These investigators also demonstrated that the expression of *C. albicans ALS* genes has host-site specific influences. In *C. glabrata*, up to 23 different genes encoding epithelial adhesins (*EPA* gene family) were already identified, and three genes, *EPA1*, *EPA6* and *EPA7*, have been shown to encode functional adhesins (Cormack et al., 1999). Alves et al. (2014b) detected expression of these three genes in reconstituted human vaginal epithelium (RHVE) infected with *C. glabrata*, with the highest value exhibited by *EPA1*. These investigators also detected all *ALS* genes in RHVE infected with *C. albicans*, with the highest expression exhibited by *ALS3* and *ALS6* and the lowest by *ALS7*. Interestingly, in RHVE co-infected with *C. glabrata* and *C. albicans* the *EPA* genes were down-regulated or absent and *ALS* genes were generally similar to those observed in single infection, with the exception of a

highly increase in *ALS3* expression. These results suggest that probably *ALS3* but not *EPA* adhesins are associated with the enhanced RHVE invasion by *C. glabrata* observed in the presence of *C. albicans*. In *C. parapsilosis*, 11 genes were identified for putative cell wall adhesins-like proteins (Butler et al., 2009) and at least three Als proteins were identified in *C. tropicalis* and *Candida dubliniensis* (Hoyer et al., 2001). However, in most NCAC species, the function of identified adhesins was still poorly studied. The absence of *in vitro* animal–human models highlights the importance to deepen and validate the information reported on those *in vitro* studies.

Biofilm formation

Adhesion of *Candida* cells to host epithelium or medical devices has been implicated as an early step in biofilm formation. Biofilms are structured communities of microorganisms, irreversibly attached to a surface, with a high degree of organization and a self-produced extracellular matrix (Douglas, 2003). Biofilms are the most prevalent growth form of microorganisms in nature, with up to 80% of all microorganisms, in the environment, existing in biofilm communities. It is also suggested that over 65% of all human infections are related to microbial biofilms (Donlan, 2002). Biofilm formation is an important virulence factor for *Candida* species as it confers unique phenotypic characteristics compared to their planktonic counterpart cells including significant resistance to antifungal agents, host defence mechanisms and physical and chemical stress (Donlan & Costerton, 2002). Furthermore, biofilm cells exhibit metabolic cooperation, community-based regulation of gene expression and the ability to withstand the competitive pressure from other organisms (Davey & O'toole, 2000; Ramage et al., 2009). The association of microorganisms into biofilms is a form of protection for their development and contributes to their survival in hostile environmental conditions (Davey & O'toole, 2000). Clinically, the most important phenotype of biofilms is their extraordinary resistance to the conventional antifungal therapy, which has been reported to be up to 1000-fold higher than of their planktonic counterpart cells (Ramage et al., 2009). However, the resistance mechanisms of *Candida* biofilms to antifungal therapy are not fully understood. It is accepted that the antifungal resistance of biofilms is a complex multifactorial phenomenon that includes alterations or overexpression of target molecules, active extrusion of antifungal agents through efflux pumps and their limited diffusion in the matrix, stress tolerance, cell density and presence of persister cells (Ramage et al., 2012). Formation of mature biofilms and subsequent production of extracellular matrix is strongly dependent on species, strains and environmental conditions (Jain et al., 2007; Silva et al., 2009). Concerning the vaginal environment, *Candida* species can form biofilms on vaginal epithelium (Harriott et al., 2010) and have high capacity to produce biofilms on IUDs promoting VVC (Chassot et al., 2008; Lal et al., 2008). *Candida* biofilms have been studied primarily on abiotic surfaces because almost all device-related infections involve growth in the form of biofilm (Dominic et al., 2007). Recently, biofilm formation on biotic surfaces has received some attention, and Harriott et al. (2010) showed for the first

time that *C. albicans* forms biofilms *in vivo* on vaginal mucosa. These investigators also reported that *Candida* biofilm formation on the vaginal mucosa *in vivo* requires regulators of biofilm formation (*BCR1*) and morphogenesis (*EFG1*), which had been previously identified as necessary genes for biofilm formation on abiotic surfaces (Nobile et al., 2006; Ramage et al., 2002). The ability of *Candida* species to form biofilms on the vaginal mucosa is an important clinical issue since the recalcitrance of these biofilms to the conventional antifungal therapy may prevent complete eradication of the microorganisms from the vaginal lumen and might explain the frequent recurrence of VVC. In addition, it has been realized that many *Candida* infections are directly linked to biofilms in which multiple species coexist, making the therapeutic management of these infections extremely difficult. Most studies are on monospecies biofilms of *Candida*, and information on mixed-species *Candida* biofilms or *Candida*-bacteria combinations is still scarce (Thein et al., 2009). Mixed-species biofilms can be difficult to both diagnose and treat, requiring complex multidrug treatment strategies. Mixed biofilms, especially *Candida*-bacteria biofilms, can cause a dilemma for clinicians because antimicrobials directed towards one species often facilitate non-targeted organisms to continue the infection (Thein et al., 2009). Studies report that both *Candida* species (Harriott et al., 2010) and vaginal bacterial pathogens as *Gardenerella vaginalis* (Scott et al., 1989; Swidsinski, 2008), which cause bacterial vaginosis (BV), have the ability to form biofilms on the vaginal mucosa. However, the formation of mixed *Candida*-bacteria biofilms on the vaginal mucosa is still understudied. Some studies have shown that approximately 20–34% of RVVC samples contain vaginal bacterial pathogens such as *Streptococcus agalactiae* and *G. vaginalis* (Buchta & Spacek, 2004; Esim et al., 2010), possibly due to mixed biofilms with *Candida* and these bacteria. Concerning mixed biofilm formation on IUDs, a survey on biofilms formed on those devices confirmed the presence of *C. albicans* as well as multiple bacterial pathogens such as *S. agalactiae*, *Escherichia coli* and *Bacteroides* species (Pál et al., 2005). Although vaginal infections are an extremely common reason for women to seek care, there is little known about the prevalence of mixed infections, particularly VVC and BV. Recently, Rivers et al. (2011) published the first article to present the prevalence of BV, VVC, yeast colonization and mixed infection. In this study, BV was diagnosed in 72.5% of the participants and VVC in 15.7%. Among women with BV, 33.1% were colonized with yeast and the overall prevalence of BV/VVC mixed infection was 4.4%. The authors suggested that the presence of infection/colonization with yeasts likely predisposes women to VVC after treatment of BV with antibiotics. In fact, some studies reveal that VVC is a common side effect of BV treatment with metronidazole or clindamycin (Bradshaw et al., 2006; Sobel & Leamna, 1998). Thus, women with BV that also have yeast residing in the vaginal ecosystem can either exhibit failure of symptom resolution from therapy targeted at one infection or development of VVC from exposure to antibiotics. The little information available on the prevalence of vaginal mixed infections is likely due to the fact that most vaginal infections are diagnosed empirically without

the aid of objective data. The lack of awareness as to the extent of this problem likely leads to under recognition of mixed infections, resulting in inadequate therapy.

Extracellular hydrolytic enzyme production

Candida species secrete several hydrolytic enzymes, which play an important role in adhesion, tissue penetration, invasion and destruction of host tissues (Schaller et al., 2005). The enzymes most frequently implicated in *Candida* pathogenicity are secreted aspartyl proteinases (Saps), but phospholipases, lipases and haemolysins are also involved in *Candida* virulence (Silva et al., 2012). Saps facilitate adhesion to host tissues and their damage and are related with changes in the host immune response (Pichová et al., 2001). To date, 10 *SAP* genes (*SAP1-10*) were identified in *C. albicans* (Odds, 2008), three (*SAPP1-3*) in *C. parapsilosis* (Merkerová et al., 2006) and at least four (*SAPT1-4*) in *C. tropicalis* (Togni et al., 1991; Zaugg et al., 2001), but in NCAC species, most of the genes remains uncharacterized. In the case of *C. glabrata*, only one study demonstrated ability of this species to produce proteinases, but the type of proteinase remains unknown (Chakrabarti et al., 1991). In contrast with other types of proteinases, Saps show proteinase activity only under acid conditions ($\text{pH} \leq 4.0$) (Williams et al., 2011). This feature is important for VVC, because the vaginal environment is acidic (pH around 4) (Sobel, 2005), providing conditions suitable for activity of Saps. Mohandas and Ballal (2011) detected higher proteinase activity in vaginal isolates than in urinary and respiratory isolates, of candidiasis-infected patients, relating Sap production and site of strain isolation. Furthermore, several 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 (Cassone et al., 1987; De Bernardis et al., 1990; Ozcan et al., 2006). It has been also demonstrated that the expression of *C. albicans* *SAP1-3* has a strong and specific correlation with VVC (Lian & Liu, 2007; Naglik et al., 2003; Schaller et al., 2003). 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 have the ability to produce extracellular phospholipases, but this ability is highly strain dependent and NCAC species produce significantly lower levels compared with *C. albicans* (Ghannoum, 2000). Mohandas and Ballal (2011) found a greater number of phospholipase producing strains in vaginal isolates of patients with candidiasis than in respiratory and skin isolates. In *C. albicans*, seven phospholipase genes have been reported (*PLA*, *PLB1-2*, *PLC1-3* and *PLD1*) (Samaranayake et al., 2006). Naglik et al. (2003) detected expression of *PLB1* and *PLB2* in vaginal washes of VVC infected women and of asymptomatic vaginal *Candida* carriers, reporting lower levels in healthy women. Furthermore, Alves et al. (2014b) detected expression of *PLB* and *PLD* gene families in RHVE infected with several *C. albicans* strains. The highest expression level was exhibited by *PLD1* indicating a potential role of this factor in RHVE damage. Lipases are involved in the hydrolysis of triacylglycerols and their activity has been associated to *Candida*

adhesion and damage of host tissues and affects immune cells (Stehr et al., 2004). In *C. albicans*, lipases are coded by 10 genes (*LIP1-10*), similar sequences were identified in *C. tropicalis* and two lipase genes were detected in *C. parapsilosis* (*CpLIP1-2*), but none in *C. glabrata* (Silva et al., 2012). These enzymes are less studied than Saps and phospholipases, especially concerning their specific association with the anatomical site of infection. Haemolysins produced by *Candida* species degrade haemoglobin, facilitating recovery of iron, being essential to survival and persistence in the host. The production of these proteins was already described in several *Candida* species, including *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis*; however, the genetic expression of haemolytic activity is poorly understood (Luo et al., 2004).

Hyphae formation

A reversible morphological alternation between unicellular yeast cells and filamentous phase (hyphae and pseudohyphae) is an important virulence factor of some *Candida* species (Silva et al., 2012). *Candida albicans* and *C. dubliniensis* have the ability to form hyphae and/or pseudohyphae, *C. parapsilosis* can generate pseudohyphae, *C. tropicalis* pseudohyphae and possibly true hyphae and *C. glabrata* grows only as blastoconidia (Silva et al., 2012). Filamentous forms give more mechanical strength, enhancing colonization and invasion of host tissues and demonstrate increased resistance to phagocytosis (Kumamoto & Vences, 2005; Silva et al., 2012). Hyphae are believed to play an important role in tissue invasion, and *in vitro* research has shown that *C. albicans* lacking hyphal formation exhibited lower ability to invade tissue compared to the wild-type strains (Jayatilake et al., 2006). The yeast–hyphal switch is affected by environmental factors and is related with other virulence factors. For instance, phospholipase D (*PLD1*) is necessary for yeast-to-hyphal transition in *C. albicans* (Hostetter, 1994) and the expression of some *SAP* genes (*SAP4-6*) occur specifically during hyphal development (Hube et al., 1997). Taylor et al. (2005) detected association of *SAP4* and *SAP5* expression with hyphal formation during vaginal candidiasis in mice. Furthermore, Alves et al. (2014b) in a study with RHVE infected with *C. albicans* detected expression of the hyphal wall protein (*HWPI*) gene, which is a specific hyphae-adhesin responsible for a covalent attachment of the yeast to host epithelial cells. Interestingly, in RHVE co-infected with *C. albicans* and *C. glabrata*, being the latter a nonhyphae producer, the investigators detected an up-regulation of *HWPI* that may have contributed for the enhanced tissue invasion by *C. glabrata* observed in the presence of *C. albicans*.

Phenotypic switching

The colonies of most *Candida* species can switch among different phenotypes at high frequency. In *C. albicans* white-phase round-ovoid cells can switch to opaque-phase cells which are elongated or bean-shaped (Anderson & Soll, 1987). It has been identified that the genes *MTLa1-2*, *WOR1-2*, *CZF1* and *EFG1* regulate the white to opaque switching (Zordan et al., 2006, 2007). Furthermore *SAP3* and *EFG1* are expressed specifically in white cells whereas *SAP1-2* are

expressed specifically in opaque cells (Soll, 1997). Comparatively to *C. albicans* the phenotypic switching of NCAC species is less understood. It has been reported that *C. glabrata* can undergo “core switching” that results in white, light brown, dark brown, very dark brown or irregular wrinkle colonies. Brockert et al. (2003) showed a predominance of dark brown colonies among vaginal isolates of patients with VVC. The phenotypic switching alters several virulence traits including hyphae formation (Anderson et al., 1989), drug resistance (Vargas et al., 2000), adhesion and Saps secretion (Morrow et al., 1992), potentially affecting survival in specific anatomical sites and promoting infection. The precise contribution of phenotypic switching to VVC is still not clear, but it has been demonstrated that vaginal isolates obtained during VVC and RVVC are in high-frequency mode of switching (Soll, 1988; Soll et al., 1987, 1989). Furthermore, in each recurrence of one patient with RVVC, different colony phenotypes were observed but DNA genotyping remained identical (Soll et al., 1989).

All virulence factors that mediate *Candida* pathogenicity are influenced by the specific environmental conditions of each anatomical site. Thus, the disturbance of the normal environment of the lower female genital tract may increase the risk of infection by *Candida* species. Next will be reviewed the knowledge about risks factors predisposing for VVC.

Risk factors for VVC

The vaginal flora is highly dynamic with a local microbial system. There is a balance between *Candida* vaginal colonization and the host environment that can be disturbed by physiological or non-physiological changes, making the colonization site favorable for the development of yeasts. Healthy women can develop VVC sporadically however this infection is often attributed to the presence of host-related and behavioral factors that disturb the vaginal environment, promoting VVC. Proposed host-related risk factors include pregnancy, hormone replacement, uncontrolled diabetes mellitus, immunosuppression, antibiotics and glucocorticoids use and genetic predispositions (Sobel, 2007) (Figure 2). Behavioral risks factors for VVC include use of oral

contraceptives, intrauterine device, spermicides and condoms, and also some sexual, hygienic and clothing habits (Patel et al., 2004; Sobel, 2007) (Figure 3).

Host factors

Pregnancy and hormone replacement therapy

Pregnancy has been considered an important risk factor for the development of VVC because several studies report high incidence of the disease in pregnant women. Table 3 shows studies published during the last years, concerning the incidence of VVC in pregnant and nonpregnant women. The epidemiologic studies have been consensual in reporting higher prevalence of the disease in pregnant women than in non-pregnant patients, although the incidence varies depending on the locations. In the last years, most studies of VVC incidence in pregnant women were made in India and Nigeria (Table 3), probably because these countries have high birth rate and thus, have interest to study pregnant-related diseases. In Indian studies (Ahmad & Khan, 2009; Neerja et al., 2006; Vijaya et al., 2014), the incidence of VVC in pregnancy ranges between 10.0% and 76.0% and in nonpregnancy between 7.7% and 31.0%. A Nigerian study (Kamath et al., 2013) reported an incidence of VVC in pregnancy and nonpregnancy of 47.7% and 20.3%, respectively, and Greek (Grigoriou et al., 2006) and Belgian (Bauters et al., 2002) studies reported a little less incidence of the disease in both conditions. Two Nigerian reports (Nwadioha et al., 2010; Shu'aibu et al., 2014) and a Kenyan one (Nelson et al., 2013) only included the incidence of VVC in pregnant women without a non-pregnant control, but all of them reported high incidence of the disease in pregnancy (Table 3). In addition, since the vaginal colonization is a prerequisite for symptomatic VVC, some studies have also studied the incidence of *Candida* colonization in non-symptomatic pregnant women. These studies reported higher prevalence of vaginal colonization by *Candida* in pregnant women than in those who were not pregnant, indicating that pregnancy increases vaginal colonization (Cotch et al., 1998; Jabeen & Siddiqi, 2014).

The high incidence of VVC in pregnancy has been attributed to the increase of sex hormones secretion in pregnancy. In fact, during pregnancy, VVC prevalence is

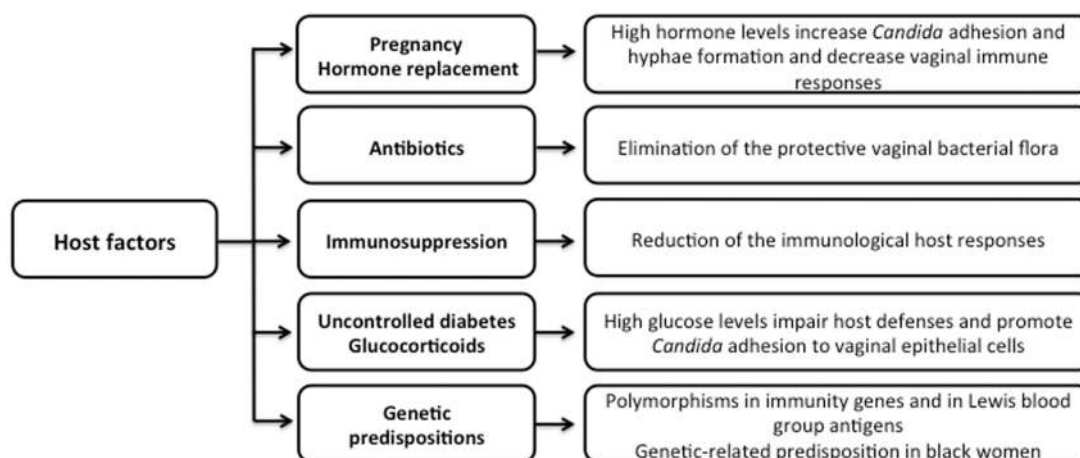


Figure 2. Host-related risk factors for vulvovaginal candidiasis and respective effects.

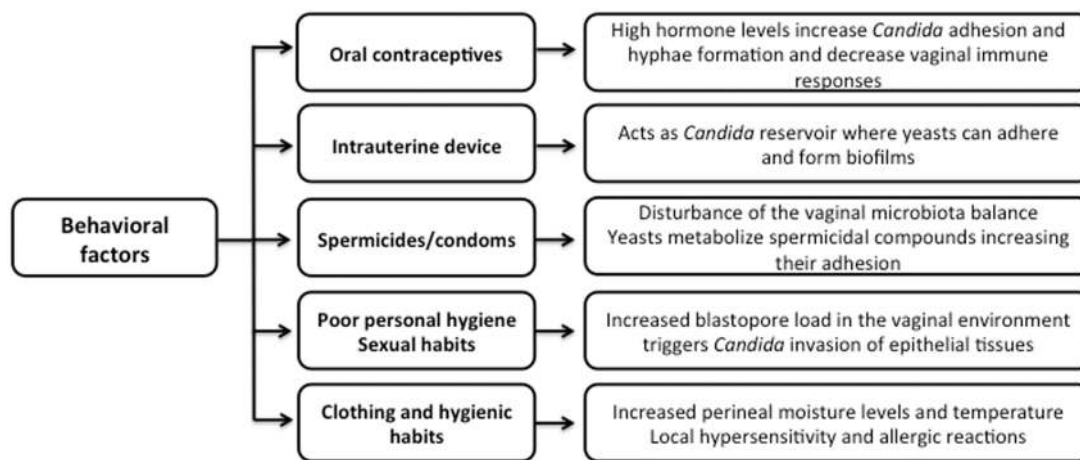


Figure 3. Behavioral risk factors for vulvovaginal candidiasis and respective effects.

Table 3. Incidence of VVC in pregnant and nonpregnant women reported in studies published from 2002 to 2014.

Country	Year(s) of study	Pregnant women (% with VVC)	Non-pregnant women (% with VVC)	References
India	2011–2012	10.0	7.7	(Vijaya et al., 2014)
India	–	28.2	7.9	(Neerja et al., 2006)
India	–	76.0	31.0	(Ahmad & Khan, 2009)
Greece	2002–2004	29.1	7.9	(Grigoriou et al., 2006)
Belgium	–	32.0	19.3	(Bauters et al., 2002)
Nigeria	2009	47.7	20.3	(Kamath et al., 2013)
Nigeria	2006–2007	40.0	Not included	(Nwadioha et al., 2010)
Nigeria	–	91.0	Not included	(Shu'aibu et al., 2014)
Kenya	2010	90.4	Not included	(Nelson et al., 2013)

higher in the last trimester, when levels of hormones are more elevated, even though symptomatic recurrences are common throughout pregnancy (Bauters et al., 2002; Nelson et al., 2013). Furthermore, in nonpregnant women the infection is more incident during the luteal phase of the menstrual cycle, which is the phase with the highest hormone secretion (Eckert, 1998; Kalo-Klein & Witkin, 1989). Kalo and Segal (1988) also demonstrated that the level of *in vitro* adherence of *C. albicans* to human vaginal exfoliated cells (VEC) has a correlation with the hormonal status of the cell donors, being higher in VEC of pregnant women and of those in luteal phase of the menstrual cycle. The hormonal dependence of VVC is also evidenced by the fact that the disease is uncommon in pre-puberty and post-menopause, except in women taking hormone replacement therapy (HRT) (Bauters et al., 2002; Tibaldi et al., 2009). HRT is used to counter adverse the consequences associated with the decrease in hormones secretion in postmenopause, such as osteoporosis, diabetes mellitus, cardiovascular disease and neurodegeneration (McNagny, 1999; Psaty, 1993); however, it has been considered a risk factor for the development of VVC. Higher incidence of VVC has been reported in women receiving HRT (26.0–29.4%) than in post-menopausal women without HRT (4–12.6%) (Bauters et al., 2002; Dennerstein & Ellis, 2001; Spinillo et al., 1997a). All authors agree that the high hormonal levels of pregnant women and of those receiving HRT are the main factors responsible for the relation of those conditions with VVC. However, emotional stress, suppression of immune system and eating habits of sugar-rich food may

also contribute to the development of VVC in those women (Sobel et al., 1998).

The two types of female sex hormones are estrogens and progestins. The most important progestin is progesterone, which is secreted by the *corpus luteum*, placenta and by adrenal cortex. During the luteal phase of the menstrual cycle, significant amounts of progesterone are secreted and during pregnancy its levels increase about 10 times. Progesterone is important in many vital actions of the woman, such as endometrial and breast development, maintenance of pregnancy, decrease in insulin action and increase in sodium excretion by the kidneys (Guyton & Hall, 2006). The main estrogen in pre-menopause is β -estradiol and in postmenopause is estrone, which has a lower estrogenic potency than β -estradiol. Significant amounts of estrogens are secreted by the ovaries and during pregnancy high amounts of these hormones are secreted by the placenta increasing about 30 times their levels. Estrogens perform many essential actions in women, including the development of secondary sex characteristics, uterine growth and conservation of the vaginal mucosa (Guyton & Hall, 2006). Despite being accepted that VVC has a hormonal dependency, the mechanisms by which progesterone and estrogens act in the disease are not fully understood. One proposed mechanism is associated with the increase in glycogen load in vaginal epithelium when the levels of progesterone and estrogens increase. The walls of vagina are lined with the pavement of epithelial cells, which produce glycogen in proportion to hormonal levels, and thus the state of the vaginal mucosa reflects female hormonal

status in different lifetime stages (Dennerstein & Ellis, 2001). The production of glycogen by hormone stimulated epithelium possibly contributes to the proliferation of *Candida* species when host hormones exceed a certain level, because glycogen provides an excellent nutritional source of carbon for *Candida* growth (Dennerstein & Ellis, 2001; Spacek et al., 2007). In addition to the effect on vaginal epithelium, it has been also proposed that sex hormones inhibit aspects of both innate and adaptive immunity at systemic or local level. In fact, studies *in vitro* with vaginal epithelial cells found that progesterone and estrogens inhibit *Candida*-specific human peripheral blood lymphocyte (PBL) responses (Kalo-Klein & Witkin, 1989, 1991) and that estradiol significantly reduces antimicrobials production (HBD2 and elafin) (Wira & Fahey, 2008). Furthermore, analyses of cervical–vaginal secretions demonstrate that chemokines and cytokines (IL-6 and IL-8), antimicrobials (HBD2 and lactoferrin) and levels of IgA and IgG antibodies are depressed by 10- to 100-fold at mid-cycle, remain depressed for 7–10 days and rise to proliferative levels in the end of the menstrual cycle (Fleetwood et al., 1984; Keller et al., 2007). In addition, Nohmi et al. (1995) reported that physiological blood level of progesterone of pregnant women clearly suppresses mice neutrophil anti-*Candida* activity.

Besides the effects on the host, it has also been shown that progesterone and estrogens have direct effects on *Candida* cells, possibly contributing to VVC. One direct effect of the hormones is the stimulation of estrogen and progesterone cytosolic receptors, which have been already identified in several *Candida* species. An estrogen-binding protein (EBP), that displays high affinity for estradiol and estrone, was identified and characterized in *C. albicans* (Skowronski & Feldman, 1989; Wagner & Johnson, 2012) and an estrogen-binding system was also detected in *C. glabrata* (Powell et al., 1984). In addition, a corticosteroid-binding protein (CBP) that exhibits high affinity for corticosterone and progesterone, but low affinity for estrogens, was identified in *C. albicans*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* (Loose et al., 1983; Skowronski & Feldman, 1989). The highest binding capacity is exhibited by *C. guilliermondii*, *C. parapsilosis* and *C. albicans* (Loose et al., 1983). Reed (1992) showed that when progesterone receptors are stimulated there is an increase in *Candida* proliferation. However, recently, Alves et al. (2014a) contradicted these results showing that the presence of progesterone reduces the ability of *C. albicans* to develop biofilms and hyphal forms (Figure 4) as well as decreases the expression levels of biofilm related genes *BCR1* and *HWPI*. These investigators also showed that *C. albicans* colonization and invasion of

RHVE decreased substantially in the presence of progesterone. In accordance with Alves et al. (2014a); Spacek et al. (2007) found lower progesterone levels in patients with RVVC than in healthy controls and Nohmi et al. (1995) showed that progesterone contribute to *C. albicans* growth form conversion from hyphal to yeast. Furthermore, Fidel et al. (2000) reported that progesterone has no effect on the induction and persistence of vaginal infection and that estrogen is the dominant reproductive hormone that supports and sustains an experimental vaginal *C. albicans* infection. In fact, *in vitro* studies show that β -estradiol increases *C. albicans* growth (Zhang et al., 2000) and directly stimulates the dimorphic transition from yeast to hyphal form also increasing hyphae length (Cheng et al., 2006; White & Larsen, 1997). Madani et al. (1994) demonstrated that estradiol inhibits the NADPH oxidase activity associated with *EBP1*, which might indicate that the inhibition of this enzyme may lead to metabolic changes favoring the yeast-hyphal conversion or blocking the inverse change. Furthermore, it has been also shown that estradiol increases *C. albicans* expression of *CDR1* and *CDR2*, which encode multidrug transporters of the ABC family, suggesting that estradiol might be also involved in *C. albicans* drug resistance (Cheng et al., 2006; Zhang et al., 2000).

Since VVC incidence is higher and cure rates are lower in conditions with high hormone levels such as pregnancy and HRT, therapy must be appropriate for these patients. VVC in pregnancy does not usually harm the unborn child but if the disease is not treated the baby can get infected (oral thrush) during delivery, which can be a very serious health problem in premature babies. Furthermore, infants with oral thrush can give rise to nipple candidiasis in breast-feeding mothers (Parveen et al., 2008). In addition, some authors report reduction of preterm birth in pregnant women treated for VVC with clotrimazole (Czeizel & Rockenbauer, 1999; Czeizel et al., 2004). It is has been also shown that a large proportion of women with RVVC first appear with the infection during pregnancy (Mitchell, 2004). Thus, in order to avert the complications associated with VVC in risk groups such as pregnant women and those with HRT early diagnosis and prompt treatment are very important.

Antibiotics

The use of antibiotics (vaginal or systemic) has been considered a risk factor for the development of VVC (Barbone et al., 1990; Geiger & Foxman, 1996). Table 4 highlights the most relevant reports that studied the

Figure 4. Scanning electron microscopy images of *C. albicans* ATCC biofilms formed in the absence (A) or presence (B) of 2 μ M of progesterone. Original magnification was $\times 1000$ and the scale bars correspond to 20 μ m (Alves et al., 2014a).

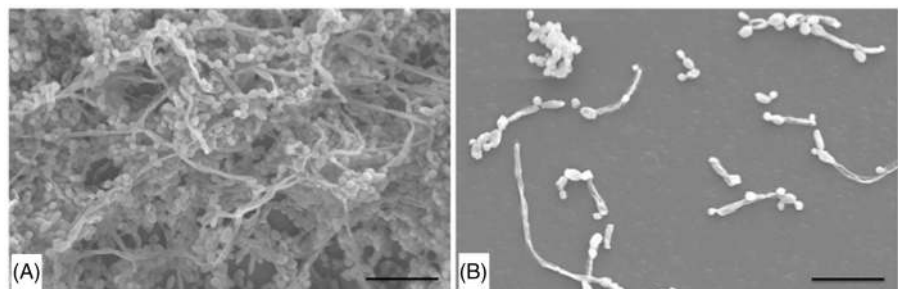


Table 4. Incidence of VVC in women that used and not used antibiotics and incidence of antibiotic use in women with and without VVC.

Country	Year(s) of study	Incidence of VVC (%)		Incidence of antibiotic use (%)		References
		Antibiotic users	Not antibiotic users	With VVC	Without VVC	
India	–	57.5	11.8	ni	ni	(Ahmad & Khan, 2009)
Greece	2002–2004	18.0	8.2	ni	ni	(Grigoriou et al., 2006)
Italy	1991–1997	55.2	41.0	19.3	11.9	(Spinillo et al., 1999)
Italy	1989–1994	45.5	39.2	18.3	11.4	(Spinillo et al., 1995)
Turkey	2009–2010	ni	ni	29.5	6.8	(Guzel et al., 2011)
Brazil	–	ni	ni	8.2	0.7	(Linhares et al., 2001)
USA	1984–1986	ni	ni	13.0	6.0	(Eckert, 1998)
Nigeria	2006–2007	ni	ni	16.0	ns	(Nwadioha et al., 2010)
Israel	–	ni	ni	23.0	ns	(Dan et al., 2003)

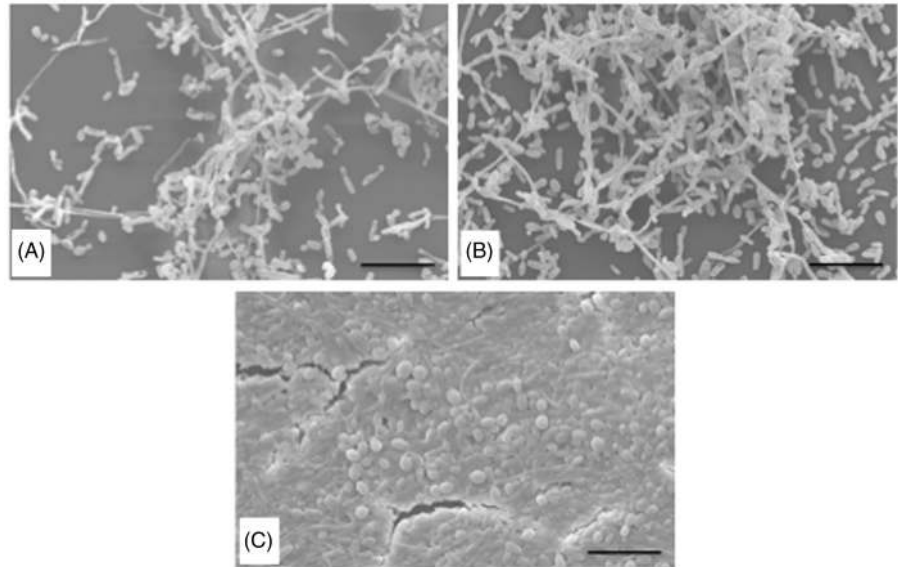
ni - not included in the study; ns - not shown in the study

prevalence of VVC in women treated and untreated with antibiotics and the incidence of antibiotic use. Studies in India (Ahmad & Khan, 2009), Greece (Grigoriou et al., 2006) and Italy (Spinillo et al., 1995, 1999) showed an increased incidence of VVC in women that have taken antibiotics than in those who have not taken antibiotics (18.0–57.5% versus 8.2–41.0%). Studies in Italy (Spinillo et al., 1995, 1999), Turkey (Guzel et al., 2011), Brazil (Linhares et al., 2001), USA (Eckert, 1998), Nigeria (Nwadioha et al., 2010) and Israel (Dan et al., 2003) showed an increased incidence of antibiotic use in women with VVC than without VVC (8.2–29.5% versus 0.7–11.9%). These epidemiologic data may have been influenced not only by the location where the study was made but also by type of antibiotic used and the duration of the treatment. In fact, the duration of antibiotic use is directly related to an increase in VVC prevalence (Spinillo et al., 1999). Moreover, broad-spectrum antibiotics (e.g. tetracycline, ampicillin and cephalosporin) are more likely to favor yeast infections compared with narrow-spectrum antibiotics (e.g. erythromycin, lincomycin) (Bluestein et al., 1991). Furthermore, it has been shown that antibiotic agents also predispose to RVVC (Guzel et al., 2011; Spinillo et al., 1995, 1999), in some cases with more significance than for sporadic acute VVC (Guzel et al., 2011). In addition, some studies found an increased incidence of asymptomatic vaginal colonization by *Candida* after an antibiotic treatment than before it (37% versus 18–21%), and a subsequent development of VVC after the antibiotic intake in 20–22% of initially colonized women (Pirota & Garland, 2006; Xu et al., 2008). These studies also report that only women already colonized with *Candida* are at risk of VVC following antibiotic treatment.

It is thought that the association of VVC and antibiotics is due to the fact that antibiotic use leads to the depletion of the vaginal bacterial microflora, which represents the dominant vaginal defense mechanism against *Candida* (Gibbs, 1987). The vaginal microbiota of healthy premenopausal woman is predominantly populated by *Lactobacillus* species, the most common of which are *L. iners*, *L. crispatus*, *L. gasseri*, *L. jenesenii*, followed by *L. acidophilus*, *L. casei*, *L. vaginalis*, *L. salivarius* (Auger & Joly, 1980; Cribby et al., 2008; Eschenbach, 1986). In fact, lactobacilli are thought to be involved in several defense mechanisms against *Candida*. One proposed mechanism is that *Lactobacillus* species compete with *Candida* species for nutrients; however, a “shoulder-to-

shoulder” survival for lactobacilli and *Candida* has been shown on an experimental basis, proving that this is not the most effective mechanism (Savage, 1969). More importantly, lactobacilli compete with *Candida* cells for adhesion sites, such as epithelial receptors, to which *Lactobacillus* has higher affinity (Boris & Barbés, 1998, 2000). Some studies have found a decreased adhesion of *C. albicans* to vaginal epithelial cells when *Lactobacillus* is present in comparison with the adhesion observed when only *Candida* is present (Boris & Barbés, 1998). Furthermore, lactobacilli secrete biosurfactants that physically decrease *Candida* binding. Velraeds et al. (1998) found a 50% decrease in the adhesion of *C. albicans* to a silicone rubber filled with a biosurfactant (surlactin) compared with the adhesion levels on silicone rubber without surlactin. Additionally, Okkers et al. (1999) showed that a bacteriocin-like peptide (pentocin TV35b) isolated from *Lactobacillus pentosus* inhibits *C. albicans* growth. Moreover, most *Lactobacillus* strains release hydrogen peroxide (H_2O_2) and fatty acids that inhibit *Candida* overgrowth and invasive hyphal formation (Boris & Barbés, 2000). Hillier et al. (2010) showed that women colonized by H_2O_2 -producing *Lactobacillus*, are less likely to have symptomatic VVC than women with non- H_2O_2 -producing *Lactobacillus*. In addition, Xu and Sobel (2003) showed that *L. delbrueckii* strains, which produce the largest amounts of H_2O_2 , have the strongest and fastest growth inhibition effect against *C. albicans*, amongst several *Lactobacillus* strains isolated from vaginas of healthy women. Moreover, it has been detected higher vaginal pH in women with *C. glabrata* infection than in those with VVC due to *C. albicans* (Ahmad & Khan, 2009; Tarry et al., 2005). In fact, a more alkaline pH occurs with concomitant bacterial vaginosis has been postulated as a risk factor for *C. glabrata* vaginal infections (Fidel et al., 1999b). In addition, increased vaginal pH and predominance of NCAC species, especially *C. glabrata*, has been detected in postmenopausal women (Fan et al., 2008b; Holland et al., 2003). Thus, the fact that *Candida* organisms can adapt to different pHs, makes them important human pathogens. In *C. albicans*, pH-regulated genes such as *PHR1* and *PHR2* have been identified. *PHR1* is only expressed at pH levels above 5.5 and its functional homolog *PHR2* is highly expressed when pH levels are below 5 and is not expressed with pH above 6 (Muhlschlegel & Fonzi, 1997). Studies *in vitro* found that both genes encode a function required for *C. albicans* morphogenesis. Deletion of *PHR1* result in

Figure 5. Scanning electron microscopy images of *C. tropicalis* ATCC biofilms formed at pH 4 (A), pH 7 (B) and pH 8 (C). Original magnification was $\times 1000$ and the scale bars correspond to $20\ \mu\text{m}$.



inability to form normal yeast or hyphal morphology at an alkaline pH, but not at an acidic pH, and these mutants lost the ability to cause systemic disease but are fully capable of causing vaginal infection. Conversely, deletion of *PHR2* result in a morphogenic defect at an acidic pH and these mutants lost the ability to cause vaginal infection but are virulent in systemic infections. Besides the morphogenic defect, both mutants also exhibit altered growth rates at the restrictive pH (De Bernardis et al., 1998; Muhlschlegel & Fonzi, 1997). In addition, our research group (results unpublished) studied planktonic cultures of *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* grown at pHs between 3 and 8, and we found that these species can proliferate in all tested pHs. We also studied the influence of pH on the capacity of *C. tropicalis* isolates to form biofilms and we found that the pH increase lead to an increased biofilm formation capacity (Figure 5) and increased hyphal length, suggesting that *Candida* species are able to adapt to vaginal environmental pH alterations.

Immunosuppression

All *Candida* infections are dependent on the host, and therefore its immune response is crucial in the host–pathogen interaction (Richardson & Rautemaa, 2009). In the vaginal environment effective anti-*Candida* defense mechanisms prevent *Candida* infection, but allow long-term colonization of *Candida* organisms as vaginal commensals (Sobel, 2007). Thus, immunosuppressive conditions such as HIV-infection (Nwadioha et al., 2010; Duerr et al., 2003), cancer chemotherapy, glucocorticoids therapy (Zitvogel et al., 2008), organ transplant, cancer, diabetes mellitus, tuberculosis (Nwadioha et al., 2010), and any chronic debilitating illness can increase the chances of developing VVC. Furthermore, it has been proposed that some women with RVVC may have a dysfunction in the normal protective immune response, acquired from early exposure to *Candida* organisms (Fidel & Sobel, 1996; Witkin, 1991).

The most studied immunosuppressive condition predisposing to VVC is the HIV infection probably because

VVC may also increase the risk of HIV acquisition (Mostad et al., 1997; Røttingen et al., 2001). It is though that up to 50–70% of HIV-infected patients develops VVC (Ferrer, 2000). Some studies have reported increased odds for vaginal *Candida* colonization (Schuman et al., 1998) and symptomatic VVC (Apalata et al., 2014; Duerr et al., 2003) in HIV-infected women than in HIV-negative patients. Moreover, Apalata et al. (2014) found a negative association between the use of highly active antiretroviral therapy and the presence of symptomatic VVC in HIV-infected women. In addition, NCAC species have been found more frequently in HIV-infected women than in non-infected patients (Corsello et al., 2003; Spinillo et al., 1997b). Nevertheless, no studies indicate that HIV-infected women with VVC are less likely to respond to the antifungal therapy or have a more severe fungal infection than HIV-negative women (Duerr et al., 2003; Schuman et al., 1997).

Cells of host's innate immunity express receptors that recognize pathogen associated molecular patterns (PAMPs) found in groups of microorganisms (Medzhitov, 2001). Some of these receptors recognize molecular patterns common to all fungi, while others bind to specific *Candida* components (Brown & Gordon, 2005; Jouault et al., 2006). The main classes of pattern recognition receptors that recognize *Candida* are toll-like receptors (TLR) and lectin-like receptors (LR) families (Netea et al., 2008). In addition to cell-membrane receptors, the circulating mannose binding lectin (MBL) recognizes and binds strongly to *Candida* surface mannans, enhancing complement activation and thus inhibiting *Candida* growth (Ip & Lau, 2004; Pellis et al., 2005). Genetic polymorphisms in recognition receptors such as dectin-1 (Ferwerda, 2009) and MBL (Babula et al., 2003; Giraldo et al., 2007) have been associated with increased susceptibility to RVVC. The cells involved in innate immune system that express cell-membrane receptors include macrophages, dendritic cells (DCs), natural killer (NK), neutrophils and epithelial cells (ECs) (Wira et al., 2005a). The role of neutrophils in anti-*Candida* protection at the vaginal level is controversial (Fidel et al., 1999a; Fulurija et al., 1996). However, macrophages, DCs and ECs are believed to play

important protective roles against VVC. Dendritic cells are scattered throughout different portions of the vagina and cervical epithelia, bind to *Candida* organisms and transmit differentiation and activation signals leading to inflammation, cell activation and induction of adaptive immunity (Liu, 2001). Regarding macrophages, their stimulation through *Candida* recognition has been shown to induce the synthesis of NO and oxygen radicals, compounds toxic for *Candida* organisms. Rosentul et al. (2009) detected lower NO concentrations in vaginal secretions of patients with RVVC than of healthy patients. Furthermore, macrophages and DCs contain cell surface receptors that recognize and bind MBL, promoting opsonization of MBL-bounded microorganisms (Babovic-Vuksanovic et al., 1999). In addition, vaginal ECs have been suggested to play a crucial role in the defense mechanisms against VVC (Barousse et al., 2005; Wira et al., 2005b). Vaginal ECs secrete immune mediators such as chemokines and cytokines, which recruit and activate immune cells of both the innate and adaptive immune systems (Cremel, 2005; Wira et al., 2005a). In addition, they also secrete molecules with potent antimicrobial activity such as human α -defensin-5 (HD5), β -defensins 1–4 (HBD1–4), elafin and SLPI (secretory leukocyte protease inhibitor) (King et al., 2000, 2003; Quayle et al., 1998). These antimicrobial peptides have been shown to be effective at inhibiting several pathogens including *Candida* organisms (Ganz, 2003; Wira et al., 2010); however, a definite anti-*Candida* role for any specific peptide in the vaginal environment has not been established.

The adaptive immunity encompasses fungal-specific defence mechanisms including destruction of specific pathogens directly or indirectly by T cells (cell-mediated immunity) and protection through specific antibodies produced by B cells (humoral immunity) (Wira et al., 2005a). Depressed T-cell mediated immunity has been suggested to be associated with increased susceptibility to VVC in women with HIV-infection, transplanted organ, under glucocorticoids therapy and cancer chemotherapy (Apalata et al., 2014; Nwadioha et al., 2010; Zitvogel et al., 2008). It has been suggested that most vaginal T-cells migrate to the vaginal epithelium in response to local antigenic stimulation and/or inflammatory chemokines (Cassone et al., 2007; Pudney et al., 2005). It is thought that the vaginal mucosa is an immunocompetent tissue capable of a compartmentalized cell-mediated response independent of systemic cell-mediated immunity (Fidel, 2005; Fidel et al., 1996). Some studies have shown that Th1 production of pro-inflammatory cytokines such as interferon- γ , may contribute to VVC protection (Carvalho et al., 2002; Kalo-Klein & Witkin, 1990). However, the protective role of Th1 cells against VVC is still controversial (Fidel et al., 1995, 1999a). In contrast to Th1 cells, Th2 cells produce anti-inflammatory cytokines such as IL-4, which inhibits macrophage-mediated anti-*Candida* responses and protective pro-inflammatory Th1-derived cytokines (Cenci et al., 1993; Essner et al., 1989). Rosentul et al. (2009) showed that a genetic propensity to an overwhelming release of anti-inflammatory Th2-derived cytokines contributes to RVVC development. In addition, Pietrella et al. (2011) showed that Th17 cells (which belong to a different lineage from that of Th1 and Th2) and the IL-17 production by them have an important role in the immune response to VVC. Similar

to T cells, B cells and immunoglobulin (Ig)-secreting plasma cells, migrate to the vaginal epithelium where they are normally absent, in response to an antigenic stimulation (Fidel & Sobel, 1996; Pudney et al., 2005). The local production of antibodies by these cells might be a quick mechanism for combating pathogenic microorganisms without the need to wait for the beginning of the systemic immune response. The exact mechanism by which antibodies may protect against *Candida* infections is unknown, but appears to include inhibition of adhesion, opsonization, neutralization of enzymes related to virulence and direct fungicidal activity (Moragues et al., 2003; Russell & Mestecky, 2002).

Immunoglobulin A (IgA) and IgG are the predominant Ig classes found in vaginal secretions, suggesting that they represent the dominant Igs in the female lower genital tract (Carvalho et al., 2003; Parr & Parr, 1994). Carvalho et al. (2003) found a marked response of IgA, IgG1 and IgG4 in vaginal washes of symptomatic women with VVC comparatively to symptomatic women without *Candida*, suggesting that the synthesis of those antibodies was stimulated by the presence of the fungus. In fact, several studies have shown that vaginal colonization or infection by *Candida* induces anti-*Candida* antibodies production (Carvalho et al., 2003; Fidel & Sobel, 1996; Silva et al., 2008), but whether these humoral responses are important for avoid VVC is still controversial. Some studies have reported normal or high titers of *Candida*-specific antibodies in vaginal secretions of women with VVC and RVVC, suggesting little to no protective role for antibodies (Fidel et al., 1997; Fidel, 2005). In contrast, other studies found low levels of anti-*Candida* antibodies in vaginal washes of women with VVC than of healthy colonized patients, proposing that the decrease of antibody levels promoted *Candida* infection (Gough et al., 1984; Silva et al., 2008). Moreover, it has been also shown that the secretor component of IgA can attach to *C. albicans* in a nonimmune way, blocking *Candida* binding to cellular receptors (Silva et al., 2008). This immunoglobulin forms complexes with *Candida* organisms, which are removed by the secretion of mucin, thus contributing to the inhibition of *Candida* adhesion to the vaginal epithelium (Russell & Mestecky, 2002; Silva et al., 2008). Nevertheless, vaginal secretory immunity can also be the cause of higher susceptibility to VVC. It has been proposed that some women could have IgE-mediated hypersensitivity contributing to the etiology and/or severity of the infection. High levels of IgE have been found in vaginal secretions of women with VVC and particularly with RVVC, compared to healthy women, suggesting an allergic sensitization to *Candida* antigens (Fan et al., 2008a; Regúlez et al., 1994; Witkin et al., 1989). In addition, hypersensitivity reactions have been also associated with eosinophils and prostaglandin E2, which are also found in higher levels in vaginal secretions of women with RVVC than of patients without RVVC (Fan et al., 2008a; Weissenbacher et al., 2009).

Uncontrolled diabetes and glucocorticoids

Diabetes mellitus (DM) has been proposed as a risk factor for the development of VVC since higher prevalence of VVC

has been found in diabetic women than in nondiabetics. The incidence of VVC in diabetic women ranges between 32 and 67.5% and in non-diabetic patients ranges between 11% and 23% (Goswami et al., 2000, 2006; Grigoriou et al., 2006). Furthermore, higher vaginal colonization by *Candida* and higher development of RVVC has been detected in diabetic patients than in non-diabetics (Bohannon, 1998; Gunther et al., 2014). It has also been shown that, a large proportion of VVC in diabetic women is due to NCAC species, especially *C. glabrata*. This species has been isolated in 50–61% of diabetic patients with VVC, whereas *C. albicans* is only detected in 29–36% of these women (Goswami et al., 2006; Peer et al., 1993; Ray et al., 2007). In addition, De Leon et al. (2002) found that most diabetic type 1 participants were vaginal colonized with *C. albicans* (56%), while *C. glabrata* was the most common isolate among type 2 participants (54%). High predominance of NCAC species in diabetic women may have important implications for VVC treatment because NCAC species are inherently less susceptible to conventional antifungal therapy. In fact, Goswami et al. (2006) reported that 81.3% of diabetic patients with VVC continued to show fungal growth following fluconazole therapy, when *C. glabrata* was the organism grown and only 45.4% of patients had persistent *C. albicans* infection. Furthermore, these investigators also found that *Candida* persistence was higher in diabetic women than in the non-diabetics control group (78.6% and 21.5% for *C. glabrata* and *C. albicans*, respectively). It has been proposed that elevated levels of glucose predispose to VVC by impairing basic mechanisms of host defense and by promoting *Candida* adhesion to vaginal cells (Hostetter, 1990). Studies *in vitro* have shown that vaginal epithelial cells from diabetic women have an enhanced capacity to bind to *C. albicans* than vaginal cells from non-diabetic patients (Kalo & Segal, 1988; Segal et al., 1984). It is thought that the excess of glucose increases the nutritive substract of *Candida* microorganisms, promoting their adhesion (Pereira et al., 1996). Furthermore, it is supposed that an isomer of glucose, a fucose (6-deoxy galactose) vaginal epithelial cell receptor, helps *Candida* adhesion to vaginal epithelial cells (Sobel et al., 1981). More importantly, *C. albicans* expresses a glucose-inducible surface protein that promotes its adhesion to vaginal epithelial cells (Hostetter, 1990). Some studies have shown that the expression of this protein is increased four- to six fold when the concentration of glucose in the growth medium is raised from 5 to 50 mM (Gilmore et al., 1988; Hostetter et al., 1990). Moreover, this protein is structurally and functionally homologous to a complement receptor on mammalian phagocytes and its increase impairs neutrophil phagocytic recognition (Gilmore et al., 1988). Furthermore, glucose binds covalently and irreversibly to the complement C3 inhibiting the attachment of this protein to *Candida* microorganisms, which is the critical determinant of phagocytic recognition (Hostetter, 1990). In addition, it has been also shown that neutrophils of diabetics are less effective than those of healthy patients in killing *C. albicans* (Wilson & Reeves, 1986). It is suggested that the inhibition of *C. albicans* killing by neutrophils in diabetic patients is due to a competition between oxidative killing and sorbitol production, for the electron donor NADPH (Wilson et al., 1987). This supposition was based

on the fact that increased concentration of glucose leads to decreased oxidative killing of *C. albicans* by neutrophils and increased presence of sorbitol (Wilson et al., 1987). In fact, it has been found that hyperglycemic environment inhibits several neutrophil functions such as phagocytosis, adhesion, chemotaxis and intracellular killing (Bohannon, 1998). Most of mechanisms responsible for these dysfunctions are not clear but they can be corrected or at least substantially improved with control of plasma glucose (Bohannon, 1998).

In addition, the use of glucocorticoids has been proposed as a risk factor for VVC (Sobel, 2007). Glucocorticoids are used to treat several human illnesses, such as, autoimmune diseases and cancer and to prevent rejection following organ transplant. However, one of their many undesirable side effects is hyperglycemia caused by increased production of glucose from amino-acids breakdown and resistance to the insulin action (Ferris & Kahn, 2012). Thus, it can be suggested that the high blood glucose levels that contribute for VVC in diabetic patients, have similar action on glucocorticoids users, leading to an increased risk of VVC development. Furthermore, it was shown that several *Candida* species possess a corticosteroid-binding protein and synthesize a fungal ligand that competes with corticosteroids for mammalian glucocorticoid receptors, leading to the speculation that exogenous glucocorticoids may directly mediate *Candida* virulence (Hostetter, 1990). In addition, the use of glucocorticoids suppress several immune reactions (which is one of the main reasons for their use), increasing the vulnerability to fungal infections (Barnes, 2011). Although the incidence of VVC in patients with glucocorticoids therapy is unknown, it has been shown that their use increases the incidence of other *Candida* infections, for instance oral candidiasis (Fukushima et al., 2003).

Genetic predispositions

Since many women develop VVC without any known predisposing factor, some groups of researchers have turned to genetic studies to reveal a possible association of VVC with genetic predispositions (Bradford et al., 2013). American studies have found that African-American women have increased prevalence of VVC (Geiger & Foxman, 1996) and are more likely to be vaginal colonized by *Candida* (Cotch, 1998) compared to Hispanic and white women. Furthermore, black women have decreased vaginal H₂O₂-producing *Lactobacillus* population, which is a protective barrier against *Candida* microorganisms (Antonio et al., 1999). Thus, a genetic-related predisposition has been proposed as a risk factor for VVC in black women. Another genetic predisposition may be a polymorphism in Lewis blood group antigens that leads to the blood group ABO-Lewis non-secretor phenotype. This status is detected with increased frequency among women with RVVC (Chaim et al., 1997; Hilton et al., 1995).

Genetic polymorphisms in innate immunity genes have been also associated with increased susceptibility to VVC and especially to RVVC, including polymorphisms in mannose-binding Lectin (MBL) gene, dectin-1 stop-codon, Interleukin-4 (IL-4) gene and NLRP3 gene (Rosentul et al., 2009). *Candida* components are initially recognized by the innate

immune system through recognition receptors, among which dectin-1 and MBL. Dectin-1 is a C-type lectin receptor expressed on the cell-membrane of innate immune cells, that amplifies TLR-induced cytokine production (e.g. IL-17, IL-6 and IL-10) (Gow et al., 2007). It was detected a mutation in an early stop-codon of dectin-1 gene that leads to the loss of the last nine aminoacids of the CDR (carbohydrate recognition domain). This mutation results in the loss of β -glucan recognition and impaired cytokine response, particularly of pro-inflammatory cytokine IL-17 (Brown, 2006). This mutation has been described in a family of patients with RVVC and onychomycosis (Ferwerda, 2009) and seems to have no correlation with susceptibility to systemic candidiasis (Plantinga et al., 2009). Regarding MBL, it is a circulating protein that binds strongly mannose and *N*-acetyl-glucosamine residues on the surface of *Candida* microorganisms, activating the complement system and promoting opsonization (Neth et al., 2000). Reduced levels of MBL and increased occurrence of genetic polymorphisms in the MBL gene were found in Chinese and Latvian women with RVVC (Babula et al., 2003; Giraldo et al., 2007; Liu et al., 2006). The decrease in circulating MBL concentrations has been shown to correlate with mutations in exon 1 of MBL gene, specially the substitution of an adenine for a guanine in codon 54, which results in impaired assembly and stability of the final MBL protein (Babula et al., 2003; Lipscombe et al., 1995; Sumiya et al., 1991). The stimulation of *Candida* recognition receptors induces the production of several cytokines, among which the anti-inflammatory IL-4 and the pro-inflammatory Interleukin-1 β (IL-1 β). IL-4 inhibits anti-*Candida* activity of Nitric Oxide (NO), which is an important effector molecule of the innate immune defense against *Candida* (Cenci et al., 1993). Thus, high production capacity of IL-4 is associated with immunosuppressive effects. It has been shown that a polymorphism in the promoter region of the IL-4 gene, consisting of a cytosine to thymine variation (C589T), leads to an increased production of IL-4 by immune cells (Burchard et al., 1999; Rosenwasser et al., 1995). The occurrence of this IL-4 gene polymorphism has been correlated with high prevalence of RVVC in some studies (Babula et al., 2005; Liu et al., 2006). Furthermore, higher concentrations of vaginal IL-4 and lower vaginal NO concentrations has been reported in patients with RVVC than in healthy women (Rosenthal et al., 2009). Regarding IL-1 β , this cytokine is processed and activated by a complex called inflammasome, which contains the NLR receptors NLRP3, ASC and caspase-1 (Rosenthal et al., 2009). Polymorphisms of the NLRP3 gene have been described and seem to have a possibly relationship with RVVC, however this potential association has not been completely clarified (Lev-Sagie et al., 2009).

Behavioral factors

Oral contraceptive pills

The use of oral contraceptive pills (OCP) has been proposed as a risk factor for VVC by some authors. However, the recognition of this contraceptive method as a risk factor is still controversial because epidemiologic data are contradictory. In fact, some studies reported higher incidence of VVC (39–58% versus 20–38%) (Cetin et al., 2007; Egbe et al., 2011; Neerja

et al., 2006) and vaginal colonization by *Candida* (58–69% versus 35–40%) (Apalata et al., 2014; Oviasogie & Okungbowa, 2009), in women taking OCP than in patients not using this method. However, other investigators did not found an association between OCP use and vaginal colonization or presence of VVC (Andrioli et al., 2009; Reed et al., 2000; Sobel et al., 2004). Nevertheless, some authors report that although they have not found increased sporadic VVC in women taking OCP, they found an increased prevalence of RVVC in these women, especially in long time OCP users, proposing the use of OCP as a predisposing factor for RVVC (Rylander et al., 2004; Spinillo et al., 1993, 1995).

The use of OCP causes anovulation, with the subsequent absence of estrogen and progesterone peaks, usually present in the normal menstrual cycle. However, OCP add constant levels of hormones that act in the host and *Candida* microorganisms, possibly contributing to VVC. Almost all OCP are combinations of synthetic estrogens and progestins. The most used estrogens are ethinyl estradiol and mestranol and the most used synthetic progestins are norethindrone, norethynodrel, etynodiol and norgestrel (Guyton & Hall, 2006). Some authors believe that hormonal effects that contribute to VVC in pregnancy are similar to those that act in women taking OCP, especially OCP containing a high hormonal dose (Bauters et al., 2002). Thus, the use of OCP may increase vaginal glycogen consequently increasing the availability of nutrient carbohydrates that support *Candida* growth (Reed, 1992). Moreover, OCP use may also increase *Candida* adhesion to vaginal epithelium by *Candida* hormone-receptors stimulation and hyphal forms induction (Cheng et al., 2006; Reed, 1992). In fact, Cheng et al. (2006) detected a similar effect of synthetic ethinyl estradiol and human β -estradiol on the increase of *C. albicans* hyphal forms. Furthermore, OCP use may also influence immune responses resulting in decreased vaginal resistance to *Candida*. Levels of lactoferrin and immunoglobulins (IgA and IgG) have been shown to be suppressed in women taking OCP for the duration of hormone exposure (Fleetwood et al., 1984). In addition, a study with women taking injectable hormonal contraceptives (DMPA – depot medroxyprogesterone acetate) found that this hormonal contraception compromises the vaginal barrier to infection (Miller et al., 2000). The hormonal dependency of VVC is well accepted in pregnancy however there is not a consensual acceptance that OCP use is a risk factor for VVC.

Intrauterine device

The use of intrauterine device (IUD) is a highly effective, safe and economic contraception method (Demirezen et al., 2005). It is a very popular way of preventing pregnancy used by millions of women worldwide and the most preferred mean of contraception in women of developing countries (Abasiattai et al., 2008; Lal et al., 2008). The IUDs are available in several shapes and sizes and are made up of a variety of materials ranging from copper to all plastic (Pruthi et al., 2003). The insertion of an IUD stimulates foreign body response or inflammatory reaction, which cause cellular/biochemical alterations in the endometrial mucosa and cervical mucus, leading to the contraceptive effects

(Lal et al., 2008; Pruthi et al., 2003). IUDs that constantly release small amounts of hormones into the uterus are also available (Lal et al., 2008; Pruthi et al., 2003). Despite being effective in preventing fertilization, some studies have shown that IUD use increases the risk of complications such as pelvic inflammations and menstrual cramps (Pruthi et al., 2003; Lee et al., 1983). More importantly, IUDs use also increases the risk of infections associated with microbes' colonization on these implanted devices, including VVC (Lal et al., 2008; Pruthi et al., 2003). Although some researchers have not found an association between VVC and IUD use (Agarwal et al., 2004; Hodoglugil et al., 2000), it has been proposed as an important risk factor for sporadic VVC and recurrent episodes by many others. In fact, several studies report higher IUD use in VVC and RVVC-infected women (13.1–43.8% and 28.1–71.4%, respectively) than in healthy women (2.9–37.6%) (Amouri et al., 2011; Guzel et al., 2011; Spinillo et al., 1995). In addition, some studies also found that VVC incidence and vaginal colonization by *Candida* is higher among IUD users than in non-users (Apalata et al., 2014; Cetin et al., 2007; Demirezen et al., 2005).

It has been suggested that the association of IUD use with VVC episodes is due to the *Candida* adhesion and biofilm formation on the surface of the device (Auler et al., 2010; Chassot et al., 2008). A biofilm is a surface-associated community of microorganisms enclosed in a self-produced matrix that confers resistance to antifungal agents, host defenses and stress (Donlan & Costerton, 2002). Pathogenic fungi can form biofilms on the inert surfaces of several implanted devices, including catheters, prosthetic cardiac valves and IUDs (Douglas, 2003; Pruthi et al., 2003). Chassot et al. (2008) showed that *C. albicans* isolates of patients with VVC can adhere strongly *in vitro* to all parts of an IUD and have high capacity to form biofilm on it. Moreover, these investigators detected high concentration of yeasts on the IUD tail, which is the segment that makes a bridge between external environment, the vagina that is colonized by yeasts and the non-colonized upper genital tract. The presence of a biofilm on the surface of IUD not only protects yeasts from the action of antifungal agents as it contributes to the microorganism's persistence, constituting a source of infection or reinfection (Auler et al., 2010; Pál et al., 2005). Some studies have shown that IUDs recovered from patients suffering from VVC or RVVC are infected by *Candida* biofilms composed of a single or multiple species (including bacteria), indicating that the presence of the biofilms on the device possibly contributed to the infection (Auler et al., 2010; Lal et al., 2008; Pruthi et al., 2003). Furthermore, Auler et al. (2010) verified through electron scanning microscopy that most microorganisms of biofilms formed on IUDs of patients with RVVC had been present on those surfaces for possibly a long time, suggesting that biofilms served as reservoirs of yeasts. These investigators also observed a larger mass of biofilm on the device of the patient with its longer use. Pál et al. (2005) also reported that biofilm formation on IUDs has a relation to duration of use and Demirezen et al. (2005) showed that women with less than 1 year of IUD use have decreased risk of developing device-related VVC. Besides acting as a *Candida* reservoir, it is also proposed that IUDs may affect the cervico-vaginal microflora by

reducing lactobacilli, an important vaginal barrier against *Candida* organisms (Demirezen et al., 2005). Furthermore, the use of IUD may predispose the cervico-flora for the more pathogenic *Candida* hyphae form and modifies or destroys the cervical mucus, which serves as a powerful barrier for ascending infections (Demirezen et al., 2005).

The association of IUD use with infections as VVC has led several authors to suggest a controlled and limited use of the IUD and a regular check of possible infections during its usage to prevent serious complications (Auler et al., 2010; Demirezen et al., 2005; Guzel et al., 2011). The IUD removal is required in many infected patients, especially in women with RVVC, in which the removal of the device has been shown to be essential to avoid the relapses (Auler et al., 2010; Parewijek et al., 1988).

Spermicides/condoms

The use of spermicides and/or condom has been associated with increased risk of VVC by some authors. Some epidemiologic data report higher incidence of VVC in condom users (40.2%) than in non-contraceptive users (37.9%) (Cetin et al., 2007) and higher use of spermicides in patients with VVC (56.3%) than in healthy women (13.5%) (Geiger & Foxman, 1996). Furthermore, it has been reported higher use of spermicide and/or condom in RVVC patients than in healthy women (21.4% versus 10%) (Amouri et al., 2011) and higher condom use in patients colonized by *Candida* than in non-colonized women (11% versus 5%) (Eckert, 1998).

It is proposed that the association between spermicides/condoms and VVC development may due to the spermicidal compound nonoxynol-9 (N9), which is the active component of many spermicidal preparations used in spermicides (creams and foams) and condoms (McGroarty et al., 1990). N-9 is a nonionic detergent that immobilizes sperm by disrupting the cell membrane and acts similarly on several pathogens, mainly bacteria and viruses, decreasing the risk of acquiring several sexually transmitted diseases (North, 1988). However, this compound upsets the ecological balance of vaginal microbiota because it also inhibits vaginal commensal organisms, specifically *Lactobacillus* species (Hooton et al., 1994; Watts et al., 1999), which represent the dominant vaginal defense mechanism against *Candida*. Furthermore, some studies showed that N-9 causes vaginal epithelium disruption, leading to genital irritation (Niruthisard et al., 1991; Roddy et al., 1993). In addition, McGroarty et al. (1990) reported that *Candida* microorganisms have the ability to metabolize the spermicidal compound N-9, which may result in altered surface characteristics, increasing *Candida* adhesion to epithelial tissues. These investigators showed that the incorporation of N-9 in adhesion assays with vaginal isolates of *C. albicans*, *C. parapsilosis* and *C. tropicalis* increase their adhesion to human epithelial cells. They proposed that N-9, which is a detergent molecule composed of a polar head and a hydrophobic tail, may enhance the adhesion of *Candida* organisms by increasing their hydrophobicity or epithelial cells hydrophobicity. In fact, it has been shown that the initial attachment of *Candida* cells to biotic and abiotic

surfaces is mediated by *Candida* surface hydrophobicity (Hazen, 1989) and that hydrophobic *Candida* cells are more virulent than hydrophilic ones (North, 1988).

Despite the use of N-9 in condoms appear to be the most probable cause of their association with VVC development, other causes have been also proposed such as, allergic reactions to latex, sensitivity of vaginal epithelial cells to condom and vaginal micro traumas with consequent disturbance of the vaginal ecosystem (Giraldo et al., 2005; Schreiber et al., 2006).

Poor personal hygiene and sexual habits

It is thought that an increase in the number of *Candida* spores in the vaginal environment triggers epithelial invasion possibly contributing to VVC (Ferrer, 2000). Thus, factors that may increase the vaginal blastopore load have been proposed as risk factors for VVC. For instance, an increased *Candida* spore load can have an intestinal reservoir source due to inappropriate personal hygiene (Ferrer, 2000). An Indian study found higher incidence of VVC in women with unsatisfactory genital hygiene (36.0%) than in those with satisfactory personal hygiene (15.6%) (Ahmad & Khan, 2009). Furthermore, an Iranian study reported low education degree in 63% of women with VVC and attributed that result to a possible less observance of genital hygiene in those women (Faraji et al., 2012). In fact, it has been shown that the gut is a possible source of vaginal colonization by *Candida* microorganisms (Holanda et al., 2007). Although it is thought that intestinal tract has a minor role in VVC (Sobel, 2007), an increase in the number of *Candida* spores in vagina promoted by poor genital hygiene, possibly contributes to VVC. However the association between inappropriate intimate hygiene and VVC has to be further studied.

In addition to poor personal hygiene, an increase in *Candida* blastopore load can be also due to exogenous *Candida* acquisition through some sexual practices, including frequent sexual intercourse and receptive orogenital intercourse. These sexual behaviors may be of importance for both primary infection and recurrent VVC episodes. Some studies have found a correlation between high frequency of monthly intercourse and vaginal *Candida* colonization (Eckert, 1998), VVC development (Foxman, 1990) and recurrent episodes (Spinillo et al., 1993, 1995). Moreover, several studies have found that VVC occurs more frequently in the third decade of life and have suggested that this may be due to an increased sexual activity in this age group (Ahmad & Khan, 2009; Ako-Nai et al., 1993; Okungbowa et al., 2003). VVC is not considered a sexually-transmitted disease because it occurs in non-sexually active women and women can be vaginal colonized by *Candida*, but nevertheless, some studies have confirmed *Candida* sexual transmission (Boatto et al., 2007; Schmid et al., 1993). Thus, high frequency of sexual intercourse may lead to an increase of vaginal *Candida* load, possibly contributing to VVC episodes. However, some authors suggest that *Candida* penile-vaginal transmission occurs in only a few cases and that, other factors also contribute to increased VVC risk in women with frequent sexual intercourse. These factors include vulvovaginal micro-lacerations that create conditions suitable for yeast to invade

tissue (Foxman, 1990; Hellberg et al., 1995) and exposition to numerous antigens, antibodies and cytokines secreted in the seminal fluid that might influence women immune reaction to *Candida* (Reed et al., 2000). Furthermore, deposition of semen (alkaline pH) in the vaginal epithelium can lead to an alteration of vaginal microbiota, local mucosa stimulation and introduction of strange bacteria to vaginal environment (Giraldo et al., 2005). Besides frequent sexual intercourse, also receptive oral intercourse has been proposed as a risk factor of both sporadic and recurrent VVC infections. In fact, some studies have found that orogenital sex is associated with increased VVC incidence (Bradshaw et al., 2005; Reed et al., 2000; Rylander et al., 2004) and with repeated episodes of VVC (Hellberg et al., 1995; Markos et al., 1992; Reed et al., 2003). It has been shown that *Candida* organisms can be transmitted from the oral cavity (Reed et al., 2000), in which they are present in one-third to one-half of adult population, leading to increased vaginal load. It has been also proposed that saliva may facilitate *Candida* adherence and growth through moistening and irritation of the vaginal mucosa (Geiger & Foxman, 1996; Reed et al., 2000) or by changing the local immune responses (Reed et al., 2000).

Other sexual practices such as receptive anal sex (Bradshaw et al., 2005; Hellberg et al., 1995), early sexual debut, casual sex partners (Hellberg et al., 1995), new sex partner (Spinillo et al., 1993), high number of lifetime sex partners (Spinillo et al., 1995) and sex during menses (Hellberg et al., 1995) have been also positively associated with VVC episodes by some studies, especially in patients with RVVC. However some researchers have not found increased risk of infection in women with some of these sexual behaviors (Barbone et al., 1990; Foxman, 1990; Reed et al., 2000). Thus, the influence of these and other sexual habits needs to be further studied for a better understanding of their role in VVC development.

Habits of clothing and hygiene

Some habits of feminine hygiene and clothing have been proposed as potential behavioral factors that predispose to VVC and RVVC. The use of tight or poorly ventilated clothing and/or use of synthetic underwear have been associated with VVC development by some authors. A Brazilian study found higher incidence of VVC in women who use tight and/or synthetic underwear (65.8%) than in women who not use those type of clothing (39.1%) (Holanda et al., 2007). Furthermore, an Italian study found a more common use of synthetic underwear in RVVC patients (36.8%) than in patients with no recent history of VVC (26.0%) (Corsello et al., 2003). In addition, an American study with RVVC-infected patients found that women who used pantyhose were more than twice as likely to report an episode of VVC during the study period, than women who not used that tight-fitting garment (40.1% versus 16.6%) (Patel et al., 2004). Thus, some authors propose that the use of well-ventilated and cotton underwear could be of value in preventing VVC; however, not all researches found an association between the use of tight clothing or synthetic underwear and VVC development (Foxman, 1990; Patel et al., 2004).

The possible association between poorly ventilated clothing and/or synthetic underwear and VVC has been attributed to increased perineal moisture levels and temperature which may contribute to *Candida* proliferation (Sobel, 2014). It has also been suggested that synthetic underwear may cause local allergy and hypersensitivity reactions, altering the vaginal milieu and possibly contributing to VVC (Neves et al., 2005). In fact, feminine hygiene practices that may trigger local hypersensitivity or allergic reactions as the use of pantyliners and vaginal douching have been also proposed as behavioral risk factors for VVC development. Patel et al. (2004) found higher incidence of VVC in patients that use pantyliners than in women who not use them (46.6 versus 14.3%). Barousse et al. (2004) found higher vaginal colonization by *Candida* in women that use solutions for vaginal douching (26%) than in women who not have this habit (20%). Furthermore, some studies found that vaginal douching is more common among VVC and RVVC patients than in healthy women (Bradshaw et al., 2005; Corsello et al., 2003; Spinillo et al., 1993). It is believed that vaginal douching not only introduces exogenous substances that may cause allergic reactions and pH alteration, but also promote mechanical cleaning of commensal bacteria, affecting the ecological balance of the vaginal cavity (Ness et al., 2003). In addition, it has been recommend for women to avoid excessive washing of vulval area and potential irritants such as perfumed soaps, bubble baths, powders or vaginal sprays, which also affect the vaginal microbiota (Watson & Calabretto, 2007).

Conclusion

Changes in the vaginal environment are generally required for the alteration of the opportunistic *Candida* organisms from commensal to pathogenic. Management of VVC involves the identification and control of risk factors that may predispose to infection. Having a risk factor for VVC increases the chance of getting the infection, but does not always lead to it. Also, the absence of any known risk factor does not necessarily avoid VVC development. Despite research advances, there are still a number of mechanisms involved in VVC and RVVC development that need to be clarified. Thus, the high incidence of VVC, its negative consequences and the increase of antifungal failure in its treatment, make it crucial to further increase our knowledge on *Candida* vaginal pathogenicity and its underlying mechanisms. Studies in this area will lead to a better understand of VVC, contributing to the identification of new targets for more efficient therapeutic approaches against this clinically relevant fungal infection.

Declaration of interest

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