Vulvovaginal candidiasis: epidemiology, microbiology and risk factors

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Vulvovaginal candidiasis: epidemiology, microbiology and risk factors

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 non-physiological 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 devices, spermicides, 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.

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 and 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 and Stafford, 1983). There is a balance
between Candida organisms and vaginal defense 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 and Fries, 2010). VVC can be classified into uncomplicated and complicated cases, a classification that has been adapted over the years. Uncomplicated VVC is characterized by fewer than four episodes per year with mild to moderate severity caused by C. albicans in apparently healthy women (Achkar and Fries, 2010). 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 presence of recognized risk factors (e.g. pregnancy, diabetes and immunosuppression) are also classified as complicated VVC (Achkar and Fries, 2010; Pappas et al., 2009).

The clinical symptoms of VVC are nonspecific and can be associated with a variety of other vaginal diseases, such as bacterial vaginosis, trichomoniasis and gonorrhea (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 antifungal 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 and are poorly effective in VVC caused by C. glabrata (Fidel et al., 1999b). In fact, complicated infections such as those caused by NCAC species or in 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 (Shirley, 2006; Sobel, 2007). VVC is also associated with
considerable direct and indirect economic costs (Foxman et al., 2000), enhanced susceptibility to human immunodeficiency virus (HIV) infection (Rottingen et al., 2001) and a potential relationship with preterm birth (Roberts et al., 2011). 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 (Shirley, 2006). Therefore, prevention, early diagnosis and prompt treatment of VVC, especially among risk groups, are essential to avert the complications.

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

Vulvovaginal candidiasis 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.,
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Microbiology of VVC

The most common Candida species associated with VVC are *Candida albicans*, *Candida 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 to 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 non-*Candida albicans* Candida (NCAC) species. In American (Richter et al., 2005; Vermitsky et al., 2008), 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 species identified in women with the disease (70.0 to 89.0%), followed by *C. glabrata* (3.4 to 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 to 90.4%), however in some Asian and African studies...
(Turkey (Cetin et al., 2007), India (Ahmad and 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 to 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 and Sobel, 1994; Otero et al., 1998; Saporiti et al., 2001; Spinillo et al., 1997b), however in recent years a change in epidemiological trends has been observed. In fact, most studies published during the last years report incidence of *C. albicans* below 85% and in some countries even below 50% (Table 2). Thus, the proportion of NCAC species causing VVC appears to be increasing, in particular *C. glabrata*. The increasing detection of NCAC species has been attributed to several factors including the widespread and inappropriate use of antifungal treatments (self-medication and prolonged antifungal therapy). These factors 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 (Vermitsky et al., 2008). Thus, the increase of these groups of patients, particularly of elderly and diabetic patients, possibly also contribute to the increasing identification of NCAC species in women with VVC (Vermitsky et al., 2008).

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 non-azole 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 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 to 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 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 and 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 which 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, \textit{EPA1}, \textit{EPA6} and \textit{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 \textit{C. glabrata}, with the highest value exhibited by \textit{EPA1}. These investigators also detected all \textit{ALS} genes in RHVE infected with \textit{C. albicans}, with the highest expression exhibited by \textit{ALS3} and \textit{ALS6} and the lowest by \textit{ALS7}. Interestingly, in RHVE co-infected with \textit{C. glabrata} and \textit{C. albicans} the \textit{EPA} genes were down-regulated or absent and \textit{ALS} genes were generally similar to those observed in single infection, with the exception of a highly increase in \textit{ALS3} expression. These results suggest that probably \textit{ALS3} but not \textit{EPA} adhesins are associated with the enhanced RHVE invasion by \textit{C. glabrata} observed in the presence of \textit{C. albicans}. In \textit{C. parapsilosis} were identified eleven genes for putative cell wall adhesins-like proteins (Butler et al., 2009) and at least three \textit{Als} proteins were identified in \textit{C. tropicalis} and \textit{C. dubliniensis} (Hoyer et al., 2001). However, in most NCAC species the function of identified adhesins was still poorly studied.

- **Biofilm formation**

Adhesion of \textit{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 \textit{Candida} species as it confers unique phenotypic characteristics compared to their planktonic counterpart cells including significant resistance to antifungal agents, host defense mechanisms and physical and chemical stress (Donlan and 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 and 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 and 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 \textit{Candida} biofilms to antifungal therapy are not fully
understood. It is accepted that the antifungal resistance of biofilms is a complex multi-factorial 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 mono-species 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 Gardnerella 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 and Spacek, 2004; Exim 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; McClelland et al., 2009; Sobel and 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, ten *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 (pH ≤ 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, SAP2 and SAP3 has a strong and specific correlation with VVC (Lian and Liu, 2007; Naglik et al., 2003; Schaller et al., 2003).

Phospholipases hydrolyse 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 (Ghanam, 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 RHVEs 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 and Vinces, 2005; Silva et al., 2012). 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 Saps 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 (*HWP1*) 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 non hyphae producer, the investigators detected an up-regulation of *HWP1* 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 and 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 et al., 1987, 1988, 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 non-pregnant 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 and 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 non-pregnancy between 7.7 and 31.0%. A Nigerian study (Kamath et al., 2013) reported an incidence of VVC in pregnancy and non-pregnancy 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 and 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 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 non-pregnant 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 and 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; Tihaldi et al., 2009). HRT is used to counter adverse the consequences associated with the decrease in hormones secretion in post-menopause, 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 to 29.4%) than in post-menopausal women without HRT (4 to 12.6%) (Bauters et al., 2002; Dennerstein and Ellis, 2001; Spiniello et al., 1997a). All authors agree that the high hormonal levels of pregnant women and of those receiving HRT are the mains responsible for the relation of
those conditions with VVC. However, emotional stress, suppression of immune system and eating habits of sugar rich containing 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 of insulin action and increase of sodium excretion by the kidneys (Guyton and Hall, 2006). The main estrogen in pre-menopause is β-estradiol and in post-menopause is estrona, 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 and 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 of 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 and 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 and 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 and Witkin, 1989, 1991) and that estradiol significantly reduces antimicrobials production (HBD2 and elafin) (Wira and 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 been also 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 and Feldman, 1989; Wagner and 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 and 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 *HWP1*. These investigators also showed that *C. albicans* colonization and invasion of RVHE 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 and 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 transition or blocking the inverse change. Furthermore, is 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 trash) 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 and 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. Although some studies failed to show association between antibiotic treatment and occurrence of VVC (Barbone et al., 1990; Geiger and Foxman 1996), many others found higher VVC prevalence in women that have taken antibiotics than in those who have not been treated with antibiotic agents (Table 4). Table 4 highlights the most relevant reports that studied the prevalence of VVC in women treated and not treated with antibiotics and/or incidence of antibiotic use in women with and without VVC. Studies in India (Ahmad and 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% vs. 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% vs. 0.7-11.9%) (Table 4). Thus, all these studies propose that antibiotic treatment is a risk factor for VVC. These epidemiologic data may have been influenced not only by the location where the study was made but also by type of antibiotic agents used and the duration of antibiotic treatment. In fact, the duration of antibiotic use is directly related to an increase of VVC prevalence (Spinillo et al., 1999) and 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% vs. 18 – 21%), and a subsequent development of VVC after the antibiotic intake in 20 – 22% of initially colonized women (Pirotta and 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; Hamad et al., 2006). 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 (Cribby et al., 2008). In healthy asymptomatic women, more than 95% of the total organisms present are Lactobacillus species which are thought to be one of the first lines of defense against VVC (Eschenbach, 1986). Auger and Joly (1980) found low numbers of lactobacilli in vaginal cultures obtained from women with VVC than from women without the disease. 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 and Barbés, 2000; Boris et al., 1998). 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 et al., 1998; Osset et al., 2001). 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. Lactobacilli also produce bacteriocins and bacteriocin-like substances that inhibit Candida growth. For instance, Okkers et al. (1999a) showed that a bacteriocin-like peptide (pentocin TV35b) isolated from Lactobacillus pentosus inhibits C. albicans growth. Moreover, most Lactobacillus strains release hydrogen peroxide (H₂O₂) and fatty acids that inhibit Candida overgrowth and invasive hypal formation (Boris and Barbés, 2000). Hillier et al. (1992) showed that women colonized by H₂O₂-producing Lactobacillus, are less likely to have symptomatic VVC than women with non-H₂O₂ producing Lactobacillus. Furthermore, Boris and Barbés (2000) detected lower presence of H₂O₂-producing population in women with VVC than in healthy women. In addition, Xu and Sobel (2003) showed that L. delbrueckii strains, which produce the largest amounts of H₂O₂, have the strongest and fastest growth inhibition effect against C. albicans, amongst several Lactobacillus strains isolated from vaginas of healthy women. The vaginal microbiota is also rich in lactic acid-
producing *Lactobacillus*, which provide adequate acidity to the vaginal environment (pH around 4-4.5), hindering the proliferation of most pathogens (Hillier et al., 1993). However, *Candida* species are exception because they proliferate in acidic environment. Patients with VVC have normal vaginal pH, while patients with other vaginal infections such as bacterial vaginosis, trichomoniasis and atrophic vaginitis have higher vaginal pH (>4.5) (Ahmad and Khan, 2009; Neerja et al., 2006). Moreover, it has been detected higher vaginal pH in women with *C. glabrata* infection than in those with VVC due to *C. albicans* (Ahmad and Khan, 2009; Tarry et al., 2005). In fact, a more alkaline pH such 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 post-menopausal women (Fan et al., 2008b; Holland et al., 2003). In these women the vaginal pH is enhanced by the hormonal depletion that leads to a reduction of glycogen production in vagina, which is essential to the vaginal bacteria anaerobic metabolism of glycogen to acid lactic (Boskey, 2001). It is known that the pH of the host niche is a significant environmental signal that determines the biological response and survival of pathogens (De Bernardis et al., 1998). 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 (Muhschlegel and Fonzi, 1997). Studies *in vitro* found that both genes encode a function required for *C. albicans* morphogenesis. Deletion of *PHR1* result in 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; Muhschlegel and 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.

Several studies have shown that vaginal *Lactobacillus* could provide colonization resistance and prevent germination of *Candida* organisms (Boris et al., 1998; Hilton, 1992; Osset et al., 2001). Thus, it is thought that
the suppression of this protective barrier due to antibiotic use may lead to VVC development. However, some studies found that the absence of vaginal lactobacilli does not increase acquisition of VVC in the absence of antibiotic intake, suggesting that the interaction between antibiotic use and Candida infection involves other mechanisms besides bacterial suppression (Hawes et al., 1996; McClelland et al., 2009). Furthermore, some studies reported that the use of vaginal or oral probiotic Lactobacillus does not prevent VVC after antibiotic treatment (Rathod et al., 2012; Spinillo et al., 1992). However, contradicting these results, some studies reported that probiotic Lactobacillus administration slows or prevents C. albicans growth (Hamad et al., 2006; Jeavons, 2003; Wagner and Johnson, 2012). Although the mechanisms involved in VVC promotion by antibiotic use are still not entirely clear, it is accepted that it is an important risk factor for the development of VVC.

- **Immunosuppression**

  All Candida infections are dependent on the host and therefore its immune response is crucial in the host-pathogen interaction (Richardson and 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 and 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; Rottingen 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 anti-retroviral 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).

Protection against potential pathogens in the female reproductive tract is provided by the innate and adaptive immunity systems. Innate immune system is recognized as the first line of defense, preventing and controlling invasion of pathogens (Wira et al., 2005a). 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 and 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 and 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-bound microorganisms (Babovic-Vuskancvic 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 surface area ranges from 65 to 108 cm² (Barnhart, 2006) and is lined by multiple layers of squamous non-keratinized epithelial cells (Farage and Maibach, 2005), which are the primary cells that Candida interact with at the vaginal mucosa. Vaginal ECs not only provide a physical barrier against pathogen entry in the lower reproductive tract but also recognize and process antigens. Furthermore, 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. It has been shown that vaginal ECs from women with RVVC exhibit reduced in vitro anti-Candida activity comparatively to ECs from healthy women (Barousse et al., 2001, 2005). Although this yet to be demonstrated in vivo, these results suggest that vaginal ECs provide an innate host resistance mechanism against Candida and that their reduced anti-Candida activity may contribute to RVVC.

Candida recognition by the innate immunity triggers a sequence of events, including cytokine and chemokine production, that lead to the activation of adaptive immunity. While the innate immune system is activated immediately after pathogen recognition, it takes several days for the adaptive system becomes functional. The adaptive immunity encompasses fungal-specific defense 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). Vaginal T-cells have been characterized and quantified in human vagina and cervix, being reported that there are about 240 T-cells per mm² of vaginal epithelial tissue (Ildgruben et al., 2003). 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 though that the vaginal mucosa is an immunocompetent tissue capable of a compartmentalized cell-mediated response independent of systemic cell-mediated immunity (Fidel et al., 1996; Fidel, 2005). Cell-mediated immunity through Th1-type T-cells response has been considered an important immunological defense mechanism against mucosal Candida infections (Fidel et al., 1997; Romano 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 and 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. (2011a) 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 and 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 and 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 and 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 and 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 non-immune 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 and 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 non-diabetics. 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 been also 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. (2002a) 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). These results showed that diabetic patients have less cure rates with fluconazole than non-diabetics, even with the same causative species. In diabetic patients, higher mycological cure of C. glabrata VVC has been achieved when they are treated with boric acid vaginal suppositories instead of oral fluconazole (Ray et al., 2007).

Diabetes mellitus is a metabolic disturbance caused by either the pancreas not producing enough insulin (type 1 DM) or the cells of the body not responding properly to the insulin produced (type 2 DM). As insulin regulates the uptake of glucose from the blood into cells of the body, diabetic patients have elevated plasma levels of glucose (Masharani and German, 2011). It has been proposed that hyperglycemia is the
major cause of increased susceptibility of diabetic patients to VVC. In fact, the risk of *Candida* vaginal colonization and symptomatic VVC is minimized in diabetic women with well-controlled glucose levels (De Leon et al., 2002; Goswami et al., 2000). Furthermore, non-diabetic patients with RVVC describe an association between excess of refined sugars consumption and exacerbation of symptomatic VVC (Donders et al., 2002). 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 and Segal, 1988; Segal et al., 1984). It is though 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 and 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).

It is though that, patients with hyperglycemia due to other factors than DM are also at high risk of developing VVC. For instance 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 and 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 and 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 amicoacids 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 onycomycosis (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, specifically 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-1β (IL-1β). 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 (Rosentul et al., 2009). Regarding IL-1β, this cytokine is processed and activated by a complex called inflammasome, which contains the NLR receptors NLRP3, ASC and caspase-1 (Rosentul 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% vs. 20-38%) (Cetin et al., 2007; Egbe et al., 2011; Neerja et al., 2006) and vaginal colonization by Candida (58-69% vs. 35-40%) (Apalata et al., 2014; Oviasogie and 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 and 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 and 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 acceptation 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 to 43.8% and 28.1 to 71.4%, respectively) than in healthy women (2.9 to 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 and 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 leaded 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; Parewijk 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 and Foxman, 1996). Furthermore, it has been reported higher use of spermicide and/or condom in RVVC patients than in healthy women (21.4% vs 10%) (Amouri et al., 2011) and higher condom use in patients colonized by *Candida* than in non-colonized women (11% vs 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 though 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 and 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; Spinillo et al., 1992), 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 and 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; Spinillo et al., 1992). 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; Spinillo et al., 1992), 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 and 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% vs.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 been also 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 vs. 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 and Calabretto, 2007).

**Concluding remarks**

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 negatives 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.

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Declaration of interest

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### 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

<table>
<thead>
<tr>
<th>Country</th>
<th>Year(s) of study</th>
<th>Symptomatic women</th>
<th>Asymptomatic women</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number With VVC (%)</td>
<td>Number Colonized (%)</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>2002</td>
<td>23</td>
<td>43.5</td>
<td>(Rosa and Rumel, 2004)</td>
</tr>
<tr>
<td>Brazil</td>
<td>2005-2007</td>
<td>121</td>
<td>47.9</td>
<td>(Andrioli et al., 2009)</td>
</tr>
<tr>
<td>Jamaica</td>
<td></td>
<td>422</td>
<td>19.6</td>
<td>(Jackson et al., 2005)</td>
</tr>
<tr>
<td>Italy</td>
<td>1996-2005</td>
<td>13014</td>
<td>18.5</td>
<td>(Hibaldi et al., 2009)</td>
</tr>
<tr>
<td>Austria</td>
<td>2000-2004</td>
<td>10463</td>
<td>39.5</td>
<td>(Pauli et al., 2006)</td>
</tr>
<tr>
<td>Greece</td>
<td>2002-2004</td>
<td>4743</td>
<td>12.1</td>
<td>(Grigorou et al., 2006)</td>
</tr>
<tr>
<td>Turkey</td>
<td>2004-2005</td>
<td>569</td>
<td>42.2</td>
<td>(Cetin et al., 2007)</td>
</tr>
<tr>
<td>India</td>
<td>2003-2004</td>
<td>601</td>
<td>18.5</td>
<td>(Mohanty et al., 2007)</td>
</tr>
<tr>
<td>India</td>
<td>2011-2012</td>
<td>300</td>
<td>17.7</td>
<td>(Vijaya et al., 2014)</td>
</tr>
<tr>
<td>Israel</td>
<td></td>
<td>208</td>
<td>35.5</td>
<td>(Dan et al., 2003)</td>
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<tr>
<td>Tunisia</td>
<td>2006-2008</td>
<td>481</td>
<td>48.0</td>
<td>(Amouri et al., 2011)</td>
</tr>
<tr>
<td>Nigeria</td>
<td></td>
<td>902</td>
<td>57.3</td>
<td>(Okanghowsa et al., 2003)</td>
</tr>
<tr>
<td>Australia</td>
<td>2003-2004</td>
<td>342</td>
<td>42.7</td>
<td>(Bradshaw et al., 2005)</td>
</tr>
</tbody>
</table>
Table 2 — Epidemiologic studies published from 2003 to 2014, concerning the distribution of Candida species in women with VVC.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year(s) of study</th>
<th>No. of subjects</th>
<th>C. albicans (%)</th>
<th>C. glabrata (%)</th>
<th>C. tropicalis (%)</th>
<th>C. parapsilosis (%)</th>
<th>C. krusei (%)</th>
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Table 3 - Incidence of VVC in pregnant and non-pregnant women reported in studies published from 2002 to 2014

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<tr>
<th>Country</th>
<th>Year(s) of study</th>
<th>Pregnant women (% with VVC)</th>
<th>Non-pregnant women (% with VVC)</th>
<th>References</th>
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Legends to figures

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).

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

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

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

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 x 1000 and the scale bars correspond to 20 µm.
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).

60x18mm (300 x 300 DPI)
Figure 2 – Host–related risk factors for vulvovaginal candidiasis and respective effects.
60x28mm (300 x 300 DPI)
Figure 3 – Behavioral risk factors for vulvovaginal candidiasis and respective effects.

60x26mm (300 x 300 DPI)
Figure 4 – Scanning electron microscopy images of C. albicans ATCC biofilms formed in absence (A) or presence (B) of 2μM of progesterone. Original magnification was x 1000 and the scale bars correspond to 20μm (Alves et al., 2014a).
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 x 1000 and the scale bars correspond to 20µm.

60x13mm (300 x 300 DPI)