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Review

Vulvovaginal candidiasis: Agents and its virulence factors

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ABSTRACT

Candida species are the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. *Candida* spp. is the second most common vaginal infection after the bacterial vaginosis. A number of risk factors have been identified in a variety of studies. Vulvovaginal candidiasis (VVC) risk factors have been identified, including genetic, intermediate age, pregnancy, uses of contraceptive pills, frequent sexual intercourse, uncontroled diabetes mellitus, contraceptive devices, and antibiotics. *C. albicans* is both a commensal and a pathogen that can exhibit yeast, pseudohyphae and hyphae morphology. The second most common species to cause VVC is *C. glabrata*, which occurs in about 5% of infections. Treatment of *Candida* spp. infections can include azoles (fluconazole) but rarely requires amphotericin B with 5 fluocytosine. This review summarizes all known clinical and experimental information about VVC with comparisons between *C. albicans* and non-Candida albicans Candida (NCAC) species and their veriulence factors.

Keyword: *Candida* spp., vulvovaginal candidiasis, biofilm formation, filamentous, antifungal.

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INTRODUCTION

There are over 150 known species of *Candida*, but only a limited number of these species are isolated from patients as infectious agents. Budding is production of small outgrowth (bud) from mother cell. The bud increases in size while it is still attached to the mother cell and eventually breaks off and form a new individual. *Candida* spp. can also produce hyphae and pseudohyphae in tissues, but this behavior is a function of both species and the involved organ. Identification of the individual species is based on standard sugar assimilation and morphological techniques (Center for Disease Control and Prevention, 2010).

The more important pathogenic species are *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* (Center for Disease Control and Prevention, 2010; Hajjeh et al., 2004; Pfaller and Diekema, 2004; Pfaller et al., 2006; Tortorano et al., 2006). The major pathogenic species of *Candida* do differ in their frequency, virulence, and clinical associations. *C. albicans* is the most common infectious agent. Between 85 and 95% of *Candida* spp. isolated from the vagina belong to the species *C. albicans* (Center for Disease Control and Prevention, 2010; Landers et al., 2004). The reminders are non-albicans spp., the most common of which is *C. glabrata*. In many part of the world, NCAC isolates notably *C. glabrata* effect 10 to 20% of women (Corsello et al., 2003; Buscemi et al., 2004). Of note, *C. glabrata* is the one species that does not produce hyphae or pseudohyphae.

EPIDEMIOLOGY

Vulvovaginal candidiasis is the second most common cause of vaginitis after bacterial vaginosis and symptomatic vaginitis is higher during pregnancy (CDC, 2010). *Candida* spp. are found in almost 24% of pregnant

and in 17% of non-pregnant women (Al-akeel et al., 2013) and *C. albicans* infection occurs in the vast majority (80 to 90%) of diagnosed true VVC cases (Boselli et al., 2004). Among the *Candida* spp. causing infections*, C. albicans, C. glabrata, C. parapsilosis,* and *C. tropicalis* account for 80 to 90% of fungal isolates encountered worldwide (Center for Disease Control and Prevention, 2010; Paulitsch et al., 2006). *C. glabrata* was also reported as the second most common agent of VVC, however, the increasing incidence of NCAC in VVC cases is not yet well established (Esmaeilzadeh et al., 2009). There is also accumulated evidence that mere *Candida*-vaginal colonization may predispose for preterm birth, and clotrimazole prophylactic-treatment during pregnancy significantly reduces the disease-incidence (Czeizel et al., 2004).

Although *C. albicans* is the most common cause of VVC (Paulitsch et al., 2006), the extensive use of azole antifungal drugs is postulated to have promoted the shifting of vaginal colonization and selection of more naturally resistant species, such as *C. glabrata* (Mathema et al., 2001; Fidel Jr et al., 1999).

Virulence factors of *Candida* spp.

Colonization of the vagina requires yeast adherence to vaginal epithelial cells*. C. albicans* is both a commensal and a pathogen that can exhibit yeast, pseudohyphae and hyphae morphology. These morphological transitions promote colonization and invasion at different anatomical sites. They also occur in other *Candida* spp. The yeast form is associated with dissemination and the hyphal form with adhesion, tissue invasion, and proteolytic activity. *C. albicans* strains seem to adhere equally well to both exfoliated vaginal and epithelial cells (Sobel, 1985).

Enzymes

C. albicans can produce and release several hydrolytic enzymes such as proteinases, phospholipases, and lipases in culture media. These enzymes play a key role in fungal metabolism and may be involved in fungal pathogenesis, causing damage to the host tissues and providing nutrients in a restricted environment.

Secreted aspartyl proteinases (SAP) elaborated by pathogenic *Candida* spp., have been identified in vaginal secretions (proteinase secretion) in women with symptomatic vaginitis but not in those with asymptomatic colonization (Al-Hedaithy, 2002). Several genes that govern proteinases production (SAP1, SAP2 and SAP3) have been cloned, and a strong correlation exists both *in vitro* and in experimental vaginitis between gene expression, aspartyl proteinase secretion, and the ability to cause disease (Schaller et al., 2003; Taylor et al., 2005).

Biofilm formation

Candida spp. can grow embedded in extracellular matrix (ECM) that is composed of carbohydrates and proteins and is referred to as biofilm. The biofilm is attached to a surface which can be biotic (the human body) or abiotic (implanted devices, medical devices such as urinary catheters and intravascular catheters e.g. intrauterine device). The consequences for health include biofilm resistance to antimicrobial agents, device failure, resistance to host defenses, and arise of persistent infections (Donlan, 2001; Kojic and Darouiche, 2004).

Biofilm generating strains of *C. albicans, C. glabrata, C. tropicalis,* and *C. parapsilosis* have been associated with considerably higher mortality (Kojic and Darouiche, 2004; Tumbarello et al., 2007; Trofa et al., 2008; Cauda, 2009; Hasan et al., 2009; Quindós et al., 2009). *Candida* spp. lives on several simple carbon and energy sources; it can grow on glucose (Seneviratne et al., 2008). *Candida* spp. grow well in the presence of oxygen, grow much better on simple substrates and is resistant to acidic environment. *Candida* biofilms consist of nearly 40% carbohydrate, with the major carbohydrate component varying between *Candida* spp. (Seneviratne et al., 2008).

After 18 to 24 h at 30°C, the bilayered structure of the *Candida* spp. biofilm consists of budding yeast, germ tubes and young hyphae. In this phase, extracellular matrix (ECM) production occurs. The ECM is mainly composed of DNA, proteins, and polysaccharides. They are composed of a mixture of cells, including yeast, pseudohyphal, and hyphal cells, and include an ECM comprising polysaccharide and protein (Seneviratne et al., 2008; Douglas, 2003; Ramage et al., 2001). Like bacterial biofilms, *C. albicans* biofilms are much more resistant than free-living planktonic cells to many antimicrobial drugs. Mature *C. albicans* biofilms have a complex three-dimensional structure and display extensive spatial heterogeneity (Ramage et al., 2001). This complex structure is an optimal environment for influx of nutrients, efflux of waste products and ramification of water channels (Ramage et al., 2006).

Recent studies suggest that specific adherence events may also be mediated by cell surface proteins such as those encoded by members of ALS (Agglutinin- Like Sequence). *Candida* biofilm formation has been shown to evolve in three distinct development phases: early (0 to 11 h), intermediate (12 to 30 h), and mature (38 to 72 h) (Jabra-Rizk et al., 2004). Most biofilim formation occurs more frequently in isolates of *C. krusei* followed by *C. tropicalis, C. kefyr, C. guilliermondii, C. parapsilosis, C. glabrata*, and *C. albicans* (Al-akeel et al., 2013; Mohamed and Al-Ahmadey, 2013). In contrast, Hawser and Douglas (1994) reported that isolates of *C. parapsilosis* and *C. glabrata* were significantly less likely to produce

biofilms than the more pathogenic *C. Albicans* (Hawser and Douglas, 1994).

Filamentous

The opportunistic fungal pathogen *C. albicans* causes both superficial and systemic infections. *C. albicans* is the species most frequently encountered in infected tissues. *C. albicans* is able to grow in different morphological forms such as budding yeast (round or oval cells), and a range of filamentous forms that include true hyphae (filamentous cells without constrictions) as well as pseudohyphae (chains of elongated cells with visible constrictions) (Sudbery et al., 2004) (Figure 1).

Germ tube formation (GTF) from blastoconidia by *C. albicans* has been suggested as a potential virulence factor in their pathogenesis (Sudbery et al., 2004), since GTF is the first stage of true hyphae development. GTF is a morphological characteristic that increases the ability of the fungi to adhere and penetrate into infected tissue (Cutler, 1991). Furthermore, *Candida* isolates unable to produce germ tube seem to be less virulent (Benson et al., 2002; Saville et al., 2003). The ability to change morphology from the yeast form to hypha (and vice versa) is thought to be the primary cause of the *C. albicans* pathogenicity (Lee et al., 2005).

A variety of environmental factors (such as serum, temperature above 37°C, pH above 6.5, glucose, and low dissolved oxygen) can trigger transitions from the yeast form of growth to pseudohyphae and hyphal growth form *in vitro* (Klengel et al., 2005).

Vulvovaginal candidiasis

Vulvovaginal candidiasis (VVC) is one of the most common conditions for which women seek medical care in many part of the world. It is usually characterized by a vaginal discharge or vulvar, itching, and irritation. Vaginal symptoms are the most common reason for a patient to visit her obstetrician-gynecologist; 10 million offices are visited each years in the United State American (Karaer et al., 2005). The three major causes of lower genital tract complaints among women are bacterial vaginosis (BV), vulvovaginal candidiasis and *Trichomonas* vaginitis (Karaer et al., 2005; Dai et al., 2010). Other sexually transmitted diseases that can cause vaginitis include Chlamydia, and gonococcal cervicitis.

Vaginitis can be caused by an infection or by non-infectious causes. Bacterial vaginosis is a vaginitis caused by an overgrowth of the *Gardnerella* spp., which normally lives in the vagina. Vaginitis can also be the result of trichomoniasis, which is a sexually transmitted disease caused by a parasite. Non-infectious vaginitis is caused by exposure of female genitals to irritating substances or to allergens, a substance that a women is allergic to typical irritants and allergens include perfumed soaps, bubble both products, douches, detergents, spermicides and vaginal deodorants (Center for Disease Control and Prevention, 2010).

Fang et al. (2007) reported the prevalence of bacterial vaginosis, VVC and trichomoniasis as diagnosed by clinical tests to be 5.9, 3.1 and 2.8%, respectively (Fang et al., 2007). Furthermore, Ferris et al. (2002) reported the actual diagnosis in 95 women who self-diagnosed VVC were: VVC (34%), bacterial vaginosis (19%), mixed vaginitis (21%), normal flora (14%), trichomonas vaginitis (2%), and other (11%) (Ferris et al., 2002).

VVC is an inflammatory disease of the vulva and vagina caused by yeast. *Candida* spp. is the second most common vaginal infection after bacterial vaginosis (Dai et al., 2010). During the child-bearing years, 75% of women experience at least one episode of VVC and approximately 40 to 50% of women have repeated infection of VVC.

VVC was classified into uncomplicated and complicated cases, a classification that has been internationally accepted and adapted. Recurrent VVC is defined as at least four episodes of VVC during one year (Center for Disease Control and Prevention, 2010). VVC, especially when recurrent, is a major clinical challenge because the organisms are becoming resistant to the therapeutic agents currently used (Wei et al., 2010).

VVC is infrequently caused by *C. parapsliosis*, *C. tropicalis*, and *C. krusei*, although most species of *Candida* have been associated with condition (Shingh et al., 2002; Nyirjespy et al., 2005). VVC induced by NCAC is clinically indistinguishable from the caused by *C. albicans*; moreover, such species are often more resistant to treatment (Erdem et al., 2003).

CANDIDA SPP. IN VVC

Distribution of *Candida* spp. in VVC

The distribution of *Candida* spp. identified in women with VVC varies widely depending on the location as well as the population studies (Table 1). Typically, a single species identified, but two or more species have been found in some vaginal colonization in minority of women (2 to 5%) with complicated as well as uncomplicated VVC (Richter et al., 2005). In the USA, Europe, and Australia, *C. albicans* colonization was found to be (76 to 94%) followed by *C. glabrata* (3 to 16%) (Corsello et al., 2003; Paulitsch et al., 2006; Esmaeilzadeh et al., 2009; Wei et al., 2010; Richter et al., 2005; Spinillo et al., 1997; Holland et al., 2003; Vermitsky et al., 2008; Mahmoudabadi et al., 2010).

The overall percentage of non-albicans spp. vaginitis in these countries, continents ranges from 2.4 to 11%. While a recent all United States study of over 90,000 samples found consistent yearly distribution from 2003 to 2007 (Vermitsky et al., 2008).

Table 1. Distribution of *Candida* spp. in different regions.

Region	No. of subject	*C. albicans*	*C. glabrata*	*C. tropicalis*	*C. parapsilosis*	*C. krusei*	Other non-albicans	No of references
Italy	410	84	9	1	1	-	5	51
Italy	4228	43.1	36	12.1	0	8.6	0.6	58
K.S.A	500	59	31	4	0.6	3.2	2.2(c)	17
Australia	1087	89	7	1	1	1	1	52
Nigeria	517	20	34	18	5	-	24(b)	57
U.S (Iowa)	429	76	16	1	4	4	2	50
Austria	3184	88	3	<1	1	<1	7	11
U.S all state	93795	89	8	1	2	-	-	53
India (Aligarh)	1050	46.9	36.7	2.8	10.2	1.4	1.9(a)	55
Iran (Babol)	2000	81	3	0	0	14	3(b)	12
China (Han)	1006	77	11	5	3	4	2(a)	46
China (Tibeta)	1102	90	4	3	1	2	0	46
Iran (Ahvaz)	1473	93	3	0	0	0	3(d)	54
K.S.A (Medina)	157	80	12.5	2.5	0	0	5(a,c)	9
K.S.A (Medina)	103	61	4	25	4	0	6	33

(a) *C. kefyr*; (b) *C. guilliermondil;* (c) *S. servisiae*.; (d) *C. dubininesis*.

Relatively recent studies showed different *C. albicans* colonization rate; 76% in U.S lower (Richter et al., 2005), 77% in China, Han (Wei et al., 2010), 90% in China, Tibeta (Wei et al., 2010), 94% in Iran, Ahvas (Mahmoudabadi et al., 2010), 46.9% in India (Ahmad and Khan, 2009), 44% in Turkey (Cetin et al., 2007), 20% in Nigeria (Okungbowa et al., 2003), 80% in KSA, Medina (Al-akeel et al., 2013) and as well as 61 and 59% in KSA (Al-Hedaithy, 2002; Mohamed and Al-Ahmadey, 2013). In contrast to the case in the USA, Europe, and Australia, NCAC in particular *C. glabrata,* appear to be more commonly associated with vaginal colonization in India and Nigeria (Table 1). In Turkey (Cetin et al., 2007), India (Ahmad and Khan, 2009) and Nigeria (Okungbowa et al., 2003) due to *C. glabrata* range between 30 to 37%, but the *Candida* spp. distribution in China resembles closely the one in the United States.

In other studies, *C. albicans* was less common than non-albicans ssp. colonization rates: 80% in Nigeria (Okungbowa et al., 2003), 57% in Italy (Parazzini et al., 2000), 56% in Turkey (Cetin et al., 2007), and 65% in India (Ahmad and Khan, 2009). In the Iran, Babol studied, 14.4% *C. krusei* was the second most common of vaginal colonization but 3% *C. glabrata* colonization rate (Esmaeilzadeh et al., 2009).

Risk factors for VVC

Genetic factors

Genetic factors have an important role in determining the risk of VVC. African American women were previously reported to be at greater risk of VVC than white women (Foxman et al., 2000) and women with Lewis blood group non-secretor phenotype all suggest that there could be genetic factors the predispose individuals to colonization (Chaim, 1997). More recent polymorphism in the mannose-binding lectin gene was found to be associated with RVVC (Babula et al., 2003; Sobel, 2007). HIV positive women higher rates of vaginal colonization with *Candida*, often non-albicans spp., than HIV negative women (Spinillo et al., 1997).

Pregnancy

Pregnant women with depressed immune system

are also more likely to a higher prevalence of VVC (Al-akeel et al., 2013; Ahmad and Khan, 2009). It occurs in the last trimester of pregnancy, due to the increased amount of glycogen in the vagina and high levels of estrogen hormones. It provides a good source of carbon, which favors the growth of *Candida* spp. and germination (Babic and Hukic, 2010). Estrogen hormone also enhances adherence of yeast to vaginal epithelial cells. A cytosol receptor or binding system for female reproductive hormones has been documented in *C. albicans*, resulting in enhanced mycelia formation (Babic and Hukic, 2010). Jindal et al. (2007) and Ahmad and Khan (2009) reported statistically significant difference in incidence of VVC between pregnant and non-pregnant women (Ahmad and Khan, 2009; Jindal et al., 2007).

Antibiotics

Vulvovaginal candidiasis frequently follows use of vaginal or systemic antibiotics (Wilton et al., 2003). Antibiotics alter the normal flora of the vagina and thus allow overgrowth of *Candida* spp. After antibiotic use, the increase in vaginal colonization with *Candida* spp., mostly *C. albicans*, estimated to range from 10 to 30%, and VVC occurs in 28 to 33% cases (Pirotta et al., 2003). Antibiotics are thought to predispose women to VVC by eliminating the protective bacterial flora, thus allowing *Candida* spp. overgrowth in the gastrointestinal tract, vagina or both (Pullz et al., 2005; Pirotta and Garland, 2006). Lactobacilli play a key role in the prevention of these conditions, which protect against invasion or overgrowth of pathogenic species (Ronnqvist et al., 2006). Tarry et al. (2005), Jindal et al. (2007), Ahmad and Khan (2009) and Al-akeel et al. (2013) reported high incidences of VVC in women using antibiotics. The utilization of antibiotics may also intensify the symptoms by inactivating the defensive vaginal flora (Sobel, 1999).

Uncontrolled diabetes mellitus

Vaginal inflammation or infection, especially VVC is more disturbing in severe hyperglycemic conditions. Many studies indicate that yeast colonization rate is more frequent in diabetic women than non-diabetic (Ahmad and Khan, 2009; Malazy et al., 2007). Women with type 2 diabetes mellitus are more prone to colonization with *C. glabrata* (Donlan and Costerton, 2002). Farajii et al. (2012) reported, that VVC in the diabetic women is more prevalent than the in non-diabetic ones, and *C. albicans* was the most predominant yeast isolated (Faraji et al., 2012). Grigoriou et al. (2006) reported that diabetic women were significantly more prone to develop fungal vaginitis than non-diabetic (Grigoriou et al., 2006). Glucose can stimulate yeast development and even promote change to a more virulent stage.

Contraceptive

Some studies indicate increased VVC after the use of oral contraceptive with high estrogen content (Raad et al., 2003; Mohmoudi Rad et al., 2011). An increase in the risk of infection is repeated with vaginal contraceptive sponge, diaphragms, intrauterine contraceptive device, and condoms (Richter et al., 2005; Ahmad and Khan, 2009; Grigoriou et al., 2006). This is because of increased levels of estrogen occurring as the oral contraceptive increases the colonization of *Candida* spp. in the vagina (Ahmad and Khan, 2009; Tarry et al., 2005; Consolaro et al., 2004; Yusuf et al., 2007). Al-akeel et al. (2013) reported that oral-contraceptive users were more likely to developed VVC than non-users (Al-akeel et al., 2013).

METHODS FOR DETECTION OF VAGINAL *CANDIDA* SPP.

Several rapid identification methods have been utilized which include a wide range of typing techniques, derived from direct microscopic examination, culture, assimilation of sugar, serological, and molecular biology to identify *Candida* spp.

Direct microscopic examination

Microscopy with the use of saline preparation detects the presence of yeast cells and mycelia in approximately 30 to 50% of patients with VVC. In approximately 50% of positive microscopy of wet mount or saline preparation, yeast cells and hyphal elements can be seen (Figure 1c). Though, the sensitivity of the 10% potassium hydroxide examination is higher than saline preparation in identifying yeast cells and hyphae (Sobel, 2007). 10% KOH destroys the cellular elements and may facilitate recognition of budding yeast and hyphae, only 50 to 70% of women with VVC and sometime fail to detect non-albicans spp. (Center for Disease Control and Prevention, 2010). Staining the slide by Giemsa stain, have also been seen for yeast with or without pseudohyphae, neither *Candida* nor other organisms and a mixture of two organisms (yeast and bacteria) (Al-akeel et al., 2013; Esmaeilzadeh et al., 2009). Therefore, a culture should be obtained in patients with negative microscopy but characteristic symptoms to confirm the clinical diagnosis and avoid empiric therapy.

Culture and selective media

Vaginal culture is the most accurate method of diagnosis of VVC and is indicated if microscopy is negative but VVC is suspected or in cases of high risk for non-albicans

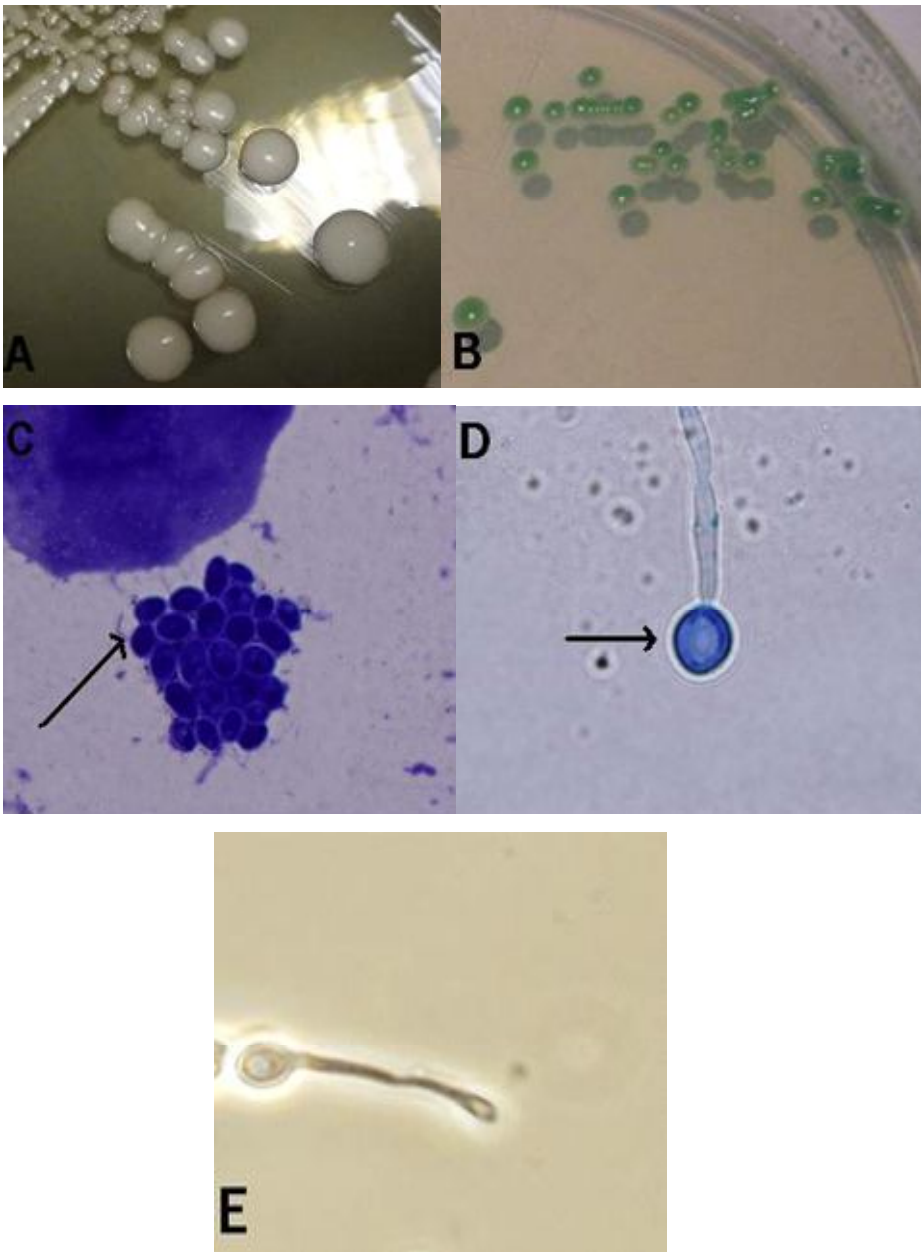

Figure 1. (A) Culture of yeast-colonies on Sabouraud Dextrose Agar, (B) *C. albicans* with green color Colonies on CHROM agar *Candida*, (C) Yeast cells seen in Giemsa slide, (D) Chlamydospores and pseudohyphae on microscopic examination *C. albicans* (lactophenol cotton blue 100X), and (E) Germ tube identified on microscopic examination of *C. albicans* (100X).

species. Specimens were inoculated into Sabouraud dextrose agar (SDA) (Figure 1A), CHROM agar medium (BD company, Belgium), incubate at 37°C for 48 h (Al-akeel et al., 2013; Mohamed and Al-Ahmadey, 2013). Enzymatic reaction methods using chromogenic substrates can accelerate the differentiation of *Candida* species. CHROM agar medium has high sensitivity and specificity in the differentiation of *Candida* spp. (33). Using this method, able to identify the following individual *Candida* spp., *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. albicans* based on their color, which also facilitates the detection of mixed infections with more than one species of *Candida* (Figure 1B). The colonies of suspected *C. albicans* are confirmed by Cornmeal agar (CMA), Sabouraud dextrose agar with cyclohexamide and germ tube test (GTT) (Al-akeel et al., 2013).

Cornmeal Agar (CMA) with 1% Tween 80 (Oxoid) for morphological examination of the production of

chlamydospores, blastospores, hyphae, and branched pseudohyphae. Typical for *C. albicans* on CMA long hyphae and produce the single terminal chlamydospores, but formed short hyphae and chlamydospores in triplets and contiguous pairs considered typical for *C. dubliniensis* on cornmeal agar (9, 57, 63, 77) (Figure 1D).

Germ tube test (GTT) for the direct identification of *C. albican* and *C. dubliniensis*. Samples were taken from all the colonies in SDA and incubated in 0.5 ml of serum at 37°C for 2 to 4 h. The cells were examined microscopically, using this method, able to identify *C. albicans* and *C. dubliniensis*, as short lateral hyphea filaments without any constrictions (Al-akeel et al., 2013; Mohamed and Al-Ahmadey, 2013) (Figure 1E).

Sugar assimilation

Analytical Profile Index (API) was used and results were correlated with hyphae on cornmeal agrar with 1% Tween 80 agar plates inoculated by the Dalmau method as recommended by the manufacturer. The API 20C (BioMereaux, France) uses disposable plastic strips containing 20 cupules. The first cupule is a negative control and second cupule contains glucose positive control. The remaining 18 cupules each contain a specific substrate that may be assimilated by the test organisms. The strips were incubated at 29 to 30°C and read at 48 to 72 h. A profile number is generated for each strip depending upon the reactions it produced Al-akeel et al. (2013) reported, only two isolates remained incorrectly identified: one *C. famata* isolate and one *C. kefyr* isolate (Al-akeel et al., 2013).

Serology and molecular diagnostic methods

Antigen detection or serologic tests as well PCR-based diagnosis are either not yet reliable or not clinically useful. *Candida* antibodies are detected using a new ELISA-based serological test (SysCan3 Candida Pathology ELISA Kit). The serological test has high sensitivity and specificity as compared with cultures for the diagnosis of VVC (Tan et al., 2003). Other methods of testing for *Candida* such as latex agglutination have not made their way into routine clinical practice (Brown et al., 2001).

The PCR methods may represent the new gold standard for detection of *Candida* spp. (Sobel, 2007). PCR offers the ability to detect *Candida* spp. within only 1h of isolates sample. This method has high sensitivity and specificity. PCR can be used not only to confirm the presence of fungi, but also when primers are chosen to selective amplify the international transcribed spacer (ITS) region of fungal ribosomal DNA. On the other hand, routine use of PCR during labor would require a significant capital outlay for other equipment, local availability of a 24 h and greater expense per test (Wei et al., 2010).

ANTIFUNGAL AGENT AND SUSCEPTIBILITY TESTING METHODS

Vulvovaginal candidiasis may be classified into complicated and uncomplicated forms (Sobel et al. 1998). Uncomplicated VVC is seen in 90% of patients and responds readily to short-course oral or topical therapy. In contrast, the complicated VVC seen in <10% of patients requires antimycotic therapy for >7 days, either daily as topical therapy or as two 150-mg doses of fluconazole administered 72 h apart (Singh et al., 2002). Azole therapy is unreliable for NCAC species. Infections with *C. glabrata*, *C. krusei* (Sobel and Vazquex, 1996) and non-albicans ssp. frequently respond to topical boric acid (600 mg/day for 14 days or topical flucytosine. Azole resistant *C. albicans* infections are extremely rare (Favel et al., 1999).

In long-term therapy, flucytosine monotherapy has been replaced by a combination of amphotericin B and flucytosine which shows favorable interactions in tests with *C. albicans* (Teseng et al., 2005; Badiee and Alborzi, 2011). In agreement with the study of Sabatelli et al. (2006), most of the detected resistant strains belong to *C. glabrata,* emphasizing its greatest potential to acquire resistance to fluconazole (Sabatelli et al., 2006). In agreement with the findings of Richter et al. (2005) our ketoconazole -susceptibility data showed that all yeast isolates were susceptible, and no observed remarkable difference in the susceptibility between *C. albicans* and non-albicans spp. (Richter et al., 2005).

Bauters et al (2002) reported that exclude 21.1% of vaginal isolates were resistant to fluconazole (Sobel et al., 2001). A second large study of 593 yeast isolates concluded that resistance to fluconazole and flucytosine was observed infrequently (3.7 and 3%, respectively), and the more resistant non-albicans spp. were more frequently isolated from recurrence VVC (Richter et al., 2005). Resistance to itraconazole was observed among *C. glabrata* (74.1%), *C. krusei* (58.3%), *S. cerevisiae* (55.6%), and *C. parasilosis* (3.4%) (Richter et al., 2005).

In vitro susceptibility testing for antifungals by the former National Committee for Clinical Standards (NCCLS), now the Clinical Laboratory Standards Institute (CLSI), subcommittee on antifungal susceptibility testing has developed and published approved methods for the broth dilution testing of yeast (M27-A2), and for the disk diffusion testing of yeasts against fluconazole (M44-A) (Al-akeel et al., 2013; Al-akeel et al., 2013; Mohamed and Al-Ahmadey, 2013). Qualitative susceptibility results are obtained 24 h sooner by the M44-A method. The E test (AB Biodisk, Solna, Sweden) is a stable agar gradient minimal inhibitory concentration (MIC) method, which has been shown to be useful in the susceptibility testing of

fungi (Al-akeel et al., 2013). Increased antifungal resistance of NCAC spp., their emergence remains a concern.

The reason for the considerable higher frequency of non-albicans isolation in some countries is unclear. Genetic, immune-based, behavioral, and nutritional factors, among some others, have to be taken into consideration.

CONCLUSION

This review summarizes the different factors that play a role in *Candida* infection, yeast species, their detection methods and antifungal susceptibility and hence may help in drawing strategies in preventing and controlling different and serious diseases that affect women and their newborns.

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