EPIDEMIOLOGY

The incidence of Candida vaginitis (CV) is poorly documented, particularly since CV is not a reportable entity. Regrettably, CV is routinely diagnosed without laboratory testing, resulting in as much as 50% misdiagnosis (1). Data on incidences where diagnostic data were based on definite clinical and mycologic findings are exceptional. Moreover, most studies have been carried out in sexually transmitted disease (STD) clinics and family planning or student health clinics which largely ignore older women and those in the private sector. Most studies suggest a CV prevalence of 5% to 15%, depending on the population studied (2). CV affects most females at least once during their lives, at an estimated rate of 70% to 75%, of whom 40% to 50% will experience a recurrence (3,4). Statistical data from England have shown a sharp increase in the annual incidence of CV, from 118 per 100,000 women to 200 per 100,000 women during the last 20 years (5). In the United States, CV is currently the second most common cause of vaginal infections, with bacterial vaginosis as the most common diagnostic entity (6). Based on a number of prescriptions written to treat yeast infections between 1980 and 1990, the incidence of CV almost doubled and these cases numbered approximately 13 million in 1990.

Point-prevalence studies indicate that Candida spp. may be isolated from the lower genital tracts of approximately 20% of asymptomatic healthy women without abnormal vaginal discharge (7). Among women with symptoms of vulvovaginitis, 30% had yeast isolated, confirming the diagnosis of CV (8). Most studies indicate that CV is a frequent diagnosis among young women, affecting as many as 15% to 30% of symptomatic women visiting a clinician. Half of all college women will have experienced at least one episode of CV by the age of 25 (3).

Between 85% to 90% of yeast strains isolated from the vagina belong to the Candida albicans species; other yeasts account for up to 15% of cases (9,10,11) (Table 1).

<table>
<thead>
<tr>
<th>Species</th>
<th>% of vaginal fungal infections</th>
</tr>
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<tbody>
<tr>
<td>Candida albicans</td>
<td>91</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>7</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>1</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>1</td>
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Candida tropicalis is isolated from 1% to 5% of subjects and may be associated with a higher rate of recurrence after standard treatment (11,12). Candida glabrata accounts for up to 10% of vaginal yeast isolates (9-11,13). Symptomatic vaginitis caused by this organism is associated with less intense itching and dyspareunia (13) than caused by other Candida spp., but the organism may be harder to eradicate with standard therapies (11,14). The relative incidence of vaginitis caused by fungi other than C. albicans appears to be increasing, accounting for up to 18% of infections in some populations (11,15). Non-C. albicans infections are associated with recurrent disease (21% versus 12% of initial infections) and with human immunodeficiency virus (21% versus 12% of infections in human immunodeficiency virus negative women), especially in those human immunodeficiency virus infected women who receive prophylaxes with imidazole or triazole (11). It is thought that the widespread use of topical antifungals, especially in short courses of treatment, may contribute to selection for non-C. albicans yeasts, which are less susceptible to these agents than C. albicans.
In earlier epidemiologic studies directed at identifying strains with specific tropism for the vagina, no such tropism was identified (16). Similarly, no evidence emerged of vaginopathic strains of *C. albicans* demonstrating greater or lesser virulence. This might explain why some women remain heavily colonized with *Candida* spp., despite being entirely asymptomatic, whereas other women develop severe symptomatic vaginitis. DNA typing has provided a more reliable and easily reproducible method of answering these questions. Using computer-assisted DNA-probe typing, Soll and co-workers (17) have presented data to support the concept of "vaginal tropism", in which *Candida* selected organisms demonstrate adaptation to unique anatomic niches that facilitate persistence and survival at certain anatomic sites, including the vagina.

*Candida* organisms are dimorphic, and may be found in humans during different phenotypic phases. In general, blastospores represent the phenotype responsible for transmission or spread of *Candida*, and are associated with asymptomatic colonization of the vagina. In contrast, germinated yeast producing mycelia most commonly constitute a tissue-invasive form of *Candida*, usually identified by the presence of symptomatic disease along with larger numbers of blastospores.

In order for species of *Candida* to colonize the vagina, they must first adhere to vaginal epithelial cells. *Candida albicans* adheres to such cells in numbers significantly higher than those of *C. tropicalis*, *C. krusei*, and *C. kefyr* (18). This may explain the relative infrequency of the latter species causing vaginitis. All *C. albicans* strains appear to adhere equally well to exfoliated vaginal and buccal epithelial cells. In contrast, there is considerable person-to-person variation in vaginal epithelial cell receptivity to *Candida* organisms in adherence assays (17, 18).

Little is known about the role of *Candidal* proteolytic enzymes, toxins and phospholipase in determining the virulence of the organisms. A secreted aspartyl proteinase generated by pathogenic *Candida* spp. has been detected and identified in vaginal secretions of women with symptomatic vaginitis, but not in those with asymptomatic colonization (19). These proteolytic enzymes with broad substrate specificity destroy free and cell-bound proteins that impair colonization and invasion. Levels of proteinase secreted by vaginal *C. albicans* isolates were greater in isolates obtained from symptomatic women than in isolates from asymptomatic carriers (19).

High frequency, heritable switching occurs in the colony morphology of most *Candida* spp. The variant phenotype switching enables *Candida* spp. to adapt to environmental factors such as drug challenges, and to escape the immune system. Although there is currently incomplete evidence that phenotypic switching occurs *in vivo* at 37°C, it is an attractive hypothesis for explaining spontaneous *in vivo* transformation from asymptomatic colonization to symptomatic vaginitis. Iron binding by *Candida* organisms has been shown to facilitate their virulence (20). The ready availability of erythrocytes and hemoglobin in the vagina creates an ideal niche for yeast possessing erythrocyte-binding surface receptors.

**PATHOGENESIS**

*Candida* organisms gain access to the vaginal lumen and secretions predominantly from the adjacent perianal area (21). Age appears to be an important factor in the overall incidence of vulvovaginal candidiasis which is seen predominantly in women of childbearing age. While the condition is extremely rare prior to menarche, the annual incidence increases dramatically toward the end of the second decade of life and peaks over the next two decades. Among college women, CV is more common among black than among white women (4) and is associated with the initiation of sexual activity (3).

High estrogen levels apparently favor overgrowth of yeasts, although such levels also promote the growth of lactobacilli (22-24). CV is more common in pregnancy and occurs in 10% of first trimester women and in 36% to 55% of women in their third trimesters (25). Symptomatic disease has eventually developed in 60% to 90% of pregnant carriers, and old inoculation studies have confirmed the increased susceptibility of pregnant women (25). High levels of reproductive hormones provide an excellent carbon source for *Candida* organisms by producing a higher glycogen content in the vaginal tissue (26). A more complex mechanism is likely, in that estrogen enhances adherence of yeast cells to the vaginal mucosa. Several investigators have demonstrated in vitro binding of female sex hormones to *Candida* organisms, as well as the capacity of certain hormones to enhance yeast mycelial formation and enhance virulence (27). Consequently, the rates of cured CV are significantly lower during pregnancy.

The onset of symptomatic CV is frequently observed during courses of systemic topical antibiotics. Broad-spectrum antibiotics, such as tetracycline and beta-lactams, are mainly responsible for exacerbation of symptoms (28). Vaginal colonization rates increase from approximately 10% to 30% (29). Antibiotics are thought to facilitate CV by eliminating the protective vaginal bacterial flora. Natural flora is thought to provide colonization resistance as well as to prevent germination and hence superficial
mucosal invasion. In particular, aerobic and anaerobic resident lactobacilli have been pinpointed as providers of this protective function. Low numbers of lactobacilli in vaginal cultures were observed in women with symptomatic CV (30). The current concept of lactobacilli-yeast cell interaction includes competition for nutrients, and lactobacilli's steric interference of receptor sites on vaginal epithelial cells for Candida organisms (31).

The incidence of CV increases dramatically in the second decade of life, corresponding with the onset of sexual activity. It peaks in the third and fourth decades then declines in females older than 40 years. Sexual transmission of CV occurs during vaginal intercourse, although the relative role of sexual and nonsexual practices in introducing CV into the lower genital region has not been apprised (16,32,33). The likelihood that sexual behavior may play a role in CV is logical, but epidemiological evidence is limited (34).

The source of Candidal infection for vaginal colonization may be initiated by the gastrointestinal tract, although this remains highly controversial (35). Species of Candida were recovered on rectal cultures from 100% of women with recurrent CV. Furthermore, the majority of Candida strains isolated from the rectum and the vagina are identical (35). However, women prone to recurrent CV are not known to suffer from perianal or rectal candidiasis. Two controlled studies using oral nystatin treatment, which reduces intestinal yeast carriage, failed to prevent symptomatic recurrence of CV (35, 37).

With respect to sexual transmission, penile colonization with Candida organisms is present in approximately 20% of male partners of women with recurrent CV (32, 33). Asymptomatic male genital colonization with species of Candida is four times more common in male sexual partners of infected women and infected partners usually carry identical strains (16, 32). Despite the aforementioned evidence indicating that sexual transmission does occur, the contribution of sexual transmission to the pathogenesis of infection remains unknown.

**CLINICAL SIGNIFICANCE**

Acute pruritus and vaginal discharge are the usual presenting complaints, but neither symptom is specific to CV and neither is invariably associated with disease. The most frequent symptom is that of vulvar pruritus. Vaginal discharge is frequently minimal. Although described as typically cottage cheese like in character, the discharge may vary from watery to homogeneously thick. Vaginal soreness, irritation, vulvar burning, dyspareunia, and external dysuria are commonly present. Odor, if present, is minimal and nonoffensive. Examination frequently reveals erythema and swelling of the labia and vulva, often with discrete pustulopapular peripheral lesions and fissure formation. Certain predisposing factors associated with increased yeast growth include glycosuria, diabetes mellitus, pregnancy, obesity, and recurrent use of antibiotics, steroids or immunosuppressive agents.

In men, Candida infection is expressed as a transient rash, erythema, and pruritus or a burning sensation of the penis that develops minutes after unprotected intercourse. The symptoms are self-limited and frequently disappear after showering.

**DIAGNOSIS**

The lack of specificity of symptoms and signs of CV precludes a diagnosis that is based solely on history and physical examination. Clinical signs and symptoms alone also should not be regarded as a satisfactory basis for diagnosis. Regrettably, both approaches are common in practice, as a myriad of infections and noninfections may cause patients to present similar signs and symptoms, hence the need for laboratory confirmation. Bergman and colleagues emphasized that a patient's symptoms are of little practical value in predicting CV (46). The most specific symptom in genital Candida infection is pruritus without discharge, and even this criterion correctly predicted CV in only 38% of patients (46).

At present, laboratory identification of Candida species requires culturing and other microscopic preparation techniques, which are time-consuming and have an inherent weakness in that they may not be species-specific. Further complicating traditional analysis is the increasing number of auxotrophs that do not grow on the media required to perform the tests (47). Diagnosis of C. glabrata vaginitis is more difficult than that of typical Candida vaginitis because of the failure of this organism to form pseudohyphae and hyphae in vivo. Accordingly, on saline and KOH microscopy, numerous budding yeasts are seen, but hypha elements are absent. There is some evidence that vaginitis with C. glabrata often occurs at a somewhat higher vaginal pH, usually at the upper limit of normal. Not infrequently, C. glabrata vaginitis coexists with bacterial vaginosis, and the higher pH of the latter may represent the link between the two entities.
Current laboratory techniques for the identification of CV include:

1. The 10% KOH preparation, with just 65% to 85% sensitivity.
2. Direct microscopy. Several studies have consistently revealed that as many as 50% of patients with culture positive symptomatic CV (responding to antifungal therapy) will have negative microscopy (21).
3. The Papanicolaou (Pap) smear, which is unreliable as a diagnostic modality, showing a positive result in only 25% of cases.
4. *Candida* cultures and the use of conventional morphological and metabolic characteristics. These are time-consuming and require several days for test results.

In order to decrease turn-around time, laboratory methods were devised for the rapid diagnosis of fungal infections which include detection of antibody (48), and cell wall mannans (49). Efforts have been directed toward molecular testing such as the use of rRNA genes for species identification.

The recent advent of Real-Time PCR technology allows for the detection of PCR amplification while the reaction is proceeding. Conventional PCR methods only allow the visualization of product at the end of the reaction, or end-point analysis. In addition to the highly specific primers that are used in a PCR reaction, Real-Time PCR utilizes a probe to enhance the sensitivity and specificity of the assay.

The validation studies performed by MDL, prior to offering testing for a specific pathogen by PCR, are used to establish the efficacy of the application (sensitivity and specificity) as well as interference studies. In interference studies, the reaction is spiked with potential amplification interfering substances (i.e., blood, mucus, genomic DNA) and perform amplification assays. The studies performed by MDL establish the ability of the PCR method to detect specific genetic sequences of a target pathogen within a given clinical specimen. Sensitivity was determined using serial dilutions of DNA extracted from known validated organisms purchased from the American Type Culture Collection (ATCC) and subcloned plasmid standards.

Specificity was determined by attempting amplification of DNA extracted from 42 known human pathogens and other validated organisms purchased from the ATCC and mixed in various cocktails. None of the organisms listed were amplified (Table 2).

For *Candida albicans*, a range of 5x10^1 to 5x10^6 copies can be detected with an r^2 value of 0.99936. A lack of interference from other substances inherent to the specimen type was determined by adding 500 ng of DNA extracted from a clinical sample that had tested negative for the pathogens in question to a dilution of plasmid standards. The addition of the extracted sample DNA did not significantly alter the detection of the target DNA. A range of 5x10^1 to 5x10^6 copies can be detected with an r^2 value of 0.99372 (Figure 1).

For *Candida tropicalis*, a range of 5x10^2 to 5x10^6 copies can be detected with an r^2 value of 0.99429. Interference from other substances inherent to the specimen type was determined by adding 500 ng of DNA extracted from a clinical sample that had tested negative for the pathogens in question to a dilution of plasmid standards. The addition of the extracted sample DNA did not significantly alter the detection of the target DNA. A range of 5x10^2 to 5x10^6 copies can be detected with an r^2 value of 0.97973 (Figure 2).

For *Candida glabrata*, a range of 5x10^2 to 5x10^6 copies can be detected with an r^2 value of 0.99980. Interference from other substances inherent to the specimen type was determined by adding 500 ng of DNA extracted from a clinical sample that had tested negative for the pathogens in question to a dilution of plasmid standards. The addition of the extracted sample DNA did not significantly alter the detection of the target DNA. A range of 5x10^2 to 5x10^6 copies can be detected with an r^2 value of 0.99933 (Figure 3).

For *Candida parapsilosis*, a range of 5x10^1 to 5x10^6 copies can be detected with an r^2 value of 0.99431. Interference from other substances inherent to the specimen type was determined by adding 500 ng of DNA extracted from a clinical sample that had tested negative for the pathogens in question to a dilution of plasmid standards. The addition of the extracted sample DNA did not significantly alter the detection of the target DNA. A range of 5x10^1 to 5x10^6 copies can be detected with an r^2 value of 0.98137 (Figure 4).
Table 2. Validated organisms purchased from the ATCC for testing specificity for *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, and *Candida parapsilosis*.

<table>
<thead>
<tr>
<th>Adenovirus</th>
<th>Candida krusei</th>
<th>HSV-2</th>
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<tbody>
<tr>
<td>Anaplasma phagocytophilum</td>
<td>Chlamydia pneumoniae</td>
<td>HTLV-I</td>
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<tr>
<td>Aspergillus fumigatus</td>
<td>Chlamydia psittaci</td>
<td>Mobiluncus curtisi</td>
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<td>Babesia microti</td>
<td>Chlamydia trachomatis</td>
<td>Mobiluncus mulieris</td>
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<td>Bacteroides fragilis</td>
<td>CMV</td>
<td>Mycoplasma fermentans</td>
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<td>Bartonella bacilliformis</td>
<td>Coxsackie Virus</td>
<td>Mycoplasma genitalium</td>
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<td>Cryptococcus neoformans</td>
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<td>Bartonella quintana</td>
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<td>Mycoplasma pneumoniae</td>
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<td>Mycoplasma salivarium</td>
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<td>Helicobacter pylori</td>
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<td>Candida glabrata</td>
<td>HHV-8</td>
<td>Trichomonas vaginalis</td>
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<td>Cryptococcus humicolus</td>
<td>HPV-16</td>
<td>Trichosporon</td>
</tr>
<tr>
<td>Candida keyfr</td>
<td>HSV-1</td>
<td>Ureaplasma urealyticum</td>
</tr>
</tbody>
</table>

Figure 1. *Candida albicans* - pCA ITS3 plasmid dilutions. Yellow lines represent plasmid alone. Black lines represent plasmid with DNA. From left to right: 5x10^6, 5x10^5, 5x10^4, 5x10^3 copies/ reaction.

Figure 2. *Candida tropicalis* - pCT ITS3 plasmid dilutions. Blue lines represent plasmid alone. Red lines represent plasmid with DNA. From left to right: 5x10^6, 5x10^5, 5x10^4, 5x10^3 copies/ reaction.

Figure 3. *Candida glabrata* - pCG ITS3 plasmid dilutions. Blue lines represent plasmid alone. Red lines represent plasmid with DNA. From left to right: 5x10^6, 5x10^5, 5x10^4, 5x10^3 copies/ reaction.

Figure 4. *Candida parapsilosis* - pCP ITS3 plasmid dilutions. Red lines represent plasmid alone. Blue lines represent plasmid with DNA. From left to right: 5x10^6, 5x10^5, 5x10^4, 5x10^3, 5x10^2 copies/ reaction.
RECOMMENDATIONS

After conventional antifungal therapy for CV, resultant negative vaginal Candida cultures once more turn positive within 30 days in 20% to 25% of women, strongly supporting the hypothesis that yeast persistence and vaginal relapse is responsible for recurrent CV (2). Strains isolated before and after therapy are of identical types in more than two-thirds of recurrences (16). Symptomatic relief after clinically successful topical therapy for symptomatic vaginitis is accompanied by a drastic reduction in the number of viable yeast cells in the vagina. Small numbers of the microorganisms persist, however, within the vaginal lumen, generally in numbers too small to be detected by conventional vaginal culture (38).

It is also possible that a small number of Candida organisms might reside temporarily within the superficial cervical or vaginal epithelial cells, only to reemerge some weeks or months later. C. glabrata is more resistant to fluconazole than C. albicans. The MIC of fluconazole for C. glabrata is 16 μg/ml, which is much higher than for C. albicans (0.25 μg/ml). C. tropicalis (1 μg/ml), and C. parapsilosis (1 μg/ml) (39). Therefore, earlier information regarding the species causing CV may help physicians to select appropriate antifungal agents and regimens to treat patients.

The specific mechanisms of antifungal resistance to the azole class of antifungal agents are not yet fully understood. It has been suggested, however, that the sterol composition of the fungal plasma membrane is altered, reducing the uptake of the antifungal agent into the cell. Recent studies with several different azoles evaluating C. albicans, C. glabrata and S. cerevisiae have demonstrated at least three known mechanisms of resistance: (1) changes in the P-450 lanosterol demethylase enzyme, (2) changes in Δ7-8-sterol desaturase, and, more recently (3), an energy-dependent drug efflux mechanism (40-43). In C. glabrata, several mechanisms of azole resistance have been identified; increased P-450-dependent ergosterol synthesis and an energy dependent efflux pump of fluconazole, possibly via a multidrug resistance-type transporter (44, 45).

The Association for Genitourinary Medicine (AGUM) and the Medical Society for the Study of Venereal Disease (MSSVD) published national guidelines for the treatment and management of candidiasis. The Centers for Disease Control and Prevention (CDC) recommendations for the treatment and management of patients can also be found in the May 10, 2002 edition of Morbidity and Mortality Weekly Report (MMWR) Vol. 51 No. RR-6 pages 45-48. (50).

REFERENCES
