Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 2 (9): 696-700, 2007
ISSN 1816-4935

Etiology of Vaginal Candidiasis in Shiraz, Southern Iran

[1]Keyvan Pakshir, [2]Maryam Yazdani and [1]Robab Kimiaghalam
[1] Department of Parasitology and Mycology, [2]Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract: Vaginal candidiasis is a common infectious disease involving genital organ. *Candida albicans* is the major cause of *Candida* vaginitis and the other species are less common but an increase of nonalbicans species has been observed. The aim of this study was to identify the etiological agents of vaginal candidiasis in Shiraz, southern Iran. A total of 183 patients suspected of *Candida* vaginitis were enrolled in our study. The specimens were collected by using vaginal swabs and cultivated on Sabouraud dextrose agar media supplemented with antibiotics. All the cultures were incubated at 35-37°C for 24-48 h. Identification of *Candida* species was performed based on Germ tube, Chlamidoconidia, Thermo tolerant tests and CHROMagar *Candida* medium. Eighty (43.71%) cases of *Candida* vaginitis were diagnosed among 183 patients suffering vaginitis. *Candida albicans* was the most dominant isolates and nonalbicans species were responsible for 21.25% of vaginal candidiasis. The frequency of *Candida* isolates were: *Candida albicans* 63 (78.75%), *Candida glabrata* 7 (8.75%), *Candida krusei* 5 (6.25%), *Candida tropicalis* 2 (2.5%) and *Candida* sp. 3 (3.75%). No species of *Candida dubliniensis* was distinguished from Candida albicans by Thermo tolerant test and Chromogenic media. Present study indicated that the rate of *Candida* vaginitis caused by nonablicans species was high. It seems that inadequate antifungal therapy and incomplete self medication could be a major cause for increasing drug resistance in nonalbicans *Candida* species.

Key words: *Candida albicans*, vaginal candidiasis, *Candida dubliniensis*, CHROMagar, Shiraz

INTRODUCTION

The most common infection treated by primary care physicians is vaginitis. It has been reported that 30-50% of vaginitis episodes are due to *Candida* sp. and that two-thirds of all women experience at last one acute episode of vaginal candidiasis during their life time (Ross *et al*., 1995). Some workers have suggested that a high percentage of women will suffer from it before the age of 25 years and approximately 75% before the age of 40 (Sobel, 1997, 1999). Vaginal candidiasis is an infectious disease involving vaginal ecosystem (Lanchares and Hernandez, 2000). It can occur in mild to severe forms, starting with the typical symptoms of itching, increased thin discharge, which later turns cheesy, marked reddening of the vagina and later stinging. About 5% of those affected suffer from chronic, recurrent vulvovaginal candidiasis, which is defined as episodes reoccurring at least four times within one year (Mendling and Seebacher, 2003). *Candida ablicans* is causative agents in 80-90% of all cases while *C. glabrata* is the second followed by *C. krusei*, *C. tropicalis*, *C. kefyr*, *C. parapsilosis*, *C. guilliermondi* which is called nonablicans (Mendling, 2003; Boselli and Seebacher, 2004; Ben-Haroush *et al*., 2004).

In recent years a change in epidemiological trends has been observed. There has been a significant increase in infections caused by non-*ablicans* species of *Candida*, particularly in recurrent cases. At

Corresponding Author: Keyvan Pakshir, Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

present, these species are found in approximately 20-30% of cases of recurrent vaginal candidiasis. Among them, *Candida glabrata* is the most common type (Lanchares and Hemandez, 2000). Since we have no information about the composition of *Candida* species causing *Candida* vaginitis in Shiraz, the aim of this study was to determine the incidence of *Candida* vaginitis (CV) and the etiological agents in premenopausal women in Shiraz, southern Iran.

MATERIALS AND METHODS

From April 2004 to March 2005, 183 patients suspected of *Candida* vaginitis which attended to gynecology clinic of Shiraz Medical School with presence of Vaginal Candidiasis symptoms (irritation, pruritis, soreness and altered discharge) were enrolled to our study. Vaginal swabs were collected from vaginal secretions and used for direct smear examination and culture. Microscopic examination of wet mount with KOH (potassium hydroxide 10%) and smear stained with Methylen blue and Giemsa were performed. The swabs were cultivated on Sabouraud dextrose agar supplemented with Penstrep-400 (Penicillin 200,000 IU L^{-1} and Streptomycin 200 mg L^{-1}), Chloramphenicol (200 mg L^{-1}) and Gentamicin (40 mg L^{-1}). The cultures were incubated at 37°C for 24-48h or until the colonies appear. Identification of *Candida* species was performed by different methods such as Germ tube test, Chlamidoconidia test (Cultivated on Corn Meal Agar plus Tween 80) and Chromogenic media, using CHROMagar *Candida* medium (Dr. A. Rambach, France).We used thermotolerant test (growth at 42-45°C) and colony color (CHROMagar *Candida* medium) for distinguishing *C. albicans* from *C. dubliniensis*.

The software package Microsoft Excel 8.0 was used for data processing.

RESULTS

A total of 80 cases from 183 patients suspected of Vaginal Candidiasis (aged 15-45) were diagnosed. The prevalence of CV was 43% and *Candida ablicans* (78.25%) was the dominant agent in this study. All *Candida albicans* isolates with positive in germ tube and Chlamidoconidia test had green colony color on CHROMagar medium (Fig. 1). The rate of nonalbicans *Candida* species was 21.25%. Three species of Candida (with presence of pseudohyphea form on corn meal agar) had no distinct color on CHROMagar medium and could not identify by this method. The frequency of *Candida* species was: *C. albicans* 63 (78.75%), *C. glabrata* 7(8.75%), *C. krusei* 5 (6.25%), *C. parapsilosis* 2 (2.5%) and un-known *Candida* 3 (3.75%) as showed in Table 1.

No species of *Candida dubliniensis* was identified in this study.

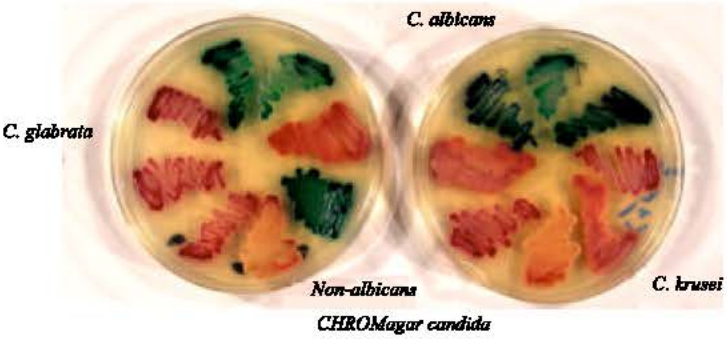

Fig. 1: Colony color on CHROMagar *Candida* medium

Table 1: Distribution of *Candida* species isolated from vaginal candidiasis

Candida species	No.	(%)
Candida albicans	63	78.75
Candida glabrata	7	8.75
Candida krusei	5	6.25
un-known *Candida*	3	3.75
Candida parapsilosis	2	2.5
Total	80	100

DISCUSSION

Vaginitis is a common gynecologic condition and accounts for more than 10 million office visits each years (Kent, 1991). Vaginal candidiasis caused 20-25% of infectious vaginitis cases, second only to the 40-45% of cases caused by bacterial vaginosis and marked by pruritis, soreness and a change in discharge, dyspareunia, vulvar erhytema, edema and fissures (Sobel, 1997, 1999). Many physicians believes that these signs and symptoms are almost suitable for diagnosing CV without any laboratory results but our study reveal that at least 53% of all patients with similar symptoms had no *Candida* vaginitis. Although Grigoriou *et al.* (2006) reported this rate in 12.1%.

It is quite known that direct microscopic examination of wet mount with potassium hydroxide and vaginal cultures on solid media is the simple and good technique for CV diagnosis (Grigoriou *et al*., 2006; Trama *et al*., 2005), so positive examination result is necessary before any therapy.

Candida isolation from the samples of vaginal exudates is very frequent finding and it maybe isolated from approximately one-fourth of symptom-free women (Trama *et al*., 2005). It has been reported that 30-50% of vaginal episodes are due to *Candida* sp. and that two-thirds of all women experience at least one acute episode of CV during their life time (Sobel, 1997; Kent, 1991). Among the *Candida* species causing infections, *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis* account for 80-90% of fungal isolates encountered worldwide. Ferror (2000) reported that *Candida ablicans* is usually the predominant yeast isolate according to 80-90% of yeast Vaginitis. *C. glabrata* is the second most species, causing approximately 5-15% of cases of CV. Over the last 30 years the incidence of the other species of *Candida*, termed non-*albicans Candida*, have steadily increased and *C. glabrata* being the most common species of this subgroup (Phillips, 2005). In this study, 21.25% of all the isolates were nonalbicans species, although this data was close to Grigoriou *et al.* (2006) in 19.8% but was not in agree with those Vrabik *et al.* (2007) and Paulitsch *et al.* (2006) studies which these rates were 8 and 12.1%, respectively. They did not confirm the higher prevalence of nonalbicans species in *Candida* vaginitis patients.

We could not identify three species of the isolates, because the chromogenic media usually used to identify clinically important *Candida* but not all kinds of the species (Odds and Bernaerts, 1994). By this reason, Saunte *et al.* (2005) could not identify four species of *Candida albicans* on CHROMagar *Candida* plates. So we highly recommend to use the other diagnostic methods such as biochemical assimilation patterns and molecular techniques for better identification of *Candida* species (Trama *et al*., 2005; Tabrizi *et al*., 2006).

Candida dubliniensis is another opportunistic pathogen that can cause both superficial and invasive infections. It is found mostly in the immunocompromised and AIDS patients and found all around the world (Sullivan and Coleman, 1998; Jabra-Rizk *et al*., 1999). Moreover, *C. dubliniensis* almost isolates from the oral cavity of HIV patients and less common in *Candida* vaginitis (Mendling and Seebacher, 2003). The most useful test for distinguishing *C. dubliniensis* from *C. albicans* is to culture them at 42-45°C which most *C. albicans* will grow (Mariano *et al*., 2003; Brito *et al*., 2006). The colonies with light green color on CHROMagar *Candida* media were considered typical for *C. albicans*, where those with a dark green color on primary isolation were initially considered atypical *C. albicans* and suspected to *C. dubliniensis* (Kirkpatrick *et al*., 1998; Momani and Qaddoomi, 2005). Gigoriou *et al.* (2006) founds that 0.5% of their patients had CV due to *C. dubliniensis* and in accord

with our methods. Us and Cengiz (2007) could identified only one strain of *C. dubliniensis* from 218 pregnant women. In this study, no species of *C. dubliniensis* was identified and we had no patients with a history of HIV infection.

Candida vaginitis is usually treated, using topical and systemic azoles (Fluconazole or Ketoconazole) and responds to antifungal treatment uniformly well, but the nonalbicans *Candida* has variable responses to conventional azoles treatment. The widespread use of azoles antifungal drugs is postulated to have promoted the shifting of vaginal colonization and selection of more naturally resistant species (Ross *et al*., 1995; Phillips, 2005; Soble *et al*., 2003). Several investigations have recently reported that the cases of sporadic and recurrent vulvovaginal candidiasis caused by nonalbicans species of *Candida* are increasing. Only a few of these studies have provided data to support this claim. In specialized clinics, more than 10% and occasionally, less than 20% of patients were infected with nonalbicans *Candida* organisms (Soble *et al*., 1998). Short-course therapy, either oral or topical may be eliminating the more sensitive *C. albicans* and selecting for more azole-resistant nonalbicans *Candida* species. Ferror (2000) pointed out that the increasing detection of nonalbicans species has been related to the widespread and inappropriate use of antimycotic treatment, self medication, long term maintenance treatments and repeated treatments for Candidosis episodes. Although Poch and Levin (2002) could not documented an association of nonalbicans species with increased use of azole antifungal agents.

This is the first study on vaginal Candidiasis in Shiraz which focused on identification of the etiological agents. This study reveals that the rate of nonalbicans *Candida* species was high and further study needs for evaluation of their sensitivity to antifungal drugs and using molecular methods for better identification.

ACKNOWLEDGMENT

We would like to express our appreciation in Dr. Kamiar Zomorodian and Sakineh Kemarloei for laboratory and scientific supports.

REFERENCES

Ben-Haroush, A., Y. Yogev and B. Kaplan, 2004. The importance of diagnostic work-up in the management of Candidal vulvovaginits. A prospective study. Clin. Exp. Obstet. Gynecol., 31; 2: 113-116.

Boselli, F., G. Chiossi, P. Garutti, A. Mattelli, M.T. Montagra and A. Spinillo, 2004. Preliminatory results of the Italian epidemiological Study on Vulvovaginitis. Minerva Ginecol., 56: 149-153.

Brito, C.A., M. Mendoza, A. Fernondez and E. Diaz, 2006. Detection of *Candida dubliniensis* in patients with Candidiasis in Caracas, Venezuela. Rev Iberoam Micol., 23: 81-84.

Ferror, J., 2000. Vaginal candidosis: Epidemiological and etiological factors. Int. J. Gyne and Obs., S21-S27.

Grigoriou, O., S. Baka, E. Makrakis, D. Hassiakos, G. Kapparos and E. Kouskouni, 2006. Prevalence of clinical Vaginal candidiasis in a university hospital and possible risk factors. Eur. J. Obstet. Gynecol. Repr. Bio., 126: 121-125.

Jabra-Rizk, M.A., A.A.M.A. Baqui, J.I. Kelley, W.A. Falkler, W.G. Merz and T.F. Meilier, 1999. Identification of *Candida dubliniensis* in a prospective study of patients in the United States. J. Clin. Microbiol., 37: 321-326.

Kent, H.L., 1991. Epidemiology of Vaginitis. Am. J. Obstet Gynecol., 65: 1168-1176.

Kirkpatrick, W.R., S.G. Revankar, R.K. Mcatee, J.L. Lopez-Ribot, A.W. Fotherqill, D.I. Mccarthy, S.E. Sache, R.A. Cantu, M.G. Rinaldi and T.F. Patterson, 1998. Detection of *Candida dubliniensis* in oropharyngeal samples from human immunodeficiency virus infected patients in North America by primary CHROMagar Canada Screening and susceptibility testing of isolates. J. Clin. Microbiol., 36: 3007-3012.

Lanchares, J. L and M.L. Hernande, 2000. Recurrent Vaginal Candidiasis changes in etiopathogenical patterns. Int. J. Gynecol and Obstet., 71: S29-S35.

Mariano, P.D.L.S., E.P. Milan, D.A. Matta and A.L. Colombo, 2003. *Candida dubliniensis* identification in Brazilian yeast stock collection. Mem inst oswaldo cruz, Rio do Janeiro. 98: 533-538.

Mendling, W. and C. Seebacher, 2003. Guideline vulvovaginal candidosis: Guideline of the German dermatological society, the German speaking mycological society and the working group for Infections and infect Immunology of the German Society for Gynecology and Obstetrics. Mycoses., 46: 365-369.

Momani, O.M. and A. Qaddoomi, 2005. Identification of *Candida dubliniensis* in a Diagnostic Microbiology Laboratory. East Mediter. Health J., 11: 366-371.

Odds, F.C. and R. Bernaerts, 1994. CHROMagar *Candida*, new differentia isolation medium for presumptive I dentification of clinically important *Candida* species. J. Clin Microbial., 32: 1923-193.

Paulitsch, A., W. Weger, G. Ginter-Hanselmayer, E. Marth and W. Buzina, 2006. A 5-year (2000-2004) epidemiological survey of *Candida* and non-*Candida* yeast species causing vulvovaginal candidiasis in Graz, Austria. Mycoses., 49: 471-475.

Phillips, A.J., 2005. Treatment of non-*albicans Candida* vaginitis with amphotericin B vaginal suppositories. Am. J. Obstet. Gynecol., 192: 2009-2013.

Poch, M.D.F. and D. Levin, 2002. High rate of vaginal infections caused by non-*C. albicans Candida* species among asymptomatic women. Med. Mycol., 40: 383-386.

Ross, R.A., M.T. Lee and A.B. Onderdonk, 1995. Effect of *Candida albicans* infection and Clotrimazole treatment on vaginal micro flora *in vitro*. American College of Obsterics and Gynecologist, 86; 6: 925-930.

Saunte, D.M., L. Kligspor, S. Jalal, J. Arnau and M.C. Arendrup, 2005. Four cases of *Candida albicans* infections with isolates developing pink colonies on CHROMagar Candida plates. Mycoses., 48: 375-381.

Sobel, J.D., 1997. Vaginitis. N Eng. J. Med., 337: 1896-903.

Sobel, J.D., 1999. Volvovaginitis in healthy women. Comp. Ther., 25: 335-346.

Soble, J.D., S. Faro, R.W. Force, B. Foxman, W.J. Ledger, P.R. Nyirjesy, B.D. Reed and P.R. Summers, 1998. Vulvovaginal candidiasis Epidemiologic, diagnostic and therapeutic considerations. Am. J. Obstet. Gynecol., 178: 203-221.

Soble, J.D., W. Chaim, V. Nagappan and D. Leaman, 2003. Treatment of vaginitis caused by *Candida glabrata*: Use of topical boric acid and flucytosine. Am. J. Obstet Gynecol., 189: 1297-1300.

Sullivan, D. and D. Coleman, 1998. *Candida dubliniensis*: Characteristics and identification. J. Clin. Microbiol., 36: 329-334.

Tabrizi, S.N., M.W. Pirotta, E. Rudland and S.M. Garland, 2006. Detection of Candida species by PCR in self-collected vaginal swabs of women after taking antibiotics. Mycoses., 49: 523-524.

Trama, J.P., E. Mordechai and M.E. Adelson, 2005. Detection and Identification of *Candida* species associated with *Candida* Vaginitis by real-time PCR and pyro sequencing. Mol. Cell. Probs., 19: 145-152.

Us, E. and S.A. Cengiz, 2007. Prevalence and phenotypic evaluation of *Candida dubliniensis* in pregnant women with Vulvovaginal candidosis in a university hospital in Ankara. Mycoses., 50: 13-20.

Vrabik, J., J. Masata, A. Jedlickova and M. Hajickova, 2007. Prospective study the prevalence of different *Candida* strains and their sensitivity to different antimycotic treatment in women with vulvovaginal candidosis. Ceska Gynekol., 72: 27-32.