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Original Article

Increased Incidence of Vulvovaginal Candidiasis Caused by *Candida glabrata* in Jordan

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SUMMARY: Due to the rising importance of *Candida glabrata* and other non-*albicans Candida* as principle human opportunistic pathogens, 356 women with abnormal vaginal discharge who attended a private obstetrics and gynecology clinic in Amman, Jordan, between January 1999 and February 2001 were examined. The isolation rate of *Candida* spp. from high-vaginal swabs was 44.9%. CHROMagar Candida and conventional mycological methods identified six isolated *Candida* spp., including *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. kefyr*. The percentages of *C. albicans*, *C. glabrata*, and *C. tropicalis* isolates were 43.1%, 32.5%, and 8.1%, respectively. *C. albicans* was isolated in combination with *C. tropicalis* and *C. krusei* in two patients. Statistical analysis of the present results clearly show an increase in the incidence of vulvovaginal candidiasis caused by *C. glabrata* in the two study periods, 1994-1996 and 1999-2001 ($P = 0.0186$). In contrast, comparing the proportions of vulvovaginal candidiasis caused by *C. albicans* in the two periods, there was no significant difference. These results may have significant clinical implications, as *C. glabrata* are innately less susceptible to most antifungal agents than *C. albicans*; these findings support viewing this organism as a major pathogen.

INTRODUCTION

Vaginal candidiasis is a widespread, common disease affecting a substantial proportion of women of childbearing age (1). The pathogenesis of this frequent clinical problem, however, remains elusive, and its incidence can be expected to continue rising, as risk factors are not diminishing. While approximately 75% of women experience a single episode of *Candida* vaginitis in their lifetime, a significant percentage of women (5-7%) experience recurrent infection (2-4). *Candida albicans* is responsible for 80-94% of vulvovaginal candidiasis (5), the remaining cases being due to other *Candida* spp. such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. kefyr* (5). In recent years, the incidence in the human population of infection with human immunodeficiency virus (HIV), organ transplantation, widespread and increased use of immunosuppressive therapy together with broad-spectrum antimycotic therapy have dramatically increased the incidence of candidiasis caused by *C. glabrata* and other non-*albicans Candida* spp. (6-10). *C. glabrata* represents the second-most common *Candida* spp. causing bloodstream infection (7,9,11). It accounts for 8% of all cases of candidemia involving patients with cancer (12), 13% of cases involving non-neutropenic patients (13,14), and 11% of cases involving patients with candidemia (7). Nosocomial yeast infections caused by *C. glabrata* have increased substantially, while yeast infections caused by *C. albicans* have decreased according to a University of Iowa Hospitals and Clinics study between the years 1987-1988 and 1993-1994 (9). Moreover, Wingard et al. (15) noted an increase in colonization with *C. glabrata* among bone-marrow transplant recipients given fluconazole as an antifungal prophylactic drug. Furthermore, *C. glabrata* accounts for 75% of fungemia cases among patients receiving fluconazole (15-18).

Several studies have suggested that the increased frequency of vulvovaginal candidiasis is caused by non-*albicans Candida* spp. (19-21). One such study demonstrated that the proportion of vulvovaginal candidiasis cases caused by non-*albicans Candida* spp. rose from 9.9% in 1988 to 17.2% in 1995 (20). Evidence regarding the selectivity of these species, especially *C. glabrata*, comes from HIV-seropositive women receiving fluconazole (4); the vaginal flora in women receiving fluconazole shifts to an increase in absolute isolation rates of *C. glabrata*, but with low attack rates of clinical vaginitis (22).

A number of investigators have emphasized the role of species other than *C. albicans* as emergent pathogens in urinary tract candidal infection (23,24). A dramatic increase in the carriage of *C. glabrata* has also been demonstrated in dentate individuals over 80 years of age; the proportion of individuals with dentures carrying *C. glabrata* in one study was greater than 50% (25). Because of differences in their susceptibility against certain antifungal drugs, changes in the frequency and distribution of *Candida* spp. are of great interest. We, therefore, felt that it would be of value to assess the frequency of isolation of various *Candida* spp. from Jordanian women with abnormal vaginal discharge during the period of 1999-2001 and to compare these results with those obtained during the period 1994-1996.

MATERIALS AND METHODS

Study subjects: High-vaginal swabs were collected from 356 women (aged between 18-52) attending obstetrics and gynecology clinics in Amman, Jordan with variable degrees of abnormal vaginal discharge between January 1999 and February 2001. After signing an informed consent to carry out the procedure, each patient completed a questionnaire about age, work, use of contraceptives, history of pelvic inflammatory disease, history of diabetes, and the use of topical

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antifungal agents and/or antibiotics. The gynecological history and examination of subjects, noted vulvovaginal symptoms (leukorrhea, burning sensation, itching, dysuria, dyspareunia), and the type (mucous, creamy, lumpy, foamy), as well as the presence or absence of odor of the vaginal secretions were also reported.

Isolation and identification of *Candida* spp.: A sample of vaginal material (posterior fornix) was obtained with the aid of a sterile plain cotton-tipped swab and was immediately inoculated into Sabouraud dextrose broth (Difco, Detroit, Mich., USA) containing 50 mgL^{-1} of chloramphenicol. Tubes were incubated at 30°C for up to 48 h, and were then cultured into Sabouraud Dextrose agar (SDA) and CHROM agar (CA) (CHROM agar, Paris, France). Following inoculation, the culture plates were incubated in air at 30°C and were inspected daily for a total of 7 days. All yeast isolates observed on CA were identified by colony morphology and pigmentation according to the manufacturer's instructions and as has been previously described (26). Isolates from CA and SDA plates were identified according to their morphologies on cornmeal agar (CMA), their formation of germ tube in serum, and, for isolates that were chlamydospore negative and/or germ-tube negative, the results of conventional biochemical and assimilation tests carried out according to established procedures (27). In cases where identification was ambiguous, the API 20C system (bioMerieux SA, Lyon, France) was employed.

Statistical analysis: The significance of differences in prevalence rates was evaluated using 2×2 contingency tables with the chi-square test; values of $P < 0.05$ were considered statistically significant.

RESULTS

A total of 356 women with variable degrees of abnormal vaginal discharge were examined between January 1999 and February 2001. Of these, 160 women (44.9%) were yeast-culture positive. The prevalence of vulvovaginal infections in the 30-40 years age group was 56.8%, followed by patients of the 20-29 years age group (50.8%) (Table 1). The prevalence of vulvovaginal infections at ≥40 and ≤19 years was 41.8% and 22.1%, respectively. Based on statistical analysis of the results, a significant difference between age groups 30-40 and ≤19 and age groups 30-40 and ≥40 in the number of vulvovaginal infections ($P = 0.000$ and 0.0266, respectively) was established. Furthermore, there was no significant difference between age groups 30-40 and 20-29 in the number of vulvovaginal infections ($P = 0.43$).

Distribution of *Candida* spp.: Using the manufacturer's guidelines for CA Candida and published criteria by Odds and Bernaerts (26), we were able to identify the following species: *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* on the basis of colony color. However, there was no difference in the sensitivity of CA and SDA. There was a 100% correlation between CA and the API test for identification of *C. albicans*, *C. tropicalis*, and *C. krusei*, and 88% for identification of *C. glabrata* and *C. parapsilosis* (confirmed by API test). A single-yeast species was cultured from 160 patients in the culture-positive population. *C. albicans* was the most frequent isolate, accounting for 43.1% of all isolates, while *C. glabrata* and *C. tropicalis* accounted for 32.5% and 8.1% of isolates, respectively (Table 2). Furthermore, *C. albicans* was isolated in combination with *C. krusei* and *C. tropicalis* in two cases.

Symptoms associated with vulvovaginal infections: The most frequent associations of the vulvovaginal symptoms were leukorrhea (36.6%), burning sensation (25.3%), and dysuria (19.7%), as presented in Table 3. The Table also shows that the percentages of patients manifesting one, two, or three symptoms of vulvovaginitis were 21.3%, 27.8%, and 25.2%, respectively. Burning sensation and leukorrhea were the

Table 1. Prevalence of vulvovaginal infections among women according to age

Age (years)	No. examined	No. infected	% infected
≤19	68	15	22.1
20-29	65	33	50.8
30-40	125	71	56.8
≥41	98	41	41.8
Total	356	160	44.9

Table 2. Proportion of vulvovaginal candidiasis caused by different *Candida* spp. during 1994-1996 and 1999- 2001

Candida spp.	Number of culture-positive (%)	
	1994-1996	1999-2001
Candida albicans	40 (51.3)	69 (43.1)
Candida glabrata	14 (17.9)	52 (32.5)
Candida tropicalis	10 (12.8)	13 (8.1)
Candida parapsilosis	2 (2.6)	6 (3.8)
Candida krusei	5 (6.4)	11 (6.9)
Candida kefyr	5 (6.4)	9 (5.6)
Candida guilliermondii	2 (2.6)	ND
Total	78 (100)	160 (100)

ND= Not detected

Table 3. Association of symptoms observed in positive cases of yeast infections

No. of symptoms claimed by the patients	Symptoms		Percentage
Asymptomatic	–		15.2
One symptom	D	4.2	21.3
	Dys	0.7	
	I	3.2	
	L	7.8	
	B	5.4	
Two symptoms	I-D	2.8	27.8
	I-Dys	1.2	
	I-B	3.2	
	L-D	2.6	
	L-Dys	3.6	
	L-I	4.9	
	B-L	6.4	
	D-B	3.1	
Three symptoms	D-I-Dys	1.2	
	L-D-Dys	2.1	25.2
	L-D-B	4.1	
	L-D-I	3.2	
	L-Dys-I	2.4	
	L-I-B	4.6	
	B-D-I	3.2	
	B-D-Dys	4.4	
Four symptoms	I-B-D-L		10.5

The data represent the percentage of total positive cases observed in the infected women. B: burning sensation, D: dysuria, Dys: dyspareunia, I: itching, L: leukorrhea.

symptoms most frequently complained of by the patients during the clinical examination; they accounted for 23% of symptoms, followed by itching and leukorrhea (17.6%). Asymptomatic cases and cases with more than three symptoms accounted for 15.2% and 10.5%, respectively, of all patients (Table 3).

Trends in vulvovaginal infections caused by yeast species: Table 2 shows the frequency distribution of *Candida* spp. in yeast-positive patients during two study periods (1994-1996 and 1999-2001); *C. albicans* was the most common species among the isolates identified to the species level during the two study periods. Overall, the proportion of vulvovaginal yeast infections caused by *C. albicans* decreased from 51.3% in 1994-1996 to 43.1% in 1999-2001. In contrast, the occurrence of *C. glabrata* was found to increase from 17.9% in 1994-1996 to 32.5% in 1999-2001. Statistical analyses indicated that in the period 1994-1996 there was a statistically significant difference between the proportion of vulvovaginal candidiasis caused by *C. albicans* and by *C. glabrata* ($P = 0.000$). Furthermore, there was no significant difference between *C. albicans* and *C. glabrata* during the 1999-2001 study period ($P = 0.05$). On the other hand, the proportions of vulvovaginal candidiasis caused by *C. albicans* during the two study periods (1994-1996 and 1999-2001) showed no significant difference. There was a significant difference; however, in the proportions of vulvovaginal candidiasis caused by *C. glabrata* in the two study periods ($P = 0.0186$), indicating a marked increase in vulvovaginal candidiasis caused by *C. glabrata* between the periods 1994-1996 and 1999-2001. The isolation rate of *C. krusei* remained at approximately 6.4%; rates of *C. tropicalis* isolation were 12.8% and 8.1% in the 1994-1996 and 1999-2001 periods, respectively. Other *Candida* spp. causing as follows: fewer than 10.1% of all vulvovaginal infections during the two study periods were *C. parapsilosis*, 3.4%; *C. kefyr* 5.9%, and *C. guilliermondii* 0.8%.

DISCUSSION

During the past two decades, several investigators have reported that the frequency of yeast infections, especially with the *Candida* spp., has increased dramatically (9,10,29). This increase has been accompanied by an increase in the number of *Candida* spp. recognized to cause disease (6,7,10,16,23). In this study, yeast was recovered from 160 (44.9%) of the 356 patients with variable degrees of abnormal vaginal discharge. These findings agree with the observations reported by other investigators that yeast can be recovered in the range of 5-53.3% in healthy women (29,30). The highest infection rate (56.8%) was detected among women in the 30-40 age group, which represents the most active sexual age. The incidence of vulvovaginal yeast infections decreased in older age groups (1,5).

Consistent with previous findings (26,31-33), there was no difference in the sensitivity of CA and SDA. We were able to identify to the species level 100% of the isolates of *C. albicans*, *C. tropicalis*, and *C. krusei* on CA, which confirms the results of other studies (26,31-33). Isolates of *C. glabrata*, the second-most common yeast isolated in our survey, presented more difficulties. Dark pink-purple colonies with a pale edge were usually *C. glabrata*, but 12% (confirmed by API 20C) did not present enough constant definitive colonial features on CA. The results of this study confirm the difficulties with *C. glabrata* identification encountered with CA reported by others (26,32), who felt that further methods were required for confirmation of *C. glabrata* identity.

C. albicans was isolated from 43.1% of the culture-positive patients, followed by *C. glabrata* 32.5%, and *C. tropicalis* 8.1% patients; these results are consistent with those presented previousy (29). The high isolation rate of *C. glabrata* from high-vaginal swab specimens has been reported by others (7,8,10,20,34). In fact, depending on the site of infection, *C. glabrata* is often the second or third most common cause of candidiasis after *C. albicans* (7). However, more recent epidemiological studies showed a mycological shift from *C. albicans* to non-*albicans Candida* spp. such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* (6,9,15,17,20,35). Spinillo et al. (20) showed that the proportion of vulvovaginal candidiasis cases caused by non-*albicans Candida* spp. rose from 9.9% in 1988 to 17.2% in 1995. All of these studies have found that *C. glabrata* has risen in incidence, and researchers have suggested that the use of fluconazole may have contributed to the increase in the isolation of *C. glabrata* and the decrease in *C. albicans* isolation. Vazquez et al. (34) reported that within 6 months of initiation of fluconazole prophylaxis, the isolation of *C. albicans* decreased by 60%, while the isolation of *C. glabrata* was >70% higher in patients assigned fluconazole than in those assigned placebo. These results are similar to those reported by other investigators (4,9,24). *C. glabrata* is of special importance because of its innate resistance to antifungal agents, specifically to azoles (3,7-9,36,37). The specific mechanisms of antifungal resistance to the azole class of antifungal agents are not yet fully understood. 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 multi-azole cross-resistance (8,34). The statistical analysis of data obtained in the present study clearly shows an increase in the incidence of vulvovaginal candidiasis caused by *C. glabrata* between the periods 1994-1996 and 1999-2001, with the results agreeing well with those of the above-mentioned studies.

This study has also revealed that the symptoms most frequently complained of by the majority of women with a positive culture for yeast are leukorrhea, a burning sensation, itching, and dysuria. Willmott (38) found that symptoms, particularly vaginal irritation, were reported by over half of patients having yeast-containing vaginal cultures. Geiger et al. (39) showed that abnormal discharge was less frequent in women with symptomatic vaginitis due to *C. glabrata* than to *C. albicans*, which may reflect the effects of a lack of hypha formation by the *C. glabrata* blastoconidia (8,10). Vaginitis due to *C. glabrata* was reported to be the main indolent with reduced inflammation and hence less dyspareunia (39). Moreover, patients with *C. glabrata* vaginitis frequently reported a burning sensation rather than itching (2,8,39).

The results presented indicate that the frequency of *C. glabrata* vaginitis has increased from 17.9% in 1994-1996 to 32.5% in 1999-2001. The described increase in the occurrence of *C. glabrata* and other non-*albicans Candida* spp. occurred some years after the introduction of fluconazole as a prophylactic agents for use by patients with vulvovaginal candidiasis.

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REFERENCES

1. Sobel, J. D. (1992): Pathogenesis and treatment of recurrent vulvovaginal candidiasis. Clin. Infect. Dis., 14 (Suppl.), S148-S153.
2. Giraldo, P., von Nowaskonski, A., Gomes, F. A., Linhares, I., Neves, N. A. and Witkin, S. S. (2000): Vaginal colonization by *Candida* in asymptomatic women with and without a history of recurrent vulvovaginal candidiasis. Obstet. Gynecol., 95, 413-416.
3. Ringdahl, E. N. (2000): Treatment of recurrent vulvovaginal candidiasis. Am. Fam. Phys., 61, 3306-3312.
4. Sobel, J. D. (1999). Limitations of antifungal agents in the treatment of *Candida* vaginitis: future challenges. Drug Resist. Updates, 2, 148-152.
5. Odds, F. C. (1988): Candidiasis of the genitalia. p.124-135. *In* F.C. Odds (ed.), Candida and Candidosis. A Review and Bibliography, 2nd ed., Balliere-Tindal, London.
6. Knoke, M., Schulz, K. and Bernhardt, H. (1997): Dynamics of *Candida* isolations from humans from 1992-1995 in Greifswald, Germany. Mycoses, 40, 105-110.
7. Pfaller, M. A. (1996): Nosocomial candidiasis: emerging species, reservoirs, and modes. Clin. Infect. Dis., 22, Suppl., 89-94.
8. Fidel, P. L., Vazquez, J. A. and Sobel, J. D. (1999): *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clin. Microbiol. Rev., 12, 80-96.
9. Berrouane, Y. F., Herwaldt, L. A. and Pfaller, M. A. (1999): Trends in antifungal use and epidemiology of nosocomial yeast infections in a university hospital. J. Clin. Microbiol., 37, 531-537.
10. Hazen, K. C. (1995): New and emerging yeast species. Clin. Microbiol. Rev., 8, 462-478.
11. Nguyen, M. H., Peacock, J. E., Morris, A. J., Tanner, D. C., Nguyen, M. L., Snydman, D. R., Wagener, M. M., Rinaldi, M. G. and Yu, V. L. (1996): The changing face of candidemia: emergence of non-*Candida albicans* species and antifungal resistance. Am. J. Med., 100, 617-623.
12. Wingard, J. R. (1995): Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. Clin. Infect. Dis., 20, 115-125.
13. Rex, J. H., Bennett, J. E., Sugar, A. M., Pappas, P. G., van der Horst, C. M., Edwards, J. E., Washburn, R. G., Scheld, W. M., Karchmer, A. W., Dine, A. P., Levenstein, M. J. and Webb, C. D. (1994): A randomized trial comparing fluconazole with amphotericin B for the treatment of candidemia in patients without neutropenia. N. Engl. J. Med., 331, 1325-1330.
14. Rex, J. H., Pfaller, M. A., Barry, A. L., Nelson, P. W. and Webb, C. D. (1995): Antifungal susceptibility testing of isolates from a randomized, multicenter trial of fluconazole versus amphotericin B as treatment of non-neutropenic patients with candidemia. Antimicrob. Agents Chemother., 39, 40-44.
15. Wingard, J. R., Merz, W. G., Rinaldi, M. G., Miller, C. B., Karp, J. E. and Saral, R. (1993): Association of *Torulopsis glabrata* infections with fluconazole prophylaxis in neutropenic bone marrow transplant patients. Antimicrob. Agents Chemother., 37, 1847-1849.
16. Abi-Said, D., Anaissie, E., Uzun, O., Raad, I., Pinzcowski, H. and Vartivarian, S. (1997): The epidemiology of hematogenous candidiasis caused by different *Candida* species. Clin. Infect. Dis., 24, 1122-1128.
17. Price, M. F., LaRocco, M. T. and Gentry, L. O.(1994): Fluconazole susceptibilities of *Candida* species and distribution of species recovered from blood cultures over a 5-year period. Antimicrob. Agents Chemother., 38, 1422-1424.
18. Rex, J. H., Rinaldi, M. G. and Pfaller, M. A. (1995): Resistance of *Candida* species to fluconazole. Antimicrob. Agents Chemother., 39, 1-8.
19. Sinofsky, F. E. (1999): Vulvovaginal candidiasis: topical versus oral therapy. The Female Patient, 24, 19-23.
20. Spinillo, A., Capuzzo, E., Gulminetti, R., Marone, P., Colonna, L. and Piazzi, G. (1997): Prevalence of and risk factors for fungal vaginitis caused by non-albicans species. Am. J. Obstet. Gynecol., 176, 138-141.
21. Sobel, J. D., Faro, S., Force, R., Foxman, B., Ledger, W. J., Nyirjesy, P. R., Reed, B. D. and Summers, P. R. (1998): Vulvovaginal candidiasis: epidemiologic, diagnosis, and the therapeutic considerations. Am. J. Obstet. Gynecol., 178, 203-211.
22. Schuman, P., Capps, L. and Peng, G. (1997): Weekly fluconazole for the treatment of mucosal candidiasis in women with HIV infection. A randomized, double blind, placebo controlled trial. Ann. Intern. Med., 126, 689-696.
23. Hazen, K. C., Theisz, G. W. and Howelly, S. A. (1999): Chronic urinary tract infection due to *Candida utilis*. J. Clin. Microbiol., 37, 824-827.
24. Febre, N., Silva, V., Medeiros, E. A. S., Wey, B., Colombo, A. L. and Fischman, O. (1999): Microbiological characteristics of yeasts isolated from urinary tracts of intensive care unit patients undergoing urinary catheterization. J. Clin. Microbiol., 37, 1584-1586.
25. Lockhart, S. R., Joly, S., Vargas, K., Swails-Wenger, J., Enger, L. and Soll, D. R. (1999): Natural defenses against *Candida* colonization breakdown in the oral cavities of the elderly. J. Dent. Res., 78, 857-868.
26. Odds, F. C. and Bernaerts, R. (1994): CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol., 32, 1923-1929.
27. Warren, N. G. and Hazen, K. C. (1995): *Candida, Cryptococcus*, and other yeasts of medical importance. p 723-737. *In* P. R. Murray, E. J. O. Baron, M. A. Pfaller, F. C. Tenover and R. H. Yolken (eds), Manual of Clinical Microbiology. American Society of Microbiology, Washington, D.C.
28. Pfaller, M. A. (1989): Infection control: opportunistic fungal infections-the increasing importance of *Candida* species. Infect. Control Hosp. Epidemiol.,10, 270-273.
29. Abu-Elteen, K. H., Abdul-Malek, A. M. M. and Abdul-Wahid, N. A. (1997): Prevalence and susceptibility of vaginal yeast isolates in Jordan. Mycoses, 40, 179-185.
30. Al-Rawi, N. and Kavanagh, K. (1999): Characterization of yeasts implicated in vulvovaginal candidiasis in Irish women. Br. J. Biomed. Sci., 56, 99-104.
31. Houang, E. T. S., Chu, K. C., Koehler, A. P. and Cheng, A. F. B. (1997): Use of CHROMagar Candida for genital specimens in the diagnostic laboratory. J. Clin. Pathol., 50, 563-565.
32. Ainscough, S. and Kibbler, C. C. (1998): An evaluation

of the cost-effectiveness of using CHROMagar for yeast identification in a routine microbiology laboratory. J. Med. Microbiol., 47, 623-628.
33. Pfaller, M. A., Houston, A. and Coffmann, S. (1996): Application of CHROMagar Candida for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida* (*Torulopsis*) *glabrata*. J. Clin. Microbiol., 34, 58-61.
34. Vazquez, J. A., Sobel, J. D., Peng, G., Steele-Moore, L., Schuman, P., Holloway, W. and Neaton, J. D. (1999): Evolution of vaginal *Candida* species recovered from human immunodeficiency virus-infected women receiving fluconazole prophylaxis: the emergence of *Candida glabrata*. Clin. Infect. Dis., 28, 1025-1031.
35. Abbas, J., Bodey, G. P., Hanna, H. A., Mardani, M., Girgawy, E., Abi-Said, D., Whimbey, E., Hachem, R. and Raad, I. (2000): *Candida krusei* fungemia. Arch. Intern. Med., 160, 2659-2664.
36. Kronvall, G. and Karlsson, I. (2001): Fluconazole and voriconazole multidisk testing of *Candida* species for disk test calibration and MIC estimation. J. Clin. Microbiol., 39, 1422-1428.
37. Chang, H. C., Chang, J. J., Chan, S. H., Huang, A. H., Wu, T. L., Lin, M. C. and Chang, T. C. (2001): Evaluation of Etest for direct antifungal susceptibility testing of yeasts in positive blood cultures. J. Clin. Microbiol., 39, 1328-1333.
38. Willmott, F. E. (1975): Genital yeasts in female patients attending a VD clinic. Br. J. Vener. Dis., 51, 119-122.
39. Geiger, A. M., Foxman, B. and Sobel, J. D. (1995): Chronic vulvovaginal candidiasis: characteristics of women with *Candida albicans*, *Candida glabrata*, and no *Candida*. Genitourin. Med., 71, 304-307.