

Original Research Article

A study on fluconazole resistance among candida species isolated from patients attending STD OPD in a tertiary care hospital

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Abstract

Back ground: Sexually transmitted infections are a significant public health problem worldwide as they cause widespread morbidity and mortality in both male & female. In developing countries, STI are the third most common public health problem in young people of reproductive age group.

Aim of the Study: This study was conducted to determine the prevalence of Candida species causing genital candidiasis in patients attending STD clinic in tertiary care hospital & to know the fluconazole resistance pattern of the isolated Candida species.

Materials and methods: KOH mount is done by adding 10% KOH to the smear made with the vaginal discharge to look for the presence of yeast cells/ hyphal elements. Gram stain is done to look for the presence of gram positive budding yeast cells.

Results: In this present study, Candida glabrata was the most common species causing vaginal candidiasis (47%) and fluconazole resistance is more among the Candida non-albicans isolates (62%). Candida albicans is the most common species isolated from male samples. Fluconazole resistance is also increasing among the Candida albicans isolates (32% in females and 30% in males).

Conclusion: Prevalence of fluconazole resistance among Candida isolates is also increasing. This is due to the inappropriate and over the counter usage of antifungal agents. The emergence of

fluconazole resistant *C. albicans* and non *albicans* species emphasises the need of species identification and antifungal susceptibility in the diagnosis and management of vaginal candidiasis.

Key words

Fluconazole resistance, *Candida albicans*, *Candida non-albicans* isolates.

Introduction

Sexually transmitted infections are a significant public health problem worldwide as they cause widespread morbidity and mortality in both male and female. In developing countries, STI are the third most common public health problem in young people of reproductive age group [1]. Vulvovaginal candidiasis is a common sexually transmitted fungal infection among females in reproductive age group. Approximately 75% of all women experience at least one episode of genital candidiasis during their lifetime and nearly half of them with multiple episodes [2]. Though genital *Candida* infection is not life threatening, it is usually associated with morbidities like itching, burning sensation, discomfort, pain, sexual dysfunction etc. [3]. Diabetes mellitus, using contraceptive devices, long term usage of antibiotics and other immunocompromised status predispose to genital candidiasis. The major cause of vulvovaginal candidiasis (85%-90%) is due to *Candida albicans* but infection due to *Candida nonalbicans* like *Candida glabrata*, *Candida tropicalis*, *Candida krusei* etc. is increasing in the last decade [4]. In a study *Candida glabrata* is the main cause VVC. In males, balanitis occurs in 10% of those attending genitourinary clinic and the most common cause is candidiasis. Resistance to antifungal agents is more common among the *Candida non albicans* and so they are very difficult to treat [5]. The increasing antifungal resistance is due to the inappropriate usage and due to the use of over the counter antifungal agents widely in developing countries. Since fluconazole is used for treating *Candida* infection very commonly, the increasing resistance to fluconazole is an emerging problem nowadays. This indicates the importance of knowing the species of *Candida* causing genital

candidiasis and about the antifungal susceptibility of *Candida* species [6].

Materials and methods

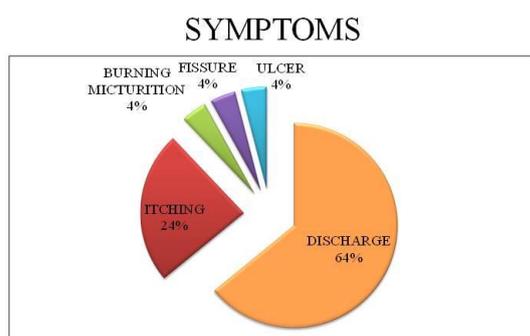
This prospective study was conducted from April 2016 to July 2016 in a tertiary care hospital in Chennai, Tamil Nadu, India. Inclusion criteria: Both males & females in the age group of 18 to 49 years who attended the STD op with symptoms of vaginal discharge/genital itching/genital burning were included in the study. In females, under speculum examination, swabs were taken from the posterior fornix. In males, cotton swab was moistened with saline and glans was rubbed to collect the discharge. Three swabs were collected, one for KOH mount, one for gram stain and one for culture of *Candida*. KOH mount is done by adding 10% KOH to the smear made with the vaginal discharge to look for the presence of yeast cells/hyphal elements. Gram stain is done to look for the presence of gram positive budding yeast cells. The swab taken for culture is inoculated in Sabouraud's dextrose agar and incubated at 37°C for 24 hours. Species identification was done with standard procedures like germ tube test, growth at 45°C, chlamyospore formation on corn meal agar. All the *Candida* cultures were inoculated in CHROM agar and incubated at 37°C for 24 hours and the species were identified by the colour of the colonies as per the manufacturer's instructions. All the culture strains were subjected to antifungal susceptibility testing with fluconazole disc 25µg by disc diffusion method as per CLSI guidelines M44-A documents.

Results

A total of 9816 patients attended the STD clinic during the month of April 2016 to July 2016. Out of them, 505 females and 41 males were

symptomatic with symptoms of vaginal discharge, itching, burning micturition, ulcer and fissure. The most common presenting symptom was vaginal discharge (64%), followed by itching in females. Vaginal swabs from 505 symptomatic females were subjected to KOH mount, Gram stain & Candida culture. 83 samples were found to be culture positive for Candida with *Candida glabrata* as the most common species (39 isolates, 47%), followed by *Candida albicans* (28 isolates, 34%). The other *Candida* species isolated are *Candida krusei* (9, 11%) and *Candida tropicalis* (7, 8%). Thus the overall prevalence of *Candida non-albicans* was 66%. Among the 55 *Candida non-albicans* isolates, 21 isolates (38%) were found to be fluconazole sensitive and 34 isolates (62%) fluconazole resistant. Among the 28 isolates of *Candida albicans* from vaginal discharge, 19 (68%) were fluconazole sensitive and 9 (32%) were fluconazole resistant. Among the 41 samples from male patients, 10 were found to be culture positive with all the 10 isolates *Candida albicans*. 70% isolates fluconazole sensitive and 30% isolates fluconazole resistant. In this present study, *Candida glabrata* was the most common species causing vaginal candidiasis (47%) and fluconazole resistance is more among the *Candida non-albicans* isolates (62%). *Candida albicans* is the most common species isolated samples from male patients. Fluconazole resistance is also increasing among the *Candida albicans* isolates (32% in females and 30% in males) (**Figure – 1, 2, 3**).

Figure – 1: Symptoms.



Discussion

This study was done to identify the *Candida* species associated with genital candidiasis and their susceptibility pattern to fluconazole in both male and female patients attending STD OP. In our study, genital candidiasis is found to be more common in females in reproductive age group of 25 to 45 years (73%). This finding is similar to the studies conducted by Ako, et al. [7] and Sehgal, et al. [8]. This may be due to the low levels of protective cervical antibodies, increased sexual activity and influence of reproductive hormones which lead to increased susceptibility to RTI [9]. The risk factors in this age group includes use of oral contraceptive pills, intra uterine devices, broad spectrum antibiotics and diabetes mellitus [10]. The rate of prevalence of vulvovaginal candidiasis is 16.4% in our study which is low when compared to other studies. In a study conducted by Srujana Mohanty et al, the isolation of yeast from vulvovaginal candidiasis was 18.5% which is similar to our study [11]. Similar results were found in some other studies from India and elsewhere with rate of *Candida* infection ranging from 20.8 to 23%. In our study, *Candida glabrata* is the most common species isolated in vaginal candidiasis (47%) with the overall prevalence of *Candida non-albicans* 66% and *Candida albicans* 34%. This is very similar to the study conducted by Mohanty, et al. [12] in which the rate of *Candida glabrata* isolated was 50.4%. *Candida glabrata* is the most common vaginal isolate in some other studies also from India, Nigeria. *Candida glabrata* is the second most common species in some regions. In an Australian study, the isolation of non-albicans species in VVC was only 11% [13] and Spinillo, et al. in an Italian study reported 17% of VVC due to non-albicans.. It was 24% in a study conducted by Richter et al. In more recent studies from India, Iran, Nigeria, Australia & Turkey, there was increasing rate of isolation non-albicans species [14]. This increase in the isolation of *Candida non-albicans* species is due to the inappropriate usage of antifungal medications, long term treatment and use of over the counter antimycotics. The inappropriate

usage of antifungal agents leads to reduction in the *Candida albicans* species and emergence of *Candida non-albicans* species [15]. This is now more common in countries like India, Iran, Nigeria, Turkey etc according to various studies. Antifungal susceptibility test was done with fluconazole which is used as the first line drug for vulvovaginal candidiasis. Our study showed that among the 55 isolates of *Candida non-albicans*, 34 isolates (62%) were found to be resistant to fluconazole and 21 isolates (38%) were fluconazole sensitive [16]. We found from this study that fluconazole resistance is emerging among *Candida non-albicans* species particularly *Candida glabrata* [19]. In a study conducted by Zahra Salehei, et al. in Iran, all the 8 isolates of *Candida glabrata* were resistant to fluconazole. In

a study by Richter et al, 67% of *Candida glabrata* from vaginal samples was non susceptible to fluconazole [7]. Among the 28 isolates of *Candida albicans* 19 (68%) were sensitive to fluconazole and 9 (32%) resistant to fluconazole [18]. In an US study among the 401 *Candida albicans* isolates recovered from recurrent vaginal candidiasis, no fluconazole resistance was found. No fluconazole resistance was found among 75 *Candida albicans* isolates from symptomatic women in an England study. In a study by Zahra Salehei from Iran, 43 of 53 isolates were resistant to fluconazole [20]. Our study results showed that fluconazole resistance is increasing among the *Candida albicans* species also when compared to the previous studies [21].

Figure – 2: Culture on Sabouraud’s agar.

CULTURE ON SABOURAUD’S DEXTROSE AGAR

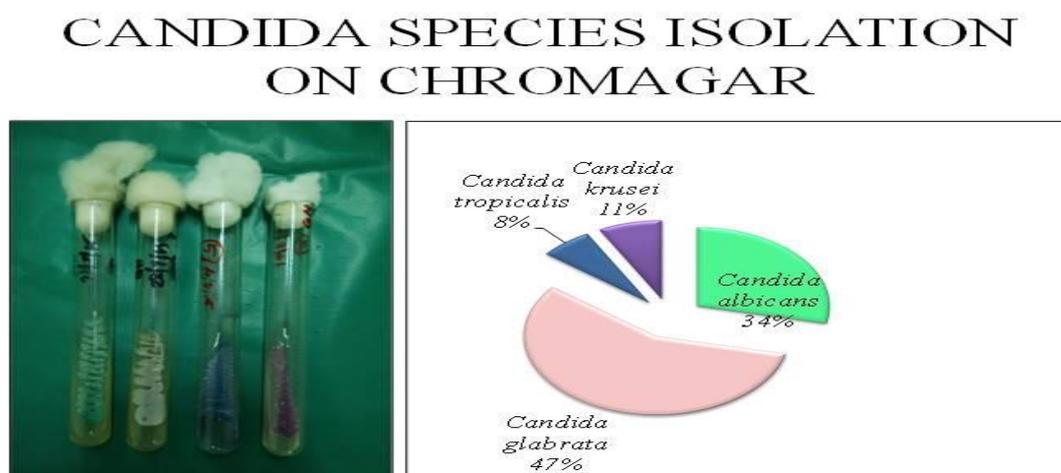


Conclusion

Culture is the gold standard for the diagnosis of vulvovaginal candidiasis. Inoculation in CHROM agar is a simple and effective method that helps in detection and speciation of different isolates *Candida*. Prevalence of *Candida non albicans* in genital candidiasis is increasing particularly in developing countries. Prevalence of fluconazole resistance among *Candida* isolates is also increasing. This is due to the inappropriate and over the counter usage of antifungal agents.

The emergence of fluconazole resistant *C. albicans* and non albicans species emphasises the need of species identification and antifungal susceptibility in the diagnosis and management of vaginal candidiasis. Treatment should be given based on the sensitivity pattern to prevent the development of resistance and recurrence. Periodic follow up and monitoring of patients is essential for cure and prevention of recurrence. Larger prospective studies should be done in future to assess the antifungal sensitivity pattern in genital candidiasis.

Figure – 3: Candida species isolation on Chromagar.



References

1. Ferrer J. Vaginal candidosis: epidemiological and etiological factors. Intl J Gynecol Obstet., 2000; 71: S 21-7.
2. Ehrstrom S, Yu A, Rylander E. Glucose in vaginal secretions before and after oral glucose tolerance testing in women with and without recurrent vulvovaginal candidiasis. Obstet Gynecol., 2006; 108(6): 1432-7.
3. Nyirjesy P, Peyton C, Weitz MV, Mathew L, Culhane JF. Causes of chronic vaginitis: analysis of a prospective database of affected women. Obstet Gynecol., 2006; 108(5): 1185-91.
4. Paulitsch A, Weger W, Ginter-Hanselmayer G, Marth E, Buzina W. A 5-year (2000-2004) epidemiological survey of Candida and non-Candida yeast species causing vulvovaginal candidiasis in Graz, Austria. Mycoses, 2006; 49(6): 471-5.
5. Jilek P, Spacek J, Buchta V, Kucera Z, Drahosova M, Forstl M, et al. [Systemic immunity in patients with recurrent vulvovaginal candidiasis]. Ceska Gynekol., 2005; 70(6): 453-9.
6. Spinillo A, Capuzzo E, Gulminetti R, Marone P, Colonna L, Piazzini G. Prevalence of and risk factors for fungal vaginitis caused by non-albicans species. Am J Obstet Gynecol., 1997; 176: 138-41.
7. Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. J Clin Microbiol., 2005; 43: 2155-62.
8. Aali BS, Tohidi A. Prevalence of Candida vaginitis among symptomatic patients in Kerman. J Qazvin Uni Med Sci., 2000; 13: 42-8.
9. Mohanty S, Xess I, Hasan F, Kapil A, Mittal S, Tolosa JE. Prevalence & susceptibility to fluconazole of Candida species causing vulvovaginitis. Indian J Med Res., 2007; 126(3): 216-9.
10. Solanki A, Mathur DR, Joshi KR. Bacterial, fungal and parasitic flora in vaginitis. J Indian Med Assoc., 1983; 81: 151-3.
11. Goswami R, Dadhwal V, Tejaswi S, Datta K, Paul A, Haricharan RN, et al. Species-specific prevalence of vaginal candidiasis among patients with diabetes mellitus and its relation to their glycaemic status. J Infect., 2000; 41: 162-6.
12. Goldacre MJ, Milne LJR, Watt B, Loudon N, Vessey MP. Prevalence of yeasts and fungi other than *Candida albicans* in the vagina of normal young

- women. Br J Obstet Gynecol., 1981; 88: 596-600.
13. Holland J, Young ML, Lee O, C-A Chen S. Vulvovaginal carriage of yeasts other than *Candida albicans*. Sex TransmInfect., 2003; 79: 249-50.
 14. Defontaine A, Bouchara JP, Declerk P, Planchenault C, Chabasse D, Hallet JN. In-vitro resistance to azoles associated with mitochondrial DNA deficiency in *Candida glabrata*. J Med Microbiol., 1999; 48(7): 663-70.
 15. Ako-Nai AK, Kassim OO, Adeniran MO, Taiwo O. A study of urinary tract infections at Ile-Ife, Nigeria. East Afr Med J, 1993; 70(1): 10-14.
 16. Sehgal SC. Epidemiology of male urethritis in Nigeria. J Trop Med Hyg., 1990; 93(2): 151-2.
 17. Sobel JD, Wiesenfeld HC, Martens M, Danna P, Hooton TM, Rompalo A, et al. Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. N Engl J Med., 2004; 351: 876-83.
 18. El- Din SS, Reynolds MT, Ashbee HR, Barton RC, Evans EGV. An investigation into the pathogenesis of vulvovaginal candidiasis. Sex Trans Infect., 2001; 71: 179-83.
 19. Omar AA. Gram stain versus culture in the diagnosis of vulvovaginal Candidiasis. East Mediterr Health J, 2001; 7: 925-34.
 20. Oviasogie FE, Okungbowa FI. *Candida* species amongst pregnant women in Benin city, Nigeria: Effect of predisposing factors. Afr J Clin Exper Microbiol., 2009; 10: 92-8.
 21. Neerja J, Aruna A, Paramjeet G. Significance of *Candida* culture in women with vulvovaginal symptoms. J Obstet Gynecol India, 2006; 56: 139-41.