

Antifungal resistance of *Candida* species isolated from Iraqi women infected with vulvovaginal Candidiasis

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الخلاصه

ان شيوع التهاب المهبل والفرج الفطري (داء المبيضات) المتسبب عن المبيضات البيضاء والاجناس الاخرى للمبيضات قد ازداد بصورة مثيرة في العقود القليلة الماضية. لا يجرى الزرع الجرثومي للافرزات المهبلية بصورة روتينيه وكذلك عدم توفر فحص الحساسيه لاجناس المبيضات لمضادات الفطرياتهدفت هذه الدراسه الى:
1- تحديد اجناس الفطريات وانتشارها بين النساء المصابات بداء المبيضات.
2- دراسه شيوع المقاومه لاجناس المبيضات المعزوله لمضادات الفطريات.
جمعت مائه مسحه مهبلية من النساء اللاتي تتراوح اعمارهن من 17-40 سنه وكان لديهن علامات واعراض داء المبيضات وراجعن العياده الاستشاريه الخارجيه لمستشفى مدينه الطب. خضعت جميع المسحات المهبلية لدراسه الفطريات كالفحص المجهرى والزرع الجرثومي وتعيين كامل للجنس بواسطه اختبار الانبوب الجرثومي وكذلك اختبار (API®) (*Candida*) واخيرا اجراء اختبار الحساسيه لمضادات الفطريات باستخدام طريقه الاقراص المنتشره المحوره مع ست من المضادات الفطريه وهي Nystatin و AmphotericinB و Fluconazole و Ketoconazole و Clotrimazole و Econazole. المبيضات البيضاء كان الجنس الاكثر تكرارا بحوالي 61.66 % يليها مبيضات الجلبراتا وعزله واحده فقط 1,6% من كل من الاجناس الثلاثه التاليه مبيضات تروبيكالس ومبيضات كروزي ومبيضات كيفيرلهذا كان تكرار ما مجموع الاجناس الاخرى للمبيضات يشكل حوالي 38.13%. كانت مقاومه عزلات المبيضات البيضاء قليله ضد Fluconazole 21,62% و Ketoconazole 8,1% و Econazole 5,4% بينما لم تلاحظ المقاومه بين المبيضات البيضاء ضد Clotrimazole كانت معظم الاجناس الاخرى للمبيضات مقاومه لمضادات الفطريات ال(azoles) خصوصا بين عزلات مبيضات الجلبراتا 75% Ketoconazole 70% و Clotrimazole 65% و Fluconazole 20% Econazole اظهرت نتائج الدراسه ان هناك زياده في تكرار ما مجموع الاجناس الاخرى للمبيضات التي تم عزلها من الزرع الجرثومي للمهبل خاصه مبيضات الجلبراتا التي تعتبر اكثر الاجناس شيوعا بعد المبيضات البيضاء. كشف اختبار الحساسيه لمضادات الفطريات ان اجناس المبيضات الاخرى خاصه مبيضات الجلبراتا مقاومه لمضادات الفطريات (azoles) على خلاف عزلات المبيضات البيضاء لذلك الزرع الجرثومي للمسحات الهبلية المأخوذه من النساء المصابات بداء المبيضات يجب ان يجرى للكشف عن الاجناس الاخرى للمبيضات.

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Abstract

The prevalence of vulvovaginal candidiasis caused by *C. albicans* and other *Candida* species has increased dramatically over the past few decades. Vaginal cultures and susceptibility testing of these *Candida* species to antifungal are not routinely obtained. Therefore the aims of this study were to

1. Determine the *Candida* species distribution among infected women with vulvovaginal candidiasis
2. Prevalence of antifungal resistance among isolated *Candida* species

One hundred vaginal swabs were collected from women their age ranged from 17-40 years old with signs and symptoms of vulvovaginitis attending Medical City Hospital. All swabs were submitted to mycological study (microscopical examination, culture, full identification by performing germ tube test and Api®-candida). Finally, susceptibility testing was carried out by using modified disc diffusion method with six antifungal agents Amphotericin B, Nystatin, Fluconazole, Ketoconazole, Clotrimazole and Econazole. *C. albicans* was the most frequent species. (61.66%) followed by *C. glabrata* (33.33%) and only one isolate (1.6%) of *C. tropicalis*, *C. krusei* and *C. kefyr*, so the frequency of overall of other *Candida* species was (38.13%). However, few resistant *C. albicans* isolates were found against Fluconazole (21.62%), Ketoconazole (8.1%) and Econazole (5.4%). While no resistant was observed in *C. albicans* against Clotrimazole. Most of other *Candida* species were resistant to azoles specially among most isolates of *C. glabrata* to Ketoconazole 75%. Clotrimazole 70%, Fluconazole 65% and Econazole 20%.

Our results showed elevated frequency of the overall of other *Candida* species particularly *C. glabrata* which the most prevalent species followed by *C. albicans*. Other *Candida* species specially *C. glabrata* were resistant to azoles in contrast to the most of *C. albicans* therefore culture from women with vulvovaginal candidiasis should be obtained to detect other *Candida* species

Key words: *Candida* species, antifungal resistance, Iraqi women

Introduction

Vulvovaginal candidiasis (VVC) remains one of the most common infections of the female genital tract, which is classified by the world health organization (WHO) as a sexually transmitted disease of frequent sexual transmission (1,2). It has been estimated that up to 75% of women will have at least one episode of candidiasis during their lives, with 40% to 50% experiencing chronic recurring episodes (1,2,3,4). *C.albicans* is the most common etiological agent of VVC but there is little evidence of significant increase in infection rate due to the other *Candida* species (spp.) such as *C.glabrata*, *C.krusie* and *C.tropicalis* (5,6). Species identification of the yeast involved in VVC is not only an important step for a better understanding of the distribution of *C.albicans* /other *Candida* spp. in different sub-populations of patients, but the data obtained can provide a very accurate view of antifungal susceptibility rates. In fact, innate or acquired resistance to available antifungal agents is now recognized among pathogenic fungi (8). It was reported that most women have tried at least one course of over-the counter antimycotic therapy as a topical and systemic azoles (e.g in cream., shampoo, powder or in washing machine detergents) (9) as well as patients who see a physician usually receive empirical therapy ; vaginal culture are not routinely obtained and susceptibility testing is rarely performed (10,11).

It has been estimated that antifungal therapy was inappropriately prescribed in 54% of culture negative cases, such unnecessary use of antifungal therapy contributes to the development of antifungal resistance and the emergence of infections associated with other *Candida* spp. and other opportunistic fungi (12). There is evidence, however of an increased azole resistant among isolates of *Candida* Spp. isolated from women with VVC, other *Candida* are generally more resistant to azoles than *C.albicans* (5). Consequently assessment of susceptibility and resistance to antifungal agents among pathogenic isolates of *C.albicans* and other *Candida* spp. recovered from women with VVC is dependent upon the use of disc diffusion method which is very simple and practical method for

performing susceptibility test on yeasts (13,14). Therefore the aims of our study were to

1. Determine the *Candida* spp. distribution among infected women with VVC
2. Prevalence of antifungal resistance among isolated *Candida* spp.

Materials and methods

Vaginal *Candida* spp. were collected from 60 out of 100 non pregnant women their age ranged from 17-40 years old with signs and symptoms suggestive of candidial vulvovaginitis that attending Medical City Hospital from June 2008 to April 2009. All vaginal swabs were submitted to mycological study, direct microscopic examination then plated on sabrouaud dextrose agar (SDA) and incubated for 72 hr. at 37C. The *Candida* isolates were identified to spp. level based on traditional methods of staining and growth morphology, germ tube testing and by the Api®-*Candida* (BioMerieux® SA).

Finally, an *in-vitro* antifungal susceptibility testing was performed on all *Candida* isolates using modified disc diffusion method, by adding 2% glucose to Mueller-Hinton agar(MHA) to accelerate growth of some spp. e.g *C. gabrata* (15).and 6.5 mm diameter disc (Bio-Rad discs) for Amphotericin B (AMB) ,Nystatin (NY), Fluconazole (FCN), Ketoconazole (KTA), Clotrimazole (CTM) and Econazole (ECN) the inocula concentration of *Candida* isolates were made from few colonies in sterile saline from an overnight subculture on SDA.

After mixing with vortex mixer the turbidity of the suspension was adjusted to match a 0.5 MacFarland turbidity standard and then diluted 1:2 with sterile saline solution. For *C.krusei* strain, inocula was equivalent to MacFarland 0.5 standard then diluted 1:10 in sterile saline solution (16). Mueller- Hinton surface was inoculated by streaking with moistened cotton swab then allowed to dry for around 15 mint, six antifungal discs were placed on surface of MHA using flamed forceps. The plate was incubated at 37C°, after 24 hr. the inhibition zones were measured in mm using a ruler.

Candida isolates classified by the disc diffusion method as susceptible, intermediate and resistant according manufactures instruction. For AMB and NY susceptible spp. were those with diameter zone >10mm ; intermediate or resistant =10mm ,for FCN susceptible spp. were those with diameter zones ≥30 mm, intermediate between 19-29mm and resistant with ≤18mm, for KTA,CTM and ECN susceptible spp. were those with diameter zone =20mm; intermediate between 10-20 mm and resistant with =10mm.

Results

During the study period it was found that 60 out of 100 of women’s vaginal swabs obtained from women with vulvovaginitis were culture positive for *Candida* spp. , 40 women’s vaginal swabs had other causes of vaginitis (Trichomoniasis, Bacterial vaginosis) were not reported in this study.

***Candida* species isolated by vaginal culture**

Table (1) illustrates the frequency of isolates of various *Candida* spp. *C.albicans* was the most frequently recovered spp. 37of 60 (61.66%) followed by *C.glabrata* 20 of 60 (33.33%) and only 1 of 60 (1.66%) of three women’s vaginal swabs were found to have different *Candida* spp. *C.tropicalis*, *C.krusie* and *C.kefyr*.

Table 1: *Candida* species isolated by vaginal culture

Candida species	frequency	
	No. of isolates	% of isolates
<i>C.albicans</i>	37	61.66
<i>C.glabrata</i>	20	33.33
<i>C.tropicalis</i>	1	1.66
<i>C.krusie</i>	1	1.66
<i>C.kefyr</i>	1	1.66
Total	60	100

Percentage of antifungal susceptibility of *Candida* species

In vitro antifungal susceptibility results of 60 *Candida* isolates are shown in table (2). All of *Candida* spp. tested were susceptible to AMB and NY (100%). The resistance was low in *C. albicans* against FCN, KTA and ECN (21.62%, 8.1% and 5.4%) respectively, while no resistance was observed in *C. albicans* against CTM. Conversely, resistance to azoles was frequent for other *Candida* spp. particularly among most isolates of *C. glabrata* against KTA, CTM, FCN and ECN (75%, 70%, 65% and 20%) respectively.

Finally, only one isolate of each of the following strains *C. tropicalis*, *C. krusei* and *C. kefyr* were resistant to azoles (FCN, KTA, CTM and ECN).

Table 2: Percentage of antifungal susceptibility of candida species

Candida species	Antifungal agents																		
	Amphotericin B			Nystatin			Fluconazole			Ketoconazole			Clotrimazole			Econazole			
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	
	SIZ 5/10	SIZ =10	SIZ =10	SIZ 5/10	SIZ =10	SIZ =10	SIZ 2/3	SIZ 1/2 2/3	SIZ 2/3	SIZ 2/3	SIZ 1/2 2/3	SIZ =10	SIZ =10	SIZ 1/2 2/3	SIZ =10	SIZ =10	SIZ 1/2 2/3	SIZ =10	SIZ-1/2 2/3
<i>C. albicans</i>	100%	-	-	100%	-	-	75.37 %	-	21.62 %	81.89 %	-	8.10 %	100%	-	-	94.59 %	-	-	5.44%
<i>C. glabrata</i>	100%	-	-	100%	-	-	15%	20%	65%	-	25%	75%	-	30%	70%	-	80%	20%	20%
<i>C. tropicalis</i>	100%	-	-	100%	-	-	-	-	100%	-	-	100%	-	-	100%	-	-	100%	100%
<i>C. krusei</i>	100%	-	-	100%	-	-	-	-	100%	-	-	100%	-	-	100%	-	-	100%	100%
<i>C. kefyr</i>	100%	-	-	100%	-	-	-	-	100%	-	-	100%	-	-	100%	-	-	100%	100%

S: Sensitive I: Intermediate R: Resistant
SIZ: Standard Inhibition Zone

Discussion

Although VVC the most common fungal disease in the world, little information is known about the distribution and etiology of candidiasis because microbiology tests are not routinely performed in laboratories (11,17)

Among the distribution of *Candida* spp. which are recovered from women's vaginal swabs, *C.albicans* was the most common spp. associated with vulvovaginitis. This finding is in agreement with other studies (8,11,18) reported that the *C.albicans* is the most predominant spp causing vaginal candidiasis that they revealed the frequency of *C.albicans* was between 61.2-70.82%, *C.glabrata* between 20.57%-31.5%, *C.tropicalis* between 1.8%-1.9% and *C.krusei* 1.8%-2.9% this may be because that *C.albicans* is a part of normal vaginal flora of reproductive age women (19), which cause *Candida* infection opportunistically due to altered conditions of the host, and at these altered conditions the fungus proliferates faster causing VVC (18). Although *C.albicans* represented the dominant spp of pathogenic yeast from women with VVC attending the outpatient clinic in Medical City Hospital, the frequency of other *Candida* spp was elevated with average of 38.13%, with *C.glabrata* as the most prevalent spp. 33.3%, this result is in accordance with many studies found that in recent years there has been a significant increase in infection caused by other *Candida* spp particularly *C.glabrata*, *C.tropicalis* and *C.krusei* they found the frequency of overall of other *Candida* spp were 34%-39%. (5,15,18).

Other study emphasized the widespread of over-the counter and alternative medicines to treat vaginal symptoms (9), on the other hand many studies were reported that widespread use of antifungal agents (azoles) appear to be associated with emerging resistance to these important antifungal agents in yeast (12,18,20) and it has been reported that the prolonged exposure to fluconazole can shift the predominant vaginal yeast flora from *C.albicans* to more intrinsically azole resistant spp.(21,22).

From the above results we can conclude that these spp which are more resistant to antifungal agents becoming more prevalent among clinical isolates from women with VVC may be due to

prolonged exposure to antifungal agents including topical agent, over-the counter, this may cause a shift in the predominant more susceptible *C.albicans* to less susceptible spp like *C.glabrata*, *C.tropicalis* and *C.krusei*.

Antifungal susceptibility testing results in our study revealed that non of Candida isolates tested were resistant to AMB and NY (Table 2) .This high AMB and NY susceptibility rate is in agreement with other studies who reported that resistant of Candida spp to AMB and other polyenes appear to be extremely uncommon (8,16,23), partly because of their irreversible fungicidal action once they are bound to the yeast cells (24).

Few resistant *C.albicans* isolates were found against FCN, ECN and KTA and no resistant was observed in *C. albicans* against CTM. While resistant to azoles were frequent for other Candida spp particularly among most isolates of *C.glabrata* to KTA, CTM, FCN and ECN and only one isolates of other Candida spp. (*C.tropicalis*,*C.krusei* and *C.kefyr*) were resistant to azole antifungal agents that used in our study.

Our finding is in agreement with results of many studies who reported that the occurrence of resistance to azole antifungal agents were greater for group of other Candida spp. especially *C.glabrata* (6,11,12,16,18). Many studies emphasized that *C.glabrata* and *C. krusei* are intrinsically less sensitive to azoles and some strains of *C.tropicalis* were resistant to azoles (5,18,25),because these organisms are haploid unlike *C.albicans* which is diploid and drug resistance is more likely to develop during treatment (25,26),on the other hand it was reported that there is evidence links empirical or prophylactic use of antifungal agents and selection for yeasts other than *C.albicans* that exhibit decrease susceptibility to azoles .Example include the emergence of *C.glabrata* and *C.krusei* infection in patients receiving prophylaxis (27).

Therefore we can suggest that the total utilization of non prescription use both systemic and topical azoles may be implicated in the emergence of resistant other Candida spp and *C. albicans* more susceptible than other Candida spp. to azoles

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