The susceptibility test of vaginal yeasts and their relationship with the age in Iraqi women

Bushra J . Mohamed* ***Amina N. AL. Thwani****

Received 4, March, 2009
Accepted 4, August, 2009

Abstract:

This study aims to isolate the pathogenic yeasts from genital tract and investigate their relationship with the age .The results clarified that the most pathogenic yeast isolated from genital tract was *Candida albicans* , also the results of *C.albicana*s isolates susceptibility test, to different antifungal revealed that they were sensitive to Miconazole, Ketoconazole and Clotrimazol and were resistant to Nystatin and Grisofulvin. The study of relationship of vaginal infection with the age showed that the incidence of infection with *Candida* was high among females age group (19-39 years).

Key words: vaginal yeasts, the age , *Candida spp*,antifungal drugs

Introduction:

In the adult female population vaginal infection is the most common reason for seeking medical attention [1] ,75% of all women suffer from at least one yeast infection during their lifetime and 50% recurrences occur in infected women(2) furthermore this infection is disturbs the natural balance of most women and it is one of the main causes of gynecologic morbidity such as infertility, ectopic pregnancy, preterm labor and chronic pelvic pain [3].

Yeast infection occurs in women regardless of their background and could be transmitted to her partner or to her child through delivery [4].

Symptoms of vaginal yeast infection include; itching burning redness stinging of urination ,abnormal discharge and pain during sexual intercourse [5].

Yeast vaginitis is a very common ailment, often precipitated by diabetes mellitus, immunity impairment, using antibiotics, pregnancy, excessive body hygiene, exposure to spermicides or hormonal changes and aging [6].

This study was designed to isolate and identify yeasts from genital tract and estimate the relationship of vaginal infection with the age.

Materials and Methods:

Vaginal swabs were randomly collected from 250 female patients who attended to the obstetric and gynecology clinics in Fatima AL-Zahra hospital in Baghdad during a period from the beginning of July 2006 to the end of March 2007, the aged of patients ranged between 19-67 years (mean age 38 years). Twenty five of control samples were collected from healthy women using sterile speculum and swabs by gynecologists.

Isolation and identification of yeasts from vaginal swabs:

Two vaginal swabs for each patient were transported to the laboratory by inoculating the swab into a sterile tube containing 3.0 ml of

*Genetic Engineering and Biotechnology Institute for Postgraduate Studies\Baghdad University

saturate transport medium (Sabourauds dextrose broth).One of the swabs was directly inoculated onto Sabourauds dextrose agar for microbiological investigation, the other swab was used for direct examination by wet mounted film and Gram stained for detection of yeasts, inoculated plats were incubated for 24-72 hrs. The isolated colonies were identified by morphological feature and biochemical tests (sugar fermentation test and carbohydrate assimilation test). Stock culture was made by inoculating single colony of the isolated yeast or bacteria into a slant of Sabourauds dextrose agar.

Identification of yeasts:

The isolated yeasts were identified as described by [7 and 8].

-Microscopic examination.

Small portion of yeast colony was transferred by sterile loop, smeared and fixed on microscopic slide for staining by Gram stain to examine cells shape and grouping [9].

-Growth on different media.

The isolated colonies were cultured on different media like Sabourauds dextrose agar (SDA), Corn meal agar (CMA) and CHROM *Candida* agar [8].

-Production of germ tube.

A small portion of the isolated colony was emulsified in one ml of sterile human serum, then incubated for 2-3 hrs at 37°C, one drop of the suspension was placed on clean slide with drop of lactophenol cotton blue then examined microscopically for the production of germ tube, the result was observed by noticing short extension shape, budding [10].

- Production of chlamydospores.

Corn meal agar medium was inoculated with single colony of the isolated yeast. inoculation was done by making 3-6 parallel cuts of 1 cm in length on surface of the media, the streaks were covered by a sterile cover slide ,the inoculated plates were incubated at 28°C up to 2 days. Examination of plates for the presence of chlamydospores was done under microscope and the result was observed by seeing round blastoconidia bunched,as described by [11] .

- Sugar fermentation test

A set of sugars consists of glucose, lactose, maltose and sucrose, which were used for identification and differentiation between *Candida* species. The test was done by inoculating tubes containing fermentation media and 2% sugar with single colony, shaking gently then incubated at 28-30°C for three days .The positive result was recorded by changing the color from red to yellow and production of CO_2 gas bubbles in Durham tube [12].

– Carbohydrate assimilation test .

The test depends on the ability of different species of yeasts to grow in various sugar solutions (glucose, lactose, trehalos, raffinose, starch and sucrose). Carbohydrate assimilation medium was poured in Petri dishes and inoculated with *Candida spp* ,then six wells were made by cork borer in the inoculated plates, each well were filled with 2% sugar and incubated plates at 30° for 2-4 days [12].

– Surface growth test.

The test was carried out by inoculating a small portion of the colony into a tube containing Subourauds dextrose broth mixed well and incubated for 24 – 72 hrs at 28-30°C. Presence of growth layer at the surface of broth was indicated a positive result **[**12].

-Antifungal Susceptibility Test:

This test was carried out by modified method of as following [13]:-

- Preparation antifungal solution by dissolved 0.0015gm of antifungal agent (Clotrimazole, Griseofulavin, Ketoconazol, Miconazol and Nystatin \Oxoid.England) in 10 ml dimetheyl sulphoxide.
- Preparation of culture media and plates; Sabouruad dextrose medium was employed and heated to 45-50° C then poured in Petri dishes on a level surface to a depth of 4 mm. When the media was solidified, the Petri dishes were placed in the incubator at 37 ºC for 15-30 min to let the excess moisture evaporated.
- *Candida* inoculum; With the sterile wire loop, the tops of 4-5 isolated a test colonies of *Candida* were picked from the original culture and introduced into tube containing 4 ml of Sabouraud dextrose broth. The broth was incubated at 37 ºC for about 2-5 hours to produce a *Candida* suspension of moderate turbidity. Its turbidity was compared to that of the recommended turbidity standard McFarland tube No. (0.5).
- Inoculation of the test plates; a sterile cotton swab was dipped into the standardized *Candida* suspension and streaked on to the upper most surface of Sabouruad agar plate in three different planes to obtain an even distribution of the inoculum. The plate lids were replaced and the inoculated plates were allowed to remain on a flat and level surface for 3-5 min to allow absorption of excess moisture.
- Five wells was made by cork borer in the inoculated plate, each well were filled with 0.1 ml of antifungal suspension.
- Within 15 min the inoculated plates were incubated at 37 ºC for 18 hours in an inverted position.
- Reading of the results; after incubation the diameters of the complete zone were noted and measured by using reflected light and ruler,the end point measured to the nearest millimeter, was taken as the area showing no visible growth.

Results and discussion:

The result showed that 71 (36.97%) for patients and 9 (42%) for control grope were positive for *Candida spp. Candida albicans* was the most prevalent pathogen of vagina was recovered from 63 (32.81%) vaginal swabs. The yeasts colonies appeared on Sabaroed dextrose agar as white, glossy, smooth and circular colonies, while on Corn meal agar as creamy and soft colonies, moreover colonies appear light to medium green on CHROM agar and appearance of growth layer at the surface of Sabourauds dextrose broth when cultured in it, as well as stained positive with Gram-stain, the cells appeared to be violet and oval shape, the other tests showed that this yeast had ability to form chalamydospore on Corn meal agar, and germ tube on human serum at 2 – 4 hrs, that confirmed that the isolates belong to *Candida albicans* as mentioned by[14].

Results in table (1) describe the ability of *Candida* isolates to ferment and assimilate different sugars. *C.albicans* isolates were able to ferment glucose and maltose during 24 hrs. but not lactose and sucrose ,as well as assimilation glucose, sucrose ,trehalose, but not lactose and raffenose. In this regard, [4] insisted on such above tests to differentiate of *Candida albicans* from other species. These results agree with [15 and 16], and with many local studies like [6, 17 and 18].

Table 1: The morphological feature and ability to ferment and assimilate different sugars of *Candida* isolates.

carbohydrate fermentation				carbohydrate assimilation					Chlamydospore formation	Surface growth	Germ tube formation	Suspected*Candida spp.*
Glu	malt	Lact	Suc	Glu	Lac	Suc	Tre	Raff				
+	+	-	-	+	-	+	+	-	+	+	+	*C. albicans*
-	+	-	+	-	+	-	+	-	-	-	-	*C. glabrata*
-	-	+	+	-	+	-	-	-	-	+With biosurfactant	-	*C. C.krusei*
-	V	-	+	-	+	-	+	+	-	–	-	*C. parapsilosis*
-	-	+	-	-	+	-	+	-	-	+ bubble	-	*C . tropicalis*

Glu= Glucose, lac= lactose, Suc= Sucrose, Tre= Trehalose, Raff= Raffinose.
, Mal= Maltose, V= variable, +=positive,-=negative

The susceptibility tests of isolates to different antifungal agents were conducted and it was found that all tested isolates were sensitive to Miconazol, Clotrimazole and Ketoconazole , while resistant to Nystatin ,Griseofulavin as shown in table (2).

Table 2 Susceptibility of random *C.albicans* isolates to different antifungal agents

Antifungal	Inhibition zone(mm)
Miconazol	**S**
Clotrimazole	**S**
Ketoconazole	**S**
Nystatin	**R**
Griseofulavin	**R**

S=Sensitive R=Resistant

When we compared present results with others we found close similarity with [19, 20 and 21].

Candidiasis is the most common cause of vaginitis in reproductive – age women. In this study most cases infected with *C.albicans* (88.73%) followed by *C.glabrata* (4.2%) and occasionally by *C.tropicalis* (2.8%),*C. krusei* (2.8%) or *C.parapsilosis* (1.4%) as shown in table(3). Vazques and Sobel [2] have demonstrated that *Candida spp*. ,other than *C.albicans* are parts of the normal flora which changed to pathogens (opportunistic pathogen) under certain conditions as low vaginal pH and increased glycogen content of vagina.

Table 3 Kinds of *Candida spp*.in vaginal swab

Candida spp.	Frequency	%
Candida albicans	63	88.73%
Candida glabrata	3	4.2%
Candida tropicalis	2	2.8%
Candida krusei	2	2. 8%
Candida parapsilosis	1	1.4%
total	71	100%

Vaginal yeast infection or vulvovaginal candidiasis is common causes of vaginal irritation .Yeasts are always present in the vagina in small numbers and symptoms only appear with overgrowth [22, 23]. Several factors are associated with increased symptomatic infection in women, including pregnancy, uncontrolled diabetes and the use of oral contraceptives or antibiotics , other factors that may increase the incidence of yeast infection include using douches, perfumed feminine hygiene

sprays and wearing tight, poorly ventilated clothing and underwear [24,25]. Infection with non –*albicans* mostly occur in immunodeficiency cases and patients treated with prophylactic [26,27]. Transmission of vaginal Candidiasis occurs not as newly acquired infection with the implicated yeast, but rather as result of imbalance of vaginal flora and this allows the yeast already present in vagina to overgrow and cause disease [28].

The results in the present work showed a high percentage of Candidal infection among the studied patients, as well as the control group, most of examined women in the control group were of sexually age group and have no clear idea about infection because have no symptoms, this observation come in accordance with [29] who concluded that 40% of vaginal Candidiasis were asymptomatic.

The relationship of vaginal infection with the age

The relationship of vaginal infection with the age was investigated in this study and the patients were grouped into four categories according to their age as shown in table(4) it was clear that incidence of infection with *Candida* was high among females age group(19-39 years), females at this age group were sexually active and therefore, the prevalence of infection is expected to be high because they acquire the infection by contact with infected consorts ,in addition most of the women at this age may be newly married , pregnant or using contraceptive pill and that increase the chance of exposure to infection more than other age groups.

These above results are in agreement with that obtained by [3] who reported that the incidence of the disease was increased in women with age group ranging between (18-40 years). The results seem that *C.albicans* decreases with age while bacterial infection increases.

Table 4 The relationship of urogental tract infection with the age

Age range	Candidal infection	Bacterial infection
19-29	27	19
30-39	26	18
40-49	11	33
50-67	**7**	27
total	71	97

Reference:

1-Oscar, M.; Philippe, M.; Michel P. G.; Jacques B.; and Dominique, S. 2000: Potent synergism of the combination of fluconazole and cyclosporine in *Candida albicans*. Antimicrobial agents chemotherapy.; 44(9), 2373 – 2381.

2-Vazques, J.A. and Sobel ,J.D.2000. Mucosal Candidiasis. Infect. Dis. Clin. N.Am.;16:793-820.

3-Warren, J. W. (1996). CLinical presentations and epidemiology candidosis. of urinary tract infections. In: Urinary tract infections: Molecular pathogenesis and clinical management. Mobley, H. L**.** T. and Warren, J. W. (eds.). ASM Press. Washington. pp 1 19-1 3 1.

4-Ping, R. 2002: Mycology critique. Mycology proficiency testing program. New York State Department of Health: 1 – 31.

5-Debbie, A. H.; Quentin, L. S.; Sanders, R. J.; Gillian, E. N; Pat, J. B.; Edwards, P. A. Sullivan and Peter, C. F. 2004: Identification of the dialyzable serum inducer of germ-tube formation in *Candida albicans*. Microbiology; 150, 3041-3049.

6-AL-Thwani ,A.N.2002. Study the role of some predisposing factors for infection with Candida albicans . Iraqi J. of Science , Vol: 43 No:12.pp 105-193 .

7-AL-Thwani ,A.N .1987.Pathological effects and immune response of *Candida albicans* and *Candida Krusei* in mice.MSc thesis submitted to the college of vet.med.-University of Baghdad.

8-Boonnaert,C.J. and Rouxhet,P.G.2000.Surface of lactic acid bacteria:relationships between chemical composition and physic-chemical properties. App. Environ. Microbial. 66:2548-2554.

9-Kreger-Van R. 1984: The Yeasts: a taxonomic study. (3rded), Elsevier Science Publishers B.V., Amsterdam, The Netherlands.

10-Evans, E. and Richardson, M. 1989: Medical mycology" a practical approach " IRI press, U.K. 23:1546-1552.

11-Nyirjesy, P. ; Seeney, S. M.; Grody, M. H.;Jordan ,C.A. and Buckley, H.R. 1995.Chronic fungal vaginitis:The Value of cultures .Amer.J. Obstet. Gynecol. ;173:820- 823 .

12-Forbes , B. A.; Sahm , D. F.; and Wessfeld , A. S. 1998. Bailey and Scott's Diagnostic Microbiology . 10th ed . Mosby Inc, St.Louis. pp. 870-951 .

13-Nothan, P.;Law,E.J.and Murph, D.F.1978.Alaboratory method for selection of topical antimicrobial agents to treat infected burn wounds.J.Burns.,4:177-187 .

14-Cheryl, G.; Maryam, G. N.; Mark, M.; Sandy, V.; Mark, S. L. and Judith, B. 2001: *Candida albicans* interacts with the septin ring in yeast and hyphal cells. Molecular Biology of the Cell; 12: 3538 – 3549.

15-Emmons , C.W.;Binford, C.H.and Utz,J.P. 1977.Candidasis .In medical mycology. Lea and Fibiger ed.2nded. Philadelphia. Ch.:14. pp:167-182 .

16-Brooks, G.F. ;Bulte, J.S. and Mores, S. A.1998. Immunology. In: Jawetz,E.;Melnick,J.L. and Adelberg, E.A.Medical Micro-biology.Middle East Edition ,Appelton and Lange .U.S.A

17-Hawar,S.N.2002. Effect of low power He – Ne laser on the viability of the pathogenic yeast *Candida albicans* isolated from different candidiasis cases. M.Sc. thesis,Iben-Alhatham education College. University of Baghdad.

18-Majeed, H. A. L. 2004 Identification and immunological study of *Candida spp*. causing vaginitis . M.Sc. thesis,College of Science for Women . University of Baghdad

19-Odds, F.C. 1985. Morphogenesis in *Candida albicans* (CRC). Critical Review in Microbiology, 131: 2581 – 2589.

20-AL-Maliky,K.R.S.2001. Otomycosis in Missan Province the etiological agents and effectiveness of five available antifungal agent . M.Sc. thesis, college of medical and health Technology .Baghdad .pp 36.

21-Liao, R.S; Rennie, R.P.and Talbot,J.A. 2003.Sublethal injury and resuscitation of *Candida albicans* after Amphtericin B treatment. Antimicrob. Agents chemother.,47(4):1200-1206 .

22-Bernhard, H.; Daniela, H.; Carol. A.; Martin, S.; Wilhelm, S. and Joseph W. 2001: The role and relevance of phospholipase D1 during growth and dimorphism of *Candida albicans*. Microbiology; 147: 879 – 889.

23-Andre, N.; Daniel, D.; Catherine, B.; Doreen, H. and Anne, M. 2002:

Transcription profiling of *Candida albicans* cells undergoing the yeast-to-hyphal transition. Mol. Bio. Cell; 13(10): 3452 – 3465.

24-Julian, R. N.; Stephen, J. C. and Bernhard, H. 2003: *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. Microbiology and Molecular Biology Reviews; 67(3), 400 – 428.

25-Forbes, D.; Eec, L.; Camer-Pescib, P. and Wardb, P. B.2001: Faecal *Candida* and diarrhea. Arch Dis Child; 84: 328 – 331.

26-Pati, M.; Jim, E.; Evelyn, E.; Robert F. and Kevin, C. 2001: Inhibition of hydrophobic protein-mediated *Candida albicans* attachment to endothelial cells during physiologic shear flow. Infection and Immunity; 69(5), 2815 – 2820.

27-Mairi, C. and Gary, B. 2004: Regulation of *Candida albicans* Morphogenesis by Fatty Acid Metabolites. Infection and Immunity; 72(11): 6206 – 6210.

28 -Eric, S.; Scott, G.; and Judith, B. 2002: A Forkhead transcription factor is important for true hyphal as well as yeast morphogenesis in *Candida albicans*. eukaryotic cell; 1(5),787–798.

29-Debbie, A. H.; Quentin, L. S.; Sanders, R. J.; Gillian, E. N; Pat, J. B.; Edwards, P. A. Sullivan and Peter, C. F. 2004: Identification of the dialyzable serum inducer of germ-tube formation in *Candida albicans*. Microbiology; 150, 3041-3049.

اختبار الحساسية للخمائر المهبلية وعلاقتها بالعمر في النساء العراقيات

بشرى جاسم محمد ***آمنه نعمه الثويني****

*معهد الهندسة الوراثية و التقنيات الاحيائيه للدراسات العليا / جامعة بغداد.

الخلاصة :

هدفت الدراسة إلى عزل الفطريات المرضية من القناة التناسلية الأنثوية و توضيح العلاقة ما بين الالتهابات المهبلية الفطرية والعمر.أو ضحت النتائج أن *Candida albicanis* هو المسبب الرئيس للالتهابات المهبلية الفطرية. فيما دلت نتائج اختبار حساسية عزلات *C. albicans* لمختلف ا لمضادات الفطرية على أن العزلات حساسة لكل من Ketoconazole , Miconazole وClotrimazol و مقاومة إلى Nystatin وGrisofulvin ، وعند دراسة علاقة العمر بالالتهابات المهبلية أكدت النتائج أن الإصابات تركزت في الفئة العمرية المحصورة ما بين (19-39) .