AL-Qadisiya Journal For Science Vol .17 No.1 Year 2012
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Evaluation of *Candida albicans* Diagnosis by using conventional PCR

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Abstract:-

This study involved evaluate conventional polymerase chain reaction (PCR) technique using kit 500/730 IC(Sacace, Italy) to detect *Candida albicans* in urine of female patients from Samawah gynaecological hospital. The evaluation performed by comparing *C. albicans* PCR direct diagnosis of clinical specimens with germ tube formation and api *Candida* system (bioMe´rieux, France). We collected 116 urine specimens of female patients in age ranging 20-50 years. The results of DNA amplification tests show 50 of these 116 specimens were positive for 500 b.p band on agarose gel. Both specificity and sensitivity of PCR diagnosis of *Candida albicans* were 100%.

Introduction:-

The opportunistic pathogen *Candida albicans* is responsible for a range of human infections. Invasive and disseminated candidiasis is a serious and often fatal complication that can occur frequently in immunocompromised patients (1). The diagnosis of invasive candidiasis is difficult because there are no specific clinical manifestations and conventional microbiological methods, there are considered to be in sufficient in both specificity and sensitivity. The PCR has been resolve of the problem for highly sensitive detection and specific identification of microorganism fingerprinting (2) .

Materials and Methods

Samples Collection: A total of 116 samples were collected from patients of Samawah gynaecological hospital. They were included 116 urine specimens of female patients in age ranging 20-50 years.

Culture of *Candida albicans*: Culture the sample on sabouraud's agar with 2-3 drops of chloramphencol syrup 250 mg/ml at37 C˚ for 24 hr.

Assay of Germ Tube Formation: Using a sterile loop, a small of pure colony of yeast was inoculation into sterile tubes containing 0.5 ml of human sera. The resulting mixture was incubated aerobically at 37 C˚ for 1-2 hr. At 15 minutes intervals, a drop of the yeast-serum mixture was place on a clean microscope slide covered with a cover slip and examined microscopically, using the x10 and x40 objective lenses. The appearance of small filaments projecting from the cell surface confirmed formation of germ tubes (3).

Api Candida System: Inoculation of the tubes was performed by adding suspension of inoculum in saline (McFrland standard of 3).After 18-24 hr. incubation at 37 C˚, the reactions were read visually without addition of reagents. The results were transfered into numerical profile which was compared with the profile index.

DNA Extraction: The DNA extraction was performed by transfer 200 µl of suspension of Candida cells to microfuge tube contains 500 µl of buffer (250 mM NaOH ; 50 mM Tris-HCl-pH 7.5; 10 mM EDTA)with heat 65 C° for 5 min., then resuspended with equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) then steps of participation with ethanol and stored at 4C° for several days or long term at – 20C°.

PCR assay: *Candida albicans* 500/730 IC (Sacace, Italy) is an *in vitro* nucleic acid amplification test for qualitative detection of Candida albicans in the biological materials. *Candida albicans* 500/730 IC test is based on three major processes; sample preparation, nucleic acid amplification of DNA using specific *Candida albicans* primers and detection of the amplified products on agarose gel. The kit contains the internal control which can be used in the isolation procedure and serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition. After prepare mixture of provided materials of kit in PCR-mix-1 transfer into the thermocycler (TC-3000,USA) when programmed 95 C˚ for 5 min as initial denaturtion step, 42 cycles: 95 C˚ for 1 min., 63 C˚ for 1 min. and 72 C˚ for I min., and 72 C˚ for 1 min. as final extension step. The sample is considered to be positive for Candida albicans DNA if the band of 500 b.p. is observed on agarose gel 2%.

Results and Discussion:-

One hundred and sixteen female patients in age ranging 20-50 years from January2009 to September2009 collected for diagnosis of *Candida albicans.* Diagnosis of *C. albicans* was based on suggestive clinical presentation, followed by germ tube formation and api *Candida* system, then they were positive samples confirmed by DNA amplification assay. Urinary Tract Infections are more prevalent in women than they are in men. The reason for that is the length of the urethra and opening to the rectum and vagina. It is estimated that 20 percent of adult women will have a least one urinary tract infection during their lifetime(4). This explains the reason why this study included all samples were from females. The studies have emphasized the role of species other than *C. albicans* as emergent pathogens in urinary tract infections (5, 6). This study showed that isolated *C. albicans* (40.4 %) most often, followed by *C. glabrata* (30.2 %), *C. krusei* (25 %), *C. tropicalis* (2.8 %), and *Geotrichum spp*. (1.6 %) as shown in table 1, which is agreement with other studies (7, 8, 3). The specific identification of yeasts provides important help in the choice of treatment, because *C. glabrata* and *C. krusei* are naturally resistant to fluconazole (9).

Table (1): Distribution of isolated yeast species in urine according to results of api system

Species	No.of specimens	% of specimens
C. albicans	47	40.4
C. glabrata	35	30.2
C. Krusei	29	25
C. tropicalis	3	2.8
Geotrichum spp	2	1.6
Total.	116	100

The present study evaluated Three diagnostic techniques used in the diagnosis of *C. albicans*. The two methods were germ tube formation and api Candida system agianst conventional PCR assay, employed as 'gold standard'. In the study all groups were females. A total of 116 urine specimens were examined by germ tube formation and api Candida system included in this study. Amplification assay of samples detected a total of 50(100%) positives, germ tube formation detected 49(42%) and api *Candida* system 47(69%) as shown in table 2. One sample positive by germ tube formation and negative by both PCR and api *Candida* methods as *C.albicans*. This one concluded *C. glabrata* deemed as false positive when reexamined germ tube formation. Two samples of the 67 deemed negative for C. albicans by germ tube formation produced 500 b.p. band by amplification assay. One of these samples gave positive for api Candida system diagnosed as *C. krusei* and other was positive by api *candida* system diagnosed as *C. albicans*.

Figure (1): Germ tube formation of *Candida albicans*

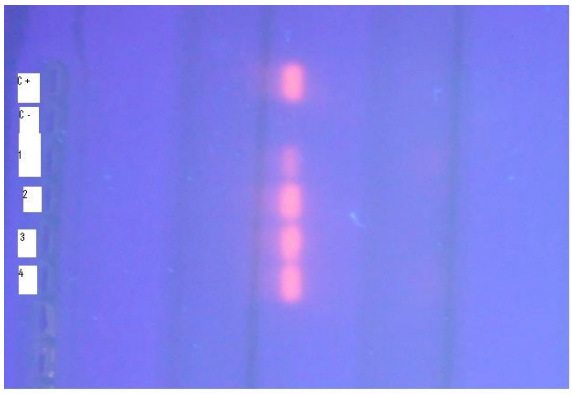

Figure (2): Agrose gel electrophoresis of PCR products(C+ positive control of amplification assay; C- Negaive control of amplification assay; 1,2,3 and 4 positive -samples of 500 b.p band)

Figure (3) : Api cadida strip

Table (2): Distribution of *Candida albicans*-positives and negatives in three methods(germ tube, api Candida and PCR)

Methods	No. tested	Female patients			
		Sample- positive		Sample-negative	
		No.	%	No.	%
Germ tube	116	49	42.2	67	57.8
Api Candida	116	47	40.5	69	59.5
PCR assay	50	50	50	0.0	0.0

Table (3) describes the results of the diagnostic methods employed. The diagnosis of *C. albicans*. using conventional PCR as gold standard to evaluate both germ tube formation and api Candida system. Both specificity and sensitivity of conventional PCR for diagnosis of *C. albicans* were 100%, while specificity and sensitivity of api Candida system were 100% and 98% ; and specificity and sensitivity of germ tube formation were 98.5% and 96%, respectively.

Table (3): Comparsion of PCR, germ tube formation and api *Candida* system for the diagnosis of *Candida albicans* .

Methods	Samples examined (No.)	positive samples detected (No.)	Specificity (%)	Sensitivity (%)
PCR assay	50	(50)	(100)	(100)
Germ tube formation	116	(49)	(97)	(92)
Api *Candida* system	116	(47)	(100)	(88.7)

The good performance of amplification assay was reflected to it is ability to detect DNA yields on agarose electrophoresis of *C. albicans* in 50 urine specimens and the good chance to avoid inhibitors of amplification because of all these urine specimens were taken from non-pregnant womens, also most inhibition was removed by storage at -20 °C overnight. This inhibition may be predicted by the presence of urinary factors, so that storage condition and rapid performance remove most of these inhibitors(10) .

Api Candida system and germ tube formation were evaluated in comparsion with the conventional PCR for identification of 50 *C. albicans* isolates. The specificity and sensitivity of api Candida were 100% and 88.7%, respectively. The api Candida system is easy, rapid to use and cheaper. The api Candida system is adapted to identify clinical *C. albicans* in the routine laboratory diagnosis. Also, It may be with same specificity and sensitivity in diagnosis

of other clinical important yeast. C. albicans in causing human diseases requires that the organism be identified from clinical specimens early enough because germ tubes develop quickly, they are used as a rapid presumptive diagnostic identification of *C. albicans*, usually within 90 minutes .

Results from this study have shown the possibility of germ tube formation by *Candida albicans* within 60-120 minutes. This present study showed that specificity and sensitivity were 97% and 92%, respectively. Although results of the germ tube formation assays included two false negative and one false positive but it has important and rapid tool for diagnosis C. albicans from other yeast.

Yeast can infect the urinary tract to cause urinary tract infection. The most common type is Candida species, which causes candidiasis. Candida frequently infects people who have an impaired immune system or a bladder catheter in place(11). Candida infections of the urinary tract or candiduria has been well docmuneted since 1890, when Schmorl first discovered the presence of candida in a patient with typhoid fever. The prevalence of candida infection and the rise in species resistant to polyene and azole drugs means that rapid detection of isolates has become increasingly important for targeted treatment, such as *Candida glabrata* has become less susceptible to fluconazole, and as *Candida krusei* is intrinsically resistant to this drug, infections by these strains may necessitate alternative treatment with amphotericin B or triaconazole(12). This study using species specific primers for the detection of *Candida albicans* from clinical isolates. The primer sets have been shown to have 100% for both specificity and sensitivity for *C. albicans*, with no cross-reaction with DNA extracts from other candida species. Currently, PCR techniques have been employed with high level of accurency by several researchers (13,14)

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تقييم تشخيص داء المبيضات الفطري *Candida albicans* بأستخدام تفاعل بلمرة السلسلةPCR

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الخلاصة :-
تضمنت الدراسة الى تقييم تقنية تفاعل بلمرة السلسلة PCR بأستخدام عدة 730/500 من الشركة الايطالية سيكيس الى تحديد داء المبيضات الفطري *Candida albicans* في عينات الادرار من مرضى اناث في مستشفى النسائية لمدينة السماوة. التقييم انجز بواسطة مقارنة التشخيص المباشر بأستخدام تقنية تفاعل بلمرة السلسلة PCR مع اختبار تكوين انبوب الانبات واختبار شريط api Candida . جمعت 116 مئة وستة عشر عينة من مرضى اناث بعمر يتراوح بين 20-50 سنة. خمسون عزلة كداء المبيضات الفطري C. albicans كانت موجبة للزرع، تكوين انبوب الانبات وشريط api Candida . اظهرت نتائج فحوصات تضخيم الحامض النووي DNA ان 50 خمسون من 116 عينة كانت موجبة لاظهار حزمة بحجم 500 زوج قاعدة على هلام الاكاروز. كانت الحساسية لتشخيص PCR لداء المبيضات الفطري *C. albicans* 100%.