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Isolation and Identification of *Candida* spp. from oral immunocompromised patients with study of virulence factors

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Abstract:-

The present study was conducted to isolation and identification of *Candida* spp. Isolated from immunocompromised patients with (Diabetes, Cancer & Kidney transplant) by different identification methods including direct examination, laboratory culture, biochemical tests and molecular method using polymerase chain reaction (PCR) technique and determine the virulence factors phenotypic to *Candida* spp. and genetic polymorphism in *Candida albicans* in secreted aspartyl proteinase (SAP2) .

During the period from October 2013 to February 2014, a total of 140 oral swab were collected from immunocompromised patients with attending to the three center in AL-Sadder Medical City (Oncology center and kidney center and Center for Diabetes and Endocrinology).In AL-Najaf Governorate, the samples were collected as following: - 110 mouth swabs from patients suffering from leukemia, prostate, stomach and bowel cancer, also 10 mouth swabs from kidney transplant patients and 20 mouth swabs from diabetes.

Introduction:-

Yeasts which are part of the genus *Candida* consist of 150-200 species **[1]**.The genus *Candida* includes ubiquitous microorganisms. They have been found on the skin, mouth, vagina and stool of animals and healthy individuals. Additionally, *Candida* infections often occur in patients with diabetes, immunocompromised patients and neutropenic patients, playing a key role in nosocomial infections **[2]**.

The genus *Candida* especially *C.albicans* were described by botanist Christine Marie Berkhout in her doctoral thesis at the University of Utrecht in 1923. The incidence of candidiasis increased following World War, with the advent of antibiotics, and then decreased again with the development of nystatin, introduced by Elizabeth Hazen and Rachel Brown **[3]**.*Candida* species are common members of the oral micro flora and are generally regarded as being commensals. However, they are able to cause a range of opportunistic infections, referred to as candidiasis **[4]**. The potential pathogenesis of *Candida* species appears to depend on many immunological and environmental host factors and strain virulence factors, including hyphae and biofilm formation, drug resistance and the production of extracellular hydrolytic enzymes**[5]**. *Candida* species are common commensals of the oral cavity, intestinal tract and vagina, with newborns being colonized soon after birth. While these species are innocuous in most individuals, under certain circumstances they can opportunistically overgrow and cause a variety of diseases**[6]**. The colonies of *Candida* spp. are cream colored to yellowish and greenish or pink and violet, grow rapidly and mature in 3 days. The texture of the colony may be pasty, smooth, glistening or dry, wrinkled and dull, depending on the species **[7]**.

There are about 165 species of Candida, but only a small number are human pathogens **[8]**. Among these, five are most frequent isolated in human infection. While *C. albicans* is the most abundant and significant species *C. tropicals*, *C.glabrata*, *C. parapsilosis*, *C.*

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krusei and *C. dubliniensis* are also isolated as causative agents of *Candida* infections **[9]** .the pathogenic stage has to resist recognition by the immune system or damaging macrophage and neutrophils **[10].**

Aim of the study:-

Based on phenotypic characteristic the precent study was desiegned to characterize *Candida* spp. isolates phenotypically and genotypically in immunocompromised patients in AL-Najaf province.

This goal was achieve by the following objectives:-

1-Isolate and identify of *Candida* spp. from oral cavity of immunocompromised patients based on traditional method (Macroscopic and Microscopic features physiological and biochemical test).
2-Determine the relationship between candidiasis in relevance to age and gender.
3-Detection of the virulence factors of *candida* spp. and Investigation the phenotypic and physiological variations.
4-Molecular diagnosis of isolates selected using simple amplified DNA technique for profiling isolates under study and some virulence factors.

Materials and Methods:-

❖ Specimens collection

The specimens were taken by sterilized cotton swabs and rubbed over the dorsal surface of the tongue,cotton swabs were aseptically dipped in sterile culture tubes containing sterile brain-heart infusion broth with chloramphenicol and were transported as soon as possible to the laboratory for incubation at 37 C˚ for 24 hours Next day , they were sub cultured on both Sabouraud's dextrose agar and CHROM agar-Candida was performed and incubated at 37C° for 1-3 days for visible growth of *C.albicans* colonies other *C.albicans* were discarded as a negative.

❖ Identification of *Candida* spp.

Identification of *Candida* spp. depended on the morphological features on culture medium, microscopic examination **[11].**Growth at 45C°**[12].**Chlamydospores formation test **[13] .**SDA agar as primary identification and final identification by CHROM agar media **[14]** urease test **[15] .**

❖ Detection of virulence factors

The virulence factors involving biofilm formation **[16]**, lipase production **[17]**, germ tube formation **[18]** and phospholipase production **[19]**.

Results and discussion:-

❖ distribution of candidiasis rate according to the immunocompromised state

The present study included collection 88 specimen's infection from 140 samples the randomly recruited immunocompromised patients, consist of cancer was more than diabetes & kidney transplant patients which was 72(81.18%), 12(13.65%) & 4(4.54%) respectively for *Candida* spp. when growth on Sabrouaud agar as in table (1).

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Table (1) distribution of candidiasis in immunocompromised patients according immunocompromised state

Immunocompromised state			Total
Cancer	Diabetes	Kidney transplants	
72(81.81%)	12(13.65%)	4(4.54%)	88(100%)

The results of distribution of *Candida* spp. among various clinical specimens in this study showed the higher percentage of *Candida* spp. obtained from cancer patients (81.81%)and lower percentage of *Candida* spp. obtained from diabetes(13.65%)and kidney transplants(4.54%)patients of total isolates ,this may caused by use of preventive therapy before the establishment of chemotherapy may decrease the incidence of oral candidiasis **[20]**,fungal infection is an important cause of morbidity and mortality in immunocompromised pediatric cancer patients **[21]** and decreased saliva flow ,smoking ,mucosal lesions ,decreased blood circulation in the mucosa due to for example radiation therapy , systemic predisposing factors are diabetes mellitus ,acquired or inborn immunodeficiency ,malignancies ,oral cancer and malnutrition **[22]**.

According to patient's age, the prevalence rate of candidiasis. In the oral cavity of adults were ranging between (5-65)years old they were divided in to seven groups as shown in table (2) the highest frequency of candidiasis was 23% at the age (45-54)years old, because of the use of denture which have been proposed as the most important predisposing factor **[23]** followed by the groups age (55-64)years old and > 65this is may due to low for immunity or not use preventive therapy **[24]**or exposed patients to fatigue due to some diseases ,which requires entry into the intensive care unit where candidiasis occurs in 1-8 percentage of patients admitted to hospitals, its occurrence is higher in intensive care units, with infection rates of approximately 10 percent.in intensive care units, candidiasis is accountable for up to 15 percent of all nosocomial infections **[25]**.

Table (2) number and percentage of candidiasis infection according to the age.

Age groups (years)	Clinical state No.%
	oral
5-14	1(2.5%)
15-24	3(8%)
25-34	4(10.2%)
35-44	6(15.3%)
45-54	9(23%)
55-64	8(20.5%)
Large than 65	8(20.5%)
Total	39(100%)

In the other hand distribution of candidiasis in immunocompromised patients according to the gender were evaluated as shown in table (3) they found that, the female patients recorded higher prevalence compared with male patients, which was (27.3%). other workers have not found any gender differences and they attributed such differences to the

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associated factors , such as level of education, monthly familial income and socioeconomic status**(26)**this result was consistent with those of other studies**(27)** .

Table (3) Numbers and percentage of candidiasis infection according to the gender.

Gender	Negative No (%)	Positive No (%)	Total of patients
Female	**22(42.3%)**	**64(72.7%)**	**86**
Male	**30(57.7%)**	**24(27.3%)**	**54**
Total	**52(100%)**	**88(100%)**	**140**

❖identification of *Candida* spp. isolates

The *Candida* spp. isolates obtained from clinical specimens were identified initially according to cultural morphology, microscopic characteristics from those isolates where all collected swabs were cultured on SDA .The colonies of *Candida* spp. are cream colored to yellowish, grow rapidly mature in 3 days the texture of the colony smooth, glistening or dry, wrinkled depending on the species in figure (1).These results are agree with **[28]**.

Figure (1) the colony of *Candida* species growth on (SDA) (37C˚for 48 hr.)

This study has been showed that using CHROM agar ,*Candida* Differential Agar appear *C. glabrata* pale pink, *C.krusei* pink, purple and *C. parapsilosis* White, pink **[29]** and *C. albicans* characterized by the green color smooth colonies and rough dark green colonies of *C. dubliniensis* **[30]** these results are agreed with **[31,32]**.

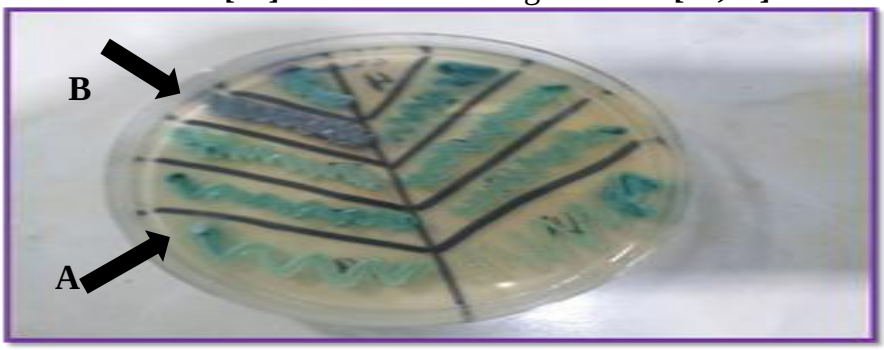

Figure (2) the colony of *Candida* on CHROM agar A-*C.albicans* B-*C.dubliniensis*.

And Urease test was used to detect presence of urease enzyme produced by different *Candida* species **[33]**. Urea agar slants were used. Also Conversion of the yellow slope to pink or red was considered positive. A negative test was reported when there was no color change observed. This result show*C. parapsilosis* (positive test) and other specie(negative test)on urea agar as shown in figure (3). These results are agreed with **[34]**.

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Figure (3)*Candida* spp. on urea agar A- *C.albicans* B-*C.parapsilosis*

The microscopic features by direct microscopic examination for identification the *Candida* spp. figure (4) germ tube.

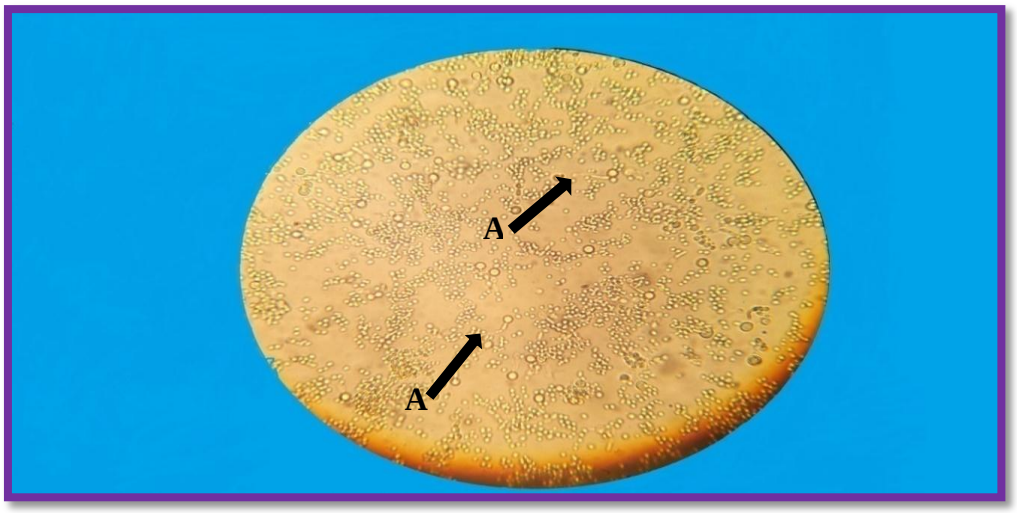

Figure (4) Micrographs showing microscopic structures of *Candida albicans* A-germ tube (40x).

❖Detection of virulence factors

The virulence factors in this study include: Germ tube, Chlamydospore, Phospholipase, and Lipase and Biofilm formation.

The results showed that isolates of *Candida albicans* produced germ tube when incubated in human serum at 37 ° C for 2.5-3 hours **[35]** while the other species from non *C.albicans* shown a negative result **[36]**.

The present study showed of *Candida albicans* also able to form Chlamydospore this agreed with **[37]** Phospholipase activities of the isolates were considered positive when a precipitation zone was visible around the colony on the plate. All *C.albicans* isolates was positive for phospholipase production. **[38]**

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Test results showed production by *Candida* isolates differ in the production of the enzyme lipase test positive by formation of a white precipitate around the colony the developing on Rhan media for 1-5 days at a temperature 30C°results in agreement with **[39]**. That could play roles in candidial adhesion,nuteient acquisition and invasion of epithelial surfaces **[40]**.

The results showed the ability of some kinds of Candida on the formation of biofilm. The result was positive in yeast *C. albicans* **[41]** and *C.dubliniensis* biofilms have 3-D structures as *C.albicans* .my results are in agreement with **[21]** the capacity of *C.albicans* to survive and for biofilm for over six months under anaerobic and nutrient limited condition was reported **[42]**biofilms have been considered as an important virulence factor in the pathogenesis of infections because biofilm associated microorganisms show an innate resistance to antibiotics, disinfectants and clearance by host defense mechanisms **[43].** As shown in figure (5) and table (4).

Figure (5)*Candida albicans* produce biofilm

Table (4) physiology and phenotype of oral Candidiasis

Candida species	Germ tube	chlamydospore	Phospholipase	Lipase	biofilms
C.albicans	+	+	+	+	+
C.krusei	-	-	+	-	-
C.parapsilosis	-	-	-	-	-
C.glabrata	-	-	-	-	-
C.dubliniensis	-	-	-	-	+

Conclusion:-

Candida albicans is the most causative agents of oral candidiasis in cancer, diabetes and kidney transplant.

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