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Isolation and identification of some types of yeast Candida spp and study of their sensitivity to some antifungals.

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Abstract :

40 samples were collected from different cases ,the samples included 22sample an oral swabs taken from new babies suffering from Candidiasis those lay in the contaminated preterm affiliated to Bent Al –Huda ,18 sample which taken from children over the age of one with mouth infection who were lying in Bent Al-Huda General Hospital pediatric department .The results of the study showed that most of the oral isolates taken from two groups of children were Candida albicans, , with 24 isolates out of 40 isolates used under the study, followed by Candida trobicalis isolates 9 Followed by isolation C. parapsilbsis 3 and C. krusei 4 isolates .The results of the study showed that most of the Candida species were sensitive to the antifungal nystatin, as it recorded the lowest resistance rate of 8% and gave the largest diameter of inhibition, followed by the fungicide flucanozole, which recorded a resistance rate of 25%, and the size of the inhibitory diameter was medium, followed by the antibiotic amphotericin B, which recorded a resistance rate of 73% and an inhibitory diameter. The antibiotic gresiofulvin was small and recorded the highest resistance rate, reaching 100%, and no areas of inhibition of the antibiotic were observed on the plate. results of the statistical analysis using the square chi showed that the total number of Candida isolates which showed that the total number of Candida isolates which showed sensitivity to Nystatine anti fungal was significantly different At a probability level of $p< 0.05$.

Keywords: yeast Candida spp , sensitivity , antifungals

عزل وتشخيص بعض أنواع خميرة Candida spp ودراسة حساسيتها لبعض مضادات الفطريات.

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خلاصة :

تم جمع 40 عينة من حالات مختلفة، شملت العينات 22 عينة مسحة فموية مأخوذة من أطفال حديثي الولادة مصابين بداء المبيضات الذين يرقدون في الخدج الملوث التابع لمدينة بنت الهدى، و18 عينة مأخوذة من أطفال فوق عمر سنة واحدة مصابين بالتهاب الفم والذين كانت ترقد في قسم الأطفال بمستشفى بنت الهدى العام. أظهرت نتائج الدراسة أن معظم العزلات الفموية المأخوذة من مجموعتين من الأطفال كانت Candida albicans، حيث بلغ عدد العزلات 24 عزلة من أصل 40 عزلة استخدمت تحت الدراسة، تليها Candida عزلات 9 trobicalis تليها عزلات 3 C. parapsilbsis و 4. C. krusei أظهرت نتائج الدراسة أن معظم أنواع المبيضات كانت حساسة لمضاد الفطريات النيستاتين، حيث سجلت أقل نسبة مقاومة بلغت 8% وأعطى أكبر نسبة مقاومة. قطر التثبيط يليه المبيد الفطري فلوكانوزول الذي سجل نسبة مقاومة 25%، وكان حجم القطر التثبيطي متوسطا، يليه المضاد الحيوي أمفوتيريسين ب الذي

سجل نسبة مقاومة 73% وقطر تثبيطي. وكان المضاد الحيوي جريسيوفولفين صغير الحجم وسجل أعلى نسبة مقاومة وصلت إلى 100%، ولم تلاحظ أي مناطق تثبيط للمضاد الحيوي على الصفيحة. أظهرت نتائج التحليل الإحصائي باستخدام مربع تشي أن العدد الكلي لعزلات المبيضات التي أظهرت حساسية لمضاد الفطريات النيستاتين كان مختلفا معنويا عند مستوى احتمال .$p<0.05$

الكلمات المفتاحية: خميرة المبيضات، الحساسية، مضادات الفطريات

Introduction :

Candidiasis yeast are considered antural flura ,where there is rash on the mucous membrane of the body and it is usually unsatisfactory ,but when pathological or immune change in the internal environment ,it turns in to fungi ,pathogenic and parastatic ,causing in this case what is known as candiasis (kayser et al ,2005).

Candida yeasts can be pathogenic when the have the appropriate conditions to transform into opportunistic yeasts,as in immunodeficiency syndrome(AIDS) ,diabetes and other chronic diseases that reduce the efficiency of the immune system (Moris ,2006)

Candidiasis is treated by using antifungal drugs such as azole and particular fluconazole ,which is the main antibiotic that is used as preventive and curative drug for sever cases of disseminated candidiasis that inhibites the enzyme lanosterol demethylase (Charlier,2009).

While the anti-nystatin acts on the cell wall of candida yeast it leads to the formation of holes in the cell wall that leads to a change in the permeability of the cell membrane ,causing the leakage of ions as a result of the death of the cell (Ghannoum,1999) Throughout the ages, different types of fungicides have been used as a treatment for candidiasis, as their use differed depending on the severity of the infection and the location of the infection. The antifungal flucanozole and nystatin were used as antibiotics to treat oral thrush. If the infection reached other parts of the digestive tract, such as the esophagus, atracozole was used, but if the infection reached the bloodstream. Antimicrobial sensitivity testing is conducted to determine which type is WS U,2020) (

Methods and materials:

Samples collection :

In the current study, 40 clinical samples were collected from Bint Al-Huda Women's and Children's Teaching Hospital in the city of Nasiriyah, (22 oral swabs were taken from children suffering from oral thrush, which were taken from newborns who were hospitalized for no more than 10 days, and 18 oral swabs were taken from children hospitalized. They are between one and three years old and suffer from oral thrush.

Isolation and identification:

The samples were grown on Sabouraud's Dextrose-Agar, which was marked with cotton swabs on the surface of the nutrient medium. Three replicates of cultivation were made on the aforementioned medium to ensure that the fungal growth was not contaminated during the cultivation process. The plates were incubated at a temperature of 37°C for 24 hours. Atlas (1995). The genus Candida was dignosed. Candida Spp and its related species, based on a set of phenotypic and biochemical specifications, as stated in (Baron et al., 1994; Murray et al., 1999), which included:

Direct microscopic examination of yeast samples: The examination was carried out based on the method (Sood, 1994; Morello et al., 2003).

Preparation of antibiotics

Three types of antifungals, nystatin and amphotericin B and Fluconazole , were used in tablet form at a concentrat ion of 10 mg.

Statistical Analysis

All results were statistically analyzed using the chi-square test and the LSD test at a probability level of $P<0.05$ (Al-Rawi, 2000**)**

Discusion&Result

Diagnosis of candida yeast

The current study showed that 40 out of 55 isolates belong to Candida yeasts, and the results were demonstrated through microscopic examination and cultural characteristics

Cultural Characteristics

The colonies growing on sabroid dextrose agar medium appeared in the form of colonies ranging in shape from oval to circular with a cream color. This is consistent with what was reported by Singh and his group (2013), who showed through his study that Candida yeasts appeared on sabroid dextrose agar medium in the form of oval, cream-colored colonies when Inoculating the dishes with the aforementioned yeast under appropriate incubation conditions.

Figure (1) Growth of Candida yeast on SDA medium at 37c

Microscopic features

Candida yeast gave a positive result for the gram stain. The yeast cells were colored blue-violet for the gram stain and appeared in the form of elongated oval or cylindrical cells in the presence of the outer peptidoglycan layer. The blue color is due to the presence of the peptidoglycan layer located in the outer cell chamber of the yeast cells, and this agrees with what was reported by Rehab and her group (2015). I found that Candida yeast cells give a positive result for the gram stain and proved that the blue color is due to The presence of an outer peptidoglycan layer.

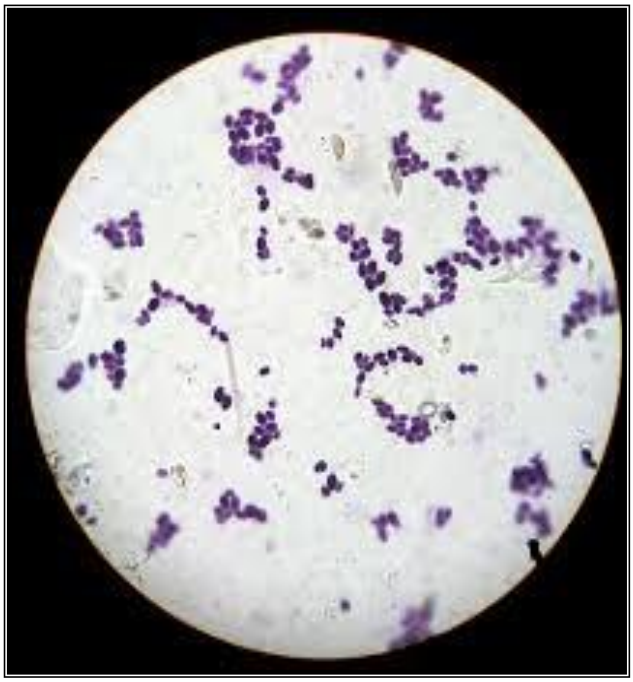
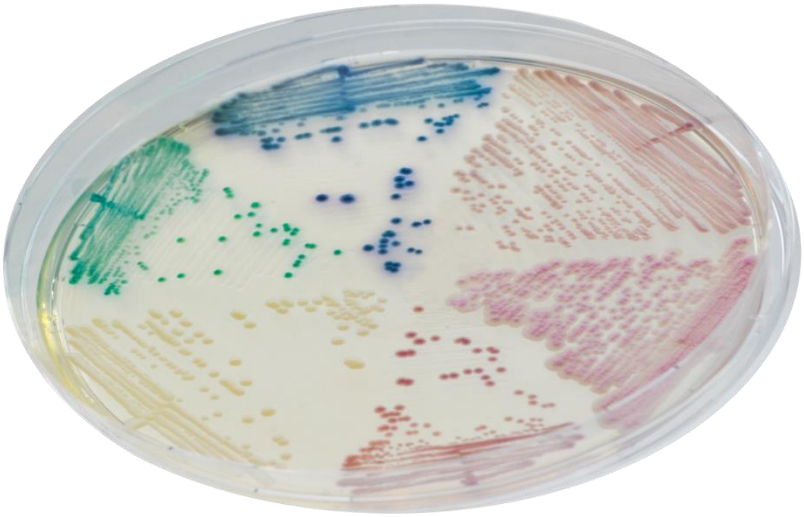


Figure (2) Candida yeast stained with Gram stain

Growth on Candida Chrom agar medium

The isolated Candida yeasts were grown on Candida chrome medium at a temperature of 37 degrees for a period of 24-28 hours, and the results showed different colors, as the Candida albicans yeasts appeared in green colonies, the Candida trobicalis species appeared in blue, while the yeasts parapsilosi is apear purple colour belonging to the krusei type appeared in apink colour .

The isolated Candida yeasts were grown on Candida chrome medium at a temperature of 37 degrees for a period of 24-28 hours, and the results showed different colors, as the Candida albicans yeasts appeared in green colonies, the Candida trobicalis species appeared in blue, while the yeasts belonging to the krusei a pink, the colonies belonging to the parapsilosis type appeared in purple, as this medium was used as a differentiation medium, and this agrees with what was stated by Raut and Varaiya (2009).



Figure(3) Growth of Candida species on chromium agar medium

Number of isolates and percentages of Candida species isolated from samples of two groups of children

The current study, which included 40 isolates of Candida yeasts out of 50 samples taken from children with oral thrush, identified 24 isolates belonging to the type Candida albicans (67%), 7 isolates belonging to Candida tropicalis 30%, 3 isolates belonging to Candida parapsilbsis 15%, and 4 isolates belonging to Candida tropicalis. Candida krusei 17%

he results of the current study showed that the isolation rate of Candida albicans is the highest among the rates of other species, and this agrees with Rehab et al 2015, as five species belonging to Candida were isolated from the oral cavity, vagina, and urine, and the species albicans was the most frequently occurring among the other species. The study also agrees with Al. Obady 2012, as different types of Candida were isolated from different samples, and Nucleus albicans came at the forefront of the isolated types

Type of Candida	Isolates taken from new born	Isolates taken from kids above	Percentage%

		one year	
Candida albicans	13	11	67%
C.tropicalis	4	5	30%
C. parapsilbsis	2	1	15%
C. krusei	3	1	17%

Susceptibility of Candida species isolates to antifungals:

The susceptibility of all Candida isolates under study was tested using four types of antifungals. Susceptibility results were determined by measuring inhibition zones based on what was stated in (Prize et al 1990). The results showed a clear difference in the susceptibility and resistance of the isolates to antifungals.

Candida yeast isolates showed resistance to the two antibiotics gesiofulvin, as the resistance to it reached 100%, as no inhibition zones appeared on the surface of the dish. Likewise, yeast isolates showed resistance to the antibiotic amphotericin B, reaching 73%, as the inhibition zones appeared with a very small diameter. As for the antibiotic FluconazoleThe resistance rate reached 25%, as the inhibition zones appeared with medium diameters. As for the antifungal Nystatin, the least resistance was recorded for Candida yeast isolates, as the inhibition zones were very clearly reduced, and the resistance rate reached 8%.

The current study showed that the antifungal Nystayin is one of the best treatment options for cases of oral thrush in children, based on the areas of inhibition obtained, followed by the antifungal Flconazole Rajaa and Sheng 2019 studied the sensitivity of the Candida isolates that he isolated from the blood and administered to some antifungals. He found that the antifungal Nystatine gave the lowest rate of resistance, reaching 14%. The current study also agrees with what was reported by Rehab 2015 through its study of the sensitivity of yeasts to antifungals nd found Also, the antifungal Nystatin was the best treatment option used, as it recorded the largest diameter of the inhibition zones and gave the lowest resistance rate.

Anti fungal	Inhibition diameter rate	Resistance ratio
Nystatin	19-35	10%
Fluconazole	10-22	25%
Amphotrcin B	8-10	73%
Gresiofulvin	0-3	100%

X2tab = 0.277 X2cal = 2.54 p< 0.05

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