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Isolation and diagnosis of some Candida species isolated from the mouths of infants in Ramadi city

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Abstract
Candida is a natural microscopic organism are yeasts that are found in the normal microorganisms of the oral cavity, opportunistic that cause diseases when conditions are created, overgrowth of yeasts leads to tissue invasion and causes diseases. One of the diseases caused by yeast overgrowth in children is oral thrush, this study was conducted at the Women's and Children's Hospital in Ramadi, where 100 oral swabs were collected from infants with oral thrush aged 1 month-2 years and then morphological and biochemical tests were conducted on positive growth isolates. The results showed that 77 isolates were positive growth by 77% and 23 isolates by 23% showed no growth. Four types of Candida were identified, *Candida albicans* with 49.35%, followed by *Candida tropicalis* with 28.57%, *Candida krusei* with 14.28% and *Candida glabrata* with 7.79%. The results also showed a statistically significant difference between the infection and the age group, where the most infected age group between 1 month-1 year by 74.36%, and the results also showed that the cases of oral fungal infection in infants are associated with artificial feeding by 90.91%.

Introduction:

Candida yeasts are commensal microorganisms in the oral cavity [1]. Yeast is both natural and pathological as Candida [2]. Which includes more than 150 species of these species there are twenty types of pathogens such as *Candida albicans* and there are other types of disease such as . [3] *C.krusei, C.glabrata*, and *C.tropicalis*. These candida are present in the body in a throwing form from birth and remain in a state of symbiosis, and despite being coexisting with humans, they are opportunistic and become pathogenic when conditions are created, including weak immune system, malnutrition, diabetes, inhaling corticosteroids, dry mouth, immunomodulatory drugs and antibiotics, which leads to an overgrowth of yeasts and thus invade the tissues, causing diseases to the body [4, 5]. One of the diseases that occur as a result of overgrowth of Candida yeasts in children is oral candidiasis, also known as oral thrush candidiasis It affects only certain groups of individuals, including newborns and infants [6]. They are white curd-like spots attached to the roof of the mouth, tongue or in the sides of the mouth [7]. the research problem is to determine the prevalence of oral fungal

infections among infants and to identify the most important factors influencing their occurrence, given the importance of prevention, early diagnosis, and appropriate treatment.

Materials and Methods

One hundred oral swabs were collected from infants at the Women's and Children's Hospital in Ramadi from September to December 1, 2024. and those aged 1 month 2 years. The specimens were grown on the medium of Sabouraud Dextrose Agar(SDA) and incubated for 28-24 hours at 37 m[8]. The growth of the developing fungal colonies was then diagnosed and their shape, size, color were observed and diagnosed based on morphological diagnostic methods and biochemical tests[5] .

Isolation and diagnosis

Swap samples were grown on the Sabouraud Dextrose Agar (SDA) medium and incubated for 28-24 hours at 37 m [8]. After that, the growth of the growing fungal colonies was reviewed, their shape, size, color were observed and diagnosed based on morphological diagnostic methods and biochemical tests.

Microscopic Test

Microscopic examination was performed to observe fungal structures such as hyphae and their forms by preparing a glass slide from the colonies growing on the medium SDA and adding a drop of lactophenol-coton blue dye. Then add the slide lid and pass it over the flame two or three times to make sure the yeast is present [9].

Germ tube composition test

This test is specific to the detection of *C. albicans*. Candida is prepared by mixing a pure colony in a tube test tube with a quantity of human serum, and incubated at 37 m from 2-4 hours after that a drop of the stuck was taken and placed on a slide glass and then examined microscopically to see the germination tube, it is noted that *C.albicans* yeast is a bacterial tube in the form of a bud growing from one side of the cell Compared to other types [10, 11].

Growth on CHRM Agar Candida medium

The medium was prepared according to the manufacturer's instructions (HIMEDIA) by dissolving 42.72 g of the medium in 1000 ml of distilled water and then heated to boil, this medium does not need to be sterilized with a closed device and is used to distinguish between Candida species according to the color of the colony, and the reason for the difference in colors between the species is due to the enzymes that are secreted from each type of these yeasts and their interaction with the base material that resides in the medium and thus each type of yeast appears to have a special color, *C. albicans* grow green, *C. tropicalis* blue, *C.krusei* dark pink to purple and *C.glabrata* light pink to cream [12].

Ethical approval

The present study was carried out in accordance with the ethical principles outlined in the Declaration of Helsinki. The study was performed following the acquisition of both verbal and written consent from the patients before collecting the samples, this study was approved by the University of Anbar (200 in December 23, 2024).

Results and Discussion

Isolation

The isolated Candida yeast species were identified based on the morphological characteristics of the colonies growing on SDA medium, where the colonies showed a convex circular shape, a smooth, shiny surface, and a creamy color [13], as shown in Figure (1).

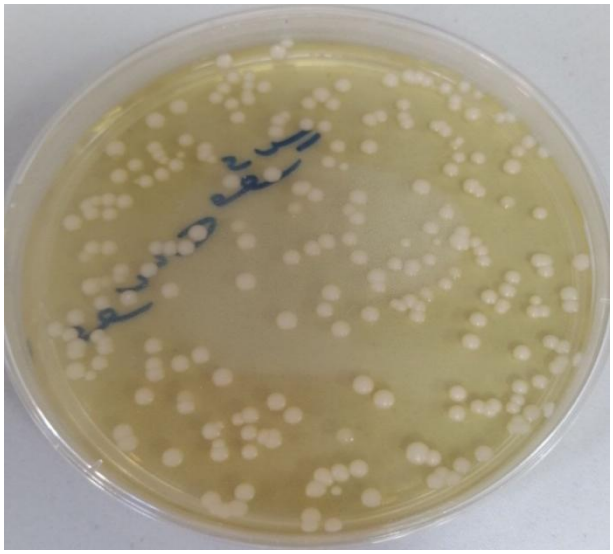

Fig. 1 *Candida yeast colonies on SDA medium.*

While microscopic examination after taking a part of the colony growing on SDA medium and placing it on a glass slide and then staining it with a drop of Lactophenol-cotton blue dye passed over the flame and the slide cover was placed and then examined under the microscope showed that all isolated species were colored blue in a spherical shape [14, 15]. Figure (2).

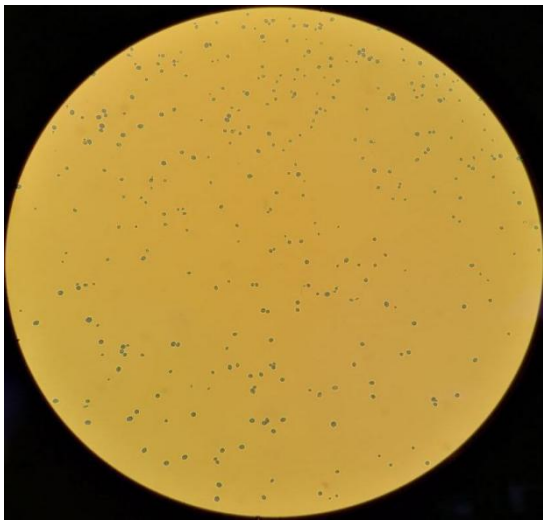

Fig. 2 *Candida* under the microscope stained with Lactophenol-cotton blue**.**

Germination Tube Formation Test

When microscopic examination of the positive isolates of this test, we observe the formation of extended filamentous structures of cells as shown in Figure (3), the test results showed that the isolates belonging to *C. albicans* have formed the germ tube and that the other types *C.krusei, C. tropicalis* and *C.glabrata* at the same conditions were not the germ tube This result is consistent with [15]. These structures work to penetrate the layer of epithelial cells and tissues to reach the bloodstream [16].

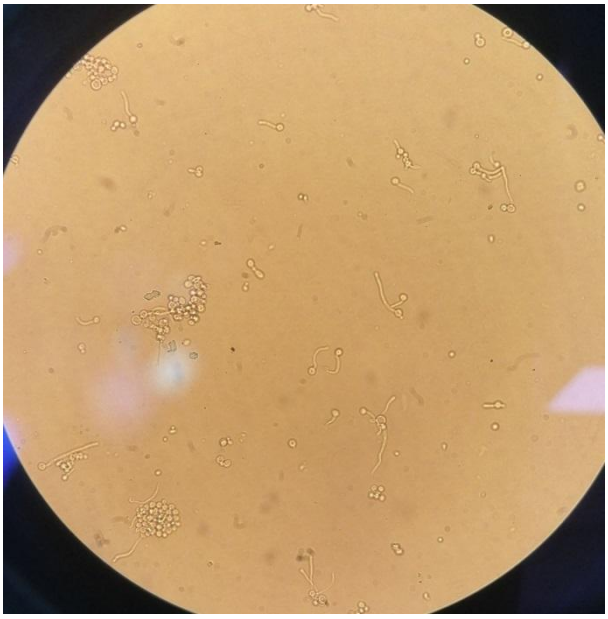

Fig .3 *Germ tube formation of C.albicans growing in human serum at 37 m for 3-2 hours (x40).*

Diagnosis of isolates by developing them on the differential medium

HiChrome Candida differential agar

After incubating Candida yeasts on the medium of Chrom agar for 24 hours at a degree of 37 ° C, colonies of different colors showed green is *C.albicans*, cream is *C.glabrata*, blue is *C.tropicalis* and purple is *C.krusei* Figure (5) and this result is consistent with [17, 18]. The reason for the difference in colors between species is due to the enzymes that are secreted from each of these yeasts and their interaction with the base material that resides in the medium, and thus each type of yeast appears to have a special color [12]. After the development of positive isolates on medium CHROM agar, four isolates were isolated out of a total of 77 positive isolated isolates from infants with oral thrush, the most common type was *C. albicans* (49.35%) and this is consistent with many studies [19, 20]. As the main cause of Candida infection, followed by *C. tropicalis*, it is the most isolated type of non-albicans Candida in this study (28.57%). Its presence in the oral environment is uncommon and its presence indicates a severe decrease in the immunity of the children from whom it was isolated. This result is consistent with the study of Alwaily, [21] who stated in his study that *C. tropicalis* is the most common type of pathogenic yeast. *C. krusei* was isolated (14.28%), followed by *C. glabrata* (7.79%), the least frequently isolated species from infants. This result is consistent with [22,23]. *C. glabrata* is less common in infants than in adults. *C. glabrata* is the least frequently isolated species in several studies because it lacks important features for primary infection. It does not possess the morphological transformation from yeast to hypha, which is important for tissue invasion and penetration. This may be due to the competitive environment in the mouth, which reduces the chances of *C. glabrata* establishing itself. However, it was placed at the top of the list of non-albicans pathogenic Candida species because it has mutated its genes to resist azole antibiotics [24].

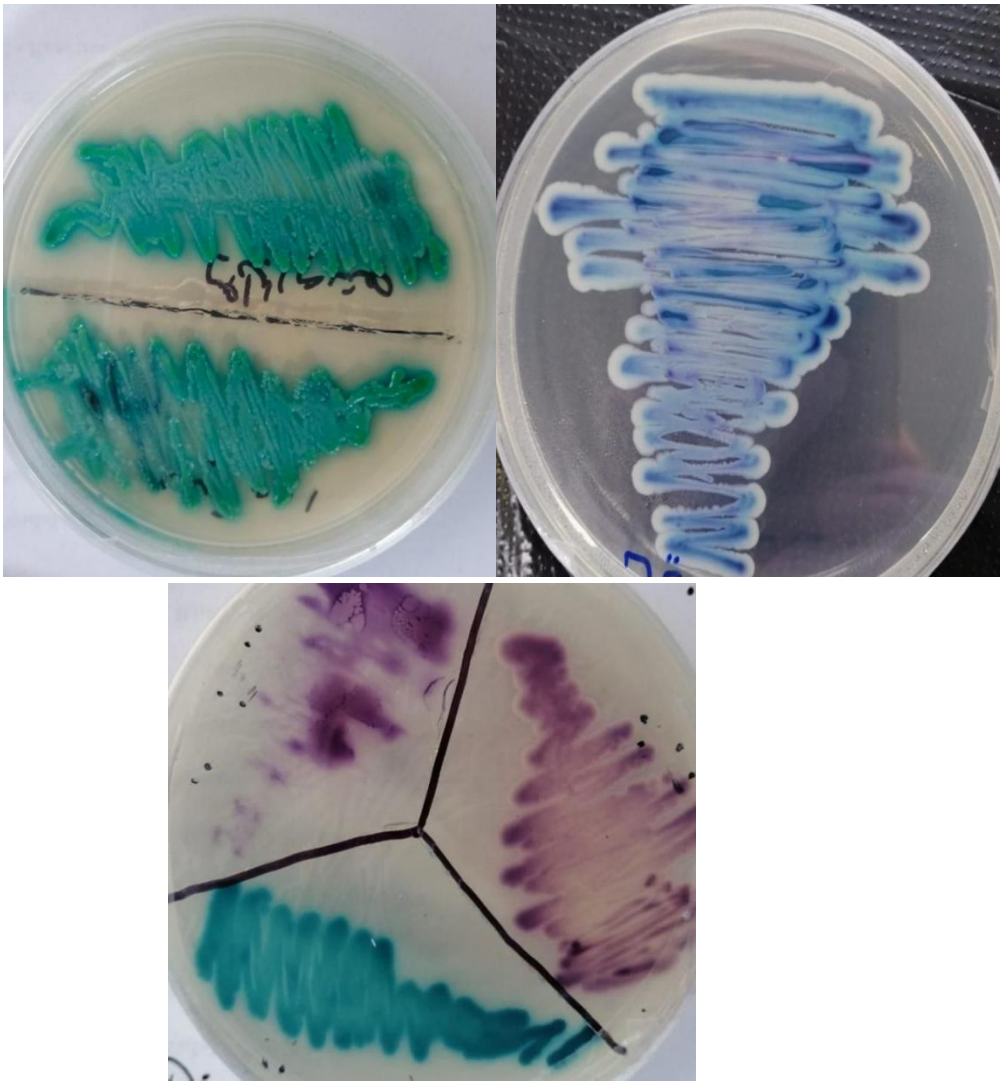

Fig .4 *Types of Candida spp. On Chrom agar medium.*

Table 1: Distribution of samples by fungal species

Sequence Type	Innate	Positive samples
1	*C. albicans*	(38) 49.35%
2	*C. tropicalis*	(22) 28.57%
3	*C. krusei*	(11) 14.29%
4	*C. glabrata*	(6) 7.79%
Total	Positive samples	77
Chi-square value- χ^2 (P-value)	---	31.312 ** (0.0001)
	**P≤0.01	

Table (2) and Figure (5) show a highly significant difference at the probability level (P<0.01) for the isolated samples. It was noted after cultivation on SDA medium that the number of positive samples was 77 samples at a rate of (77%), while the remaining samples recorded negative results at a rate of (33%).

Table 2: Number of isolated samples under study

Sequencing	Fungal specimens	Number
1	Positive samples	(77) 77.00%
2	Negative samples	(23) 23.00%
Total	---	100
Chi-square value-χ2 (P-value)	---	29.160 ** (0.0001)
	**(P≤0.01)	

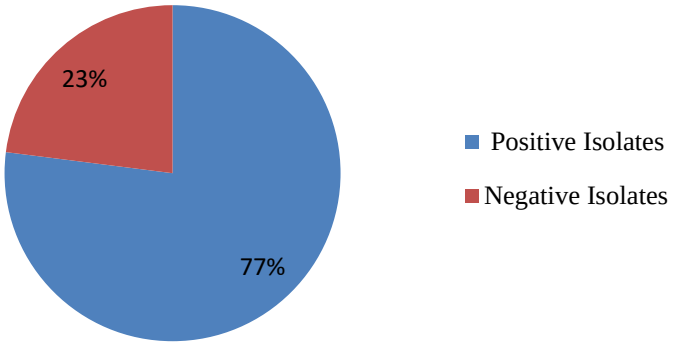

Fig. 5 *Percentage of suggestive and negative samples.*

Table (3) showed the presence of highly significant differences in the infection rate in the different age groups, as it was noted that the highest infection rate appeared in children aged between 1 month and 1 year, at a rate of 74.36%, while it gave a rate of (31.82%) at the age of 2 years, and the reason for this may be due to the low immunity of the newborn child.

Table 3. Distribution of isolates according to age groups

Age Group	Positive samples	Negative samples
1 Month-Year	(16) 68.18%	(57) 74.36%
One Year – 2 Years	(7) 31.82%	(20) 25.64%
Total	23	78
Total	100	
Chi-square value-χ2	2.909 NS	18.512 **
(P-value)	(0.0881)	(0.0001)
	** (P≤0.01).	

As this study showed in Table 4 A statistically significant difference was observed between the type of breastfeeding and the increase in cases of Candida yeast infection. We showed that most of the positive samples were in children who received artificial feeding at a rate of 90.91%, and the percentage of positive cases in infants who received breastfeeding was 3.90% and in the case of mixed feeding it was 5.19%. This indicates that the type of breastfeeding is a very important factor for the health status of infants. The results are consistent with [25], as infants who depend on artificial feeding became more susceptible to infection compared to children who depend on

breastfeeding due to the presence of proteins that are very important for the infant's immunity, Lysozyme and Lactoferrin.

Table 4. Distribution of isolates according to the type of lactation

Type of lactation	Positive samples	Negative samples
Normal	(2) 8.70%	(3) 3.90%
industrial	(19) 82.61%	(70) 90.91%
Natural + Artificial	(2) 8.70%	(4) 5.19%
Total	23	77
Chi-square value-χ2	25.386 **	116.05 **
(P-value)	(0.0001)	(0.0001)
**(P≤0.01)		

Table (5) shows the collection of 77 positive samples, 17 of which were for children suffering from kidney failure, pneumonia, anemia, and high urea. The study showed a statistically significant difference between chronic diseases and the distribution of positive isolates at the probability level (P<0.05), which is consistent with the results of [26]. This is perhaps due to the high frequency of their hospitalization and exposure to hemodialysis or tracheal intubation, as this increases the likelihood of transmission of the infection to them because it is opportunistic. If the opportunity arises in individuals with weak immunity, it causes oral candidiasis, which is why Candida infection was called "the disease of the sick" [27].

Table 5: Distribution of isolates according to type of chronic disease

Chronic diseases	Number of positive samples(%)
Kidney failure	(1) 5.88%
Pneumonia	(8) 47.06%
Anemia	(6) 35.29%
Urea height	(2) 11.76%
Total	17
Chi-square value-χ2	7.706 *
(P-value)	(0.050)
* (P≤0.05(.	

Conclusion

The study was interested in knowing the prevalence of oral fungi among infants in the Women's and Children's Hospital in Ramadi. The results revealed the presence of four types of candida yeasts, *C.albicans* were the most common and prevalent by 49.35%, followed by *C.krusei* by 14.29%, *C. tropicalis* by 28.57% and *C.glabrata* by 7.79%. The study also showed that newborns are the most vulnerable to infection and that the type of artificial feeding recorded the highest rates of infection at 90.91%.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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عزل وتشخيص بعض أنواع فطريات الكانديدا المعزولة من أفواه الأطفال في مدينة الرمادي

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الخلاصة:

الكانديدا هي كائنات مجهرية طبيعية عبارة عن خميرة توجد في الكائنات الحية الدقيقة الطبيعية في تجويف الفم، وهي انتهازية تسبب الأمراض عندما تتوفر الظروف، ويؤدي النمو الزائد للخميرة إلى غزو الأنسجة والتسبب في الأمراض. ومن الأمراض التي يسببها النمو الزائد للخميرة عند الأطفال مرض القلاع الفموي، أجريت هذه الدراسة في مستشفى النساء والأطفال في الرمادي، حيث تم جمع 100 مسحة فموية من الرضع المصابين بداء القلاع الفموي الذين تتراوح أعمارهم بين شهر واحد وسنتين ومن ثم أجريت اختبارات مورفولوجية وكيميائية حيوية على عزلات النمو الإيجابي. أظهرت النتائج أن 77 عزلة كانت إيجابية النمو بنسبة 77٪ و 23 عزلة بنسبة 23٪ لم تظهر أي نمو. تم التعرف على أربعة أنواع من الكانديدا، *Candida albicans* بنسبة 49.35٪، تليها *Candida krusei* بنسبة 28.57٪، %, *Candida tropicalis* بنسبة 14.28٪، *Candida glabrata* بنسبة 7.79٪. وأظهرت النتائج أيضا وجود فروق ذات دلالة إحصائية بين الإصابة والفئة العمرية حيث كانت الأكثر إصابة الفئة العمرية بين 1 شهر-1 سنة بنسبة 74.36%، كما أظهرت النتائج أن حالات الإصابة الفطرية الفموية عند الرضع مرتبطة بالرضاعة الصناعية بنسبة 90.91%.