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Candida albicans Infection and Genotypes identification among children less than 5 years old

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A B S T R A C T

The most common yeast infecting humans are the genus *Candida. Candida albicans*, which are involved in the major opportunistic yeast infection in the world candidiasis, but continue to be the most common among the species of the genus. While this yeast is responsible for around 50-90% of human candidiasis. *Candida albicans* is a common opportunistic pathogen that has attracted growing interest in both clinical and basic biology. The aim of this study is to isolation and identification, with molecular detection of *C.albicans* genotypes from children in AlZahraa Hospital in Diyala city by routine laboratory procedure. Two hundred oral and dermal samples of children less than five years old referred to ALZahraa Hospital, during the period (October 2022 to February 2023). were collected by direct microscopic examination, cultured on Sabouraud dextrose agar and microscopic examination of colonies and sub cultured on CHROM agar which is selective media to identify *candida species.* The result of this regarding to baby information oral *Candida* spp. showed that the percentage of UTI in the oral samples was (20%), the percentage of GIT (40%) and the percentage of oral thrush (40%). In addition to the above we note the percentage of *C.albicans* in the dermal samples was (42%) and percentage of oral thrush (6%) and percentage of diaper rash (50%). Also the percentage of UTI in the mix samples was (12%) and percentage of GIT (44%) and the percentage of diaper rash (44%). For identification of *C.albicans* genotypes, the PCR amplification revealed two genotypes (A) and (A, C) a single amplification product size (450 bp) for 15 isolates conceder as A genotype and single amplification product size (840 bp) for 1 isolate and called B genotype in addition to type of two amplification sizes (450 and 840 bp) for 4 isolates and this categorized as C genotype.

Introduction

The available methods for diagnosing fungal elements in human body fluids include routine mycological methods such as direct microscopic examination (observation of hyphae or pseudohyphae) and culture in specific fungal media, serological methods such as ELISA's latex antigen test, galactomannan, and mannan antigen or

antibody detection, and molecular methods such as nested PCR, Real-time PCR and PCR ELISA.

Determining the sensitivity of isolated fungi to antifungal agents can help improve systemic mycosis clinical management (Predari *et al.,* 2007).

C. Albicans can be presumptively identified using simple, rapid, and inexpensive methods such as germ tube or colorimetric tests, as well as selective chromogenic agar media (Freydiere *et al.,* 2001).

The clinical diagnosis of candidiasis involves various molecular methods. Species of candida can be carefully distinguished at species level. One of these approaches is the determination of 25S ribosomal DNA (rDNA), which has been used for many years (Ngwogu and Otokunefor, 2007).

The genotyping was based on the presence or absence of a DNA tag, which codes the C. dividing ribosomal 26S RNA. Albicans in four groups: A (*C. albicans* – 450 bp), B (*C. albicans* – 840 bp), C (*C. stellatoidea* – 840 bp) and D (*C. dubliniensis* – 1080 bp) and the other methods are candida detection based on polymerase chain reaction, MALDI-TOF MS and candida detection microarray of DNA (Mccullough *et al.,* 1999).

Polymerase chain reaction (PCR)Different Candida DNA markers such as the 5.8S rRNA genes, the 18S rRNA gene, the small subunit rRNA gene, the non-coding internal transcribed spacer (ITS) of rRNA genes, and the lanosterol demethylase gene were used to detect Candida species in PCR amplification (Kabir and Ahmad, 2013).

Materials and Methods

Samples were collected from AlZahraa hospital, Diyala province 200 babies of age less than 5years old fora period from October 2022 to march 2023. A furthermore history of the baby, baby information and age, body weight as well as a universal physical examinations of all babies were ensure. An intensive dermatological examination was conducted to record physiological and pathological signs in baby skin and mouth. Special emphasis was laid on simple non-invasive laboratory analysis for example as scraping for Candida sp., pus swaps for bacterial culture smear from pustules for grams stain as indicated and culturing of collected samples on Sabourauds dextrose agar (SAD). To enhance the cultivation the agar were filled with (0.005)g L of chloramphenicol vial. Oral and dermal samples which were collected by cotton swabs were streaked on the agar plates and incubated at 30 °C for 48hrs to isolate the pure fungal colony (*Candida* sp.) after the incubation period the isolates were examined for their shape, color, size and consistency. Finally they were stored in the refrigerator to keep for the culture. Chromogenic agar Candida (CAC) this method was prepared according to Ibrahim *et al.,* (2017).

Results and Discussion

Candida albicans- specific primer pairs (CA-INT-L and CA-INT-R) were able to amplify the region of the 25S rDNA gene. Polymerase Chain Reaction amplification shows two genotypes (A), and type (A, C) in the [Figure 1]. Results revealed that single amplification product size (450bp) for 15 isolates, and this categorize isolates as genotype A of the *C. albicans*; and that it was most upper among the other genotype of *C. albicans*, single amplification product size (840 bp) this categorize isolates as genotype B of the *C. albicans* there were 1 result for this product size and amplification providing two sizes (450 and 840 bp) for 4 isolates, and this categorized isolates as genotype C of the *albicans*, as it appears in [Table 1].

Candida albicans genotypes identified according the specific primer pairs used to detect the 25S rRNA were CA-INT-L (ATA AGG GAA GTC GGC AAA ATA CCG TAA) and CAINT-R (CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT) (McCullough *et al.,* 1999).

These results are agreed with Hameed (2018) show that *Candida albicans* genotype A (450bp) for 20 isolates is the most frequent genotype in patients followed by genotype B (840 bp) for 2 isolates and C (450 and 840 bp) for 2 isolates. Shows that are *C. albicans* genotype A is the most frequent genotype in patients followed by genotype B and C with the equal rate.

In Najaf governorate, Haydar *et al.,* (2013) showed that 21(95.45%) isolates belonged to the genotype A and 1 (4.54%) isolates belonged to the genotype B of the *C albicans*. The results agree with previous studies of Melahat and Ilknur (2010), which showed genotype A of the *C. albicans* predominant in clinical samples, while genotype B, less frequent. In China, Bii, *et al.,* (2009) was found that the rate of genotype A, B, and C of the *C.albicans* from children with early childhood carriers and carriers free children were (61.2%), (15.5%) and

(23.3%). In Northern Ireland, Xu *et al.,* (2002) found that rate of genotype A (71.5%); B (9.5); C (9.5%) and D (9.5%) from patients with blood borne Candidiasis in Hospital of the Belfast. In addition, it was found that rate of genotype A (66.7%); B (16.7%); C (11.1%) and D (5.1%) that collected of the several parts of the body (throat, groin, anus. bronchi, sputum, catheter urine and endotracheal tube) attending Belfast City Hospital.

Also these results are agreed with Rasheed (2002) find that *Candida albicans* genotype A (450bp) was that the most frequent genotype in patients followed by genotype C (450 and 840 bp) this study showed that *C. albicans* genotype A (25) (83.3%) sample was the most frequent genotype in patients followed by genotype C (5) (16.6%) sample, but there were no results for genotype B can be found.

The frequency and distribution of the genotypes in this study also agreed with the results of a previous study in Iraq by Samaka *et al.,* (2016) who isolated *C. albicans* strains from the mouth of cancer patients and they found that genotype A was the predominant (78.6%), followed by genotypes B and C, and agreed with Taher *et al.,* (2017) in their study which found that (66.7%) genotype A was higher genotypes.

According to the baby information e.g. (health, disease, type of feeding) for UTI in the oral samples are collected from questionnaire was (20) % and the GIT (40)% and the percentage of oral thrush (40)%, also it showed that the percentage of GIT in the dermal samples was (42%), the percentage of oral thrush (6%) and the percentage of diaper rash (50)%.

UTI results in the mix samples was (12%) and the percentage of GIT (44%), the % of diaper rash was (44%) as show in table 1.

For UTI group in the oral samples the *Candida albicans* percentage was 2 % while *Candida* non-albicans was 4% then the negative percentage was 14%, also GIT samples the percentage of *C. albicans* was 3 %, non-albicans 9% and for negative samples was 8 %.

The oral thrush group recorded 8% for *C. albicans* samples while non-albicans less 7 % finally negative samples revealed 5 % only.

The dermal samples, UTI group demonstrated zero %, for both *albicans* and non-*albicans* samples only 1 % the negative recorded.

GIT group demonstrated 12 % for *C. albicans* and 14 % for non-albicans then 16 %for negative samples,oral thrush group resulted in very little 2 % only for total (albicans, non-albicans & negative).

In addition to above the diaper rash group albicans samples recoded 6% as well as 18 % for C. non-albicans and 26 % for the negative samples (Table-1)

Fungal infections still cause a major health problem all over the world and especially affect children of all ages. Therefore, several studies have been conducted on various economic, control and therapeutic epidemiological characteristics of this infection (Seebacher *et al.,* 2008).

In studies by Bassiri-Jahromi and Sberna, female patients were less affected by fungal infections than male patients (Seebacher *et al.,* 2008). On the other hand, several studies have indicated the higher incidence of superficial and cutaneous fungal infections in men compared to women (Sardi *et al.,* 2012).

The results also were similar to Sousa *et al.,* (2011) who found in their study that males (32.0%) and females (67.7%) and it was also non-significant in their study, and the result similar to Sanitá coworkers (Miklić *et al.,* 2010) who found that the number of females were more than male with non-significant differences among the groups. Also the result agreed with Premkumar coworkers in their study that showed that females were higher infected (Male 47.5, Female 52.5).

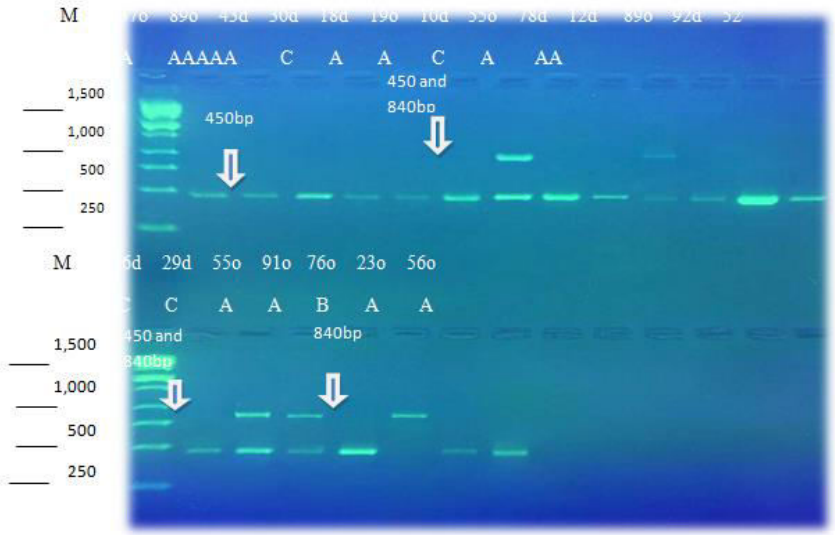
Table.1 *Candida* genotypes. DNA products generated through the primer pairs CA-INT-L and CA-INT-R.

***C. albicans* genotypes**	**Sizes(bp)**	**No. of isolates**
genotype A	**450bp**	15 (%)
genotype B	**840**	1
genotype C	**450 and 840**	4(%)

Table.2 Candida infection rate among patients according baby information

Group				Result			Total	p-value
				C.albican	*C.non albican*	negative		
Oral	Baby information	UTI	Count	1	2	7	10	0.086 NSD
			% of Total	2.0%	4.0%	14.0%	20.0%	
		GIT	Count	3	9	8	20	
			% of Total	6.0%	18.0%	16.0%	40.0%	
		oral thrush	Count	8	7	5	20	
			% of Total	16.0%	14.0%	10.0%	40.0%	
	Total		Count	12	18	20	50	
			% of Total	24.0%	36.0%	40.0%	100.0%	
Dermal	Baby information	UTI	Count	0	0	1	1	0.727 NSD
			% of Total	.0%	.0%	2.0%	2.0%	
		GIT	Count	6	7	8	21	
			% of Total	12.0%	14.0%	16.0%	42.0%	
		oral thrush	Count	1	1	1	3	
			% of Total	2.0%	2.0%	2.0%	6.0%	
		diaper rash	Count	3	9	13	25	
			% of Total	6.0%	18.0%	26.0%	50.0%	
	Total		Count	10	17	23	50	
			% of Total	20.0%	34.0%	46.0%	100.0%	
Mix	Baby information	UTI	Count	3	1	2	6	0.635 NSD
			% of Total	6.0%	2.0%	4.0%	12.0%	
		GIT	Count	5	10	7	22	
			% of Total	10.0%	20.0%	14.0%	44.0%	
		diaper rash	Count	5	10	7	22	
			% of Total	10.0%	20.0%	14.0%	44.0%	
	Total		Count	13	21	16	50	
			% of Total	26.0%	42.0%	32.0%	100.0%	

Figure.1 Agarose gel electrophoresis (2%) for 1.5 hr at 5volt/cm of Candida albicans genotypes. DNA products generated through The primer pairs CA-INT-L and CA-INT-R, stained with Diamond nucleic acid. lane M: Molecular marker (100bp), lanes A: genotype A; lanes B: genotype B; lanes C: genotype C.



But this study was disagreed with that have males higher than females (34 (56.5%) -23 (40.4%)) respectively.

The present study conclude that *C. albicans* genotype A is the most frequent genotype in patients followed by genotype B and C with the equal rate. In addition to this study found that male children are more infected by *Candida albicans* than female children.

Author Contribution

Raghad Hameed Khaleefa: Investigation, formal analysis, writing—original draft. Zainab R. Hameed: Validation, methodology, writing—reviewing. Luma Taha Ahmed:—Formal analysis, writing—review and editing. Adnan Y. Khather: Investigation, writing—reviewing. Dawood S. Hameed: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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