University of Thi-Qar Journal of Science (UTJsci)
E-ISSN:2709-0256, ISSN Print: 1991-8690 Volume (10), No.1, June 2023

Identification of *Candida* species isolated from oral pediatric and Identification of its sensitivity to some antifungals

1st Rieam Sami kheon
Dept. of Analysis Pathology / College of Science/University of Thi-Qar
Nasiriyah/ Iraq
Scipath21p2@sci.utq.edu.iq

2nd Diyaa. A. Aljaza
Dept. of Analysis Pathology / College of Science/University of Thi-Qar
Nasiriyah/ Iraq
diyaamt71@utq.edu.iq

Abstract—**In newborns, *Candida* are responsible for the common oral thrush and rash in skin folds and in the diaper area. Before the advent of modern sanitary measures and topical antifungal treatments, infants died from dehydration due to this disease. Oral thrush is more likely to occur in infants and older adults due to reduced immunity. The study aimed to shed light on the relationship between Thrush and *Candidia* and to molecular identification of *Candidia* spp. isolated from Thrush. One hundred samples of Oral swabs were collected from pediatric with Thrush at ages ranging from (1day-12 years). All samples cultured on Sabouroud dextrose agar; Mannitol salt agar and Malt extract agar. Several tests were used to identify the types of isolated yeasts, including growth on Chromogenic agar medium, as well as biochemical and molecular tests using primers ITS1, ITS4. The Results found that a six species of *Candida* were isolated on Chromogenic: *C. albicans, C. glabrata, C. tropicalis, C. dubliniensis, C. krusei and C. kefyr*, Also, showed the number of yeasts that were genetically identification by molecular diagnosed was five species of yeasts as follows 2 isolates *C. tropicalis* from 22% and one isolate for each of the following types *C. albicans, C. kefyr, C. dubliniensis* and *Candida* species with 11% of all isolates where *C. albicans* showed the highest percentage. The results of the statistical analysis of the isolates showed that the most affected age group of pediatric was (one month -1 year) in the rate of 24%. Antifungal susceptibility was studied, as the results indicated that all *Candida* isolates were sensitive to Itraconazole, while all *Candida* isolates were resistant to fluconazole Furthermore, most species of *Candida* were resistant to Nystatin and Amphotericin B, except for three type's *C. krusei, C. glabrata,* and *C. kefyr.* The most common pathogen in pediatric is *Candida* spp. In pediatric, the results indicated that the best treatment to eliminate *Candida* Itraconazol, Clotrimazol, Nystatin, and Amphotricine B Nystatin respectively.**

Key Words: **Thrush, *pediatric, PCR, Candida Spp., ITS-rDNA, Antifungal Susceptibility***

I. Introduction

Thrush is a fungal infection caused by *Candida albicans* or other *Candida* species in 95% of cases[1]. Other *Candida* species include *C. glabrata, C. tropicalis, C. parapsilosis, C.* krusei, *C. dubliniensis* and *C.* guilliermondii [2].

According to Riordan [3]. thrush in newborns is also known as oropharyngeal candidiasis (OPC), pseudomembranous oral candidiasis, or Monilia. When *Candida* spp. overgrow in the oral mucosa's superficial epithelium, an infection with symptoms develops. This typically appears as white plaque in the baby's mouth and/or tongue.

Candida spp. is opportunistic pathogens that colonize the skin and mucosal surfaces of healthy individuals as commensals [4]. Species of the genus *Candida* are found in their natural hosts, including human.

According to Hajjeh [5]. the genus *Candida* is defined by its oblong or oval form and bipolar bud method of reproduction. As yeast or oval spherical cells, *Candida* can develop on living tissue or culture media. Emerging yeast cells are cream-white in color, range in size from 3 to 6 microns, and have the potential to generate pseudohyphae [6]. Recently, methods have been developed for the purpose of diagnosing *Candida* species, such as the growth test on Chromagar *Candida* medium, where species grow on this medium in a range of different colors after an incubation period of 24 hours at 37°C [7].

in addition to several differential tests. Such as the formation of spores, [8]. The most common cause of superficial injuries to the nails and skin, a hot or humid environment of the body is important in the overgrowth of these yeasts because they stimulate their growth like skin folds in people with obesity, such as between the toes hand. perineal and genitocrural [9]. *C. albicans* is the main human pathogen among *Candida* yeast species. It is considered one of the yeasts that coexist in healthy people and is

https://doi.org/10.32792/utq/utjsci/v10i1.1038

widespread in most environments as well as other species belonging to the genus *Candida* namely *C. krusei, C. glabrata, C. paraphsilosis, C. dubliniensis* and *C. tropicalis* [10]. The current study-aimed to; To know the correlation between Thrush and *Candidia* and determine the predominant spp, To isolation of *Candidia* spp. from oral cavity also to molecular identification of *Candidia* spp. and study *Candida* resistance of selective antifungal isolated. from pediatric attended Bint Al- Huda Hospital in Thi-Qar.

II. METERALS AND METHODS

A. Sample Collection

A hundred samples were collected from pediatrics suffering from oral Candidiasis of different ages and (20) samples from pediatrics are not infected that attended the Bint Al Huda Hospital in Thi Qar Governorate between September 2022 and November 2022. Oral samples were collected using a sterile cotton swab with transport media. The oral swab samples were transported to the Microbiology Laboratory, and cultured within the 3h of the collection.

B. Isolation *Candida* spp.

All samples were cultured on three mediums: malt extract agar (MEA) ,sabouroud dextrose agar (SDA), and mannitol salt agar (MSA) it which was made according to manufacture instruction. [11]. All plates were cultured has been examined for pasty, smooth white and creamy colonies, The colony morphology was examined after cultivation and incubation on MSA. SDA.MSA.

C. Identification

Macroscopically and Microscopically Identification *Candida* spp. The colonies' morphological traits, such as size, color, and morphology of the colonies and creation of the germ tube, were investigated. An a-Pasteur pipette was used to transfer 0.5 mL of serum to a tiny micro-centrifuge tube, and a colony of yeast was transferred via a sterile-wire loop and emulsified in the serum. This test was performed to evaluate whether yeast had germinated. A drop of the serum was placed on a slide for examination under a microscope with a 40_X magnification to see the *Candida* germ tube after incubation at 37°C for 2-3 hours, but no longer after mixing. In comparison to the yeast cell they come from, germ tubes are three to four times longer and half as wide. There was no shrinking between the yeast cell and the germination tube[12].

D. Chrom Agar *Candida*

This medium was for the rapid identification of *Candida* spp. from mixed cultures in clinical and non-clinical samples. This medium was prepared according to the manufacturer's instructions. The *Candida*-colonies were first-recognized by colonial color when-compared to standard color images provided-by the manufacturer and also-exhibited after 72 hours of incubation [13].

E. APi *Candida* Identification Kit

The identification tag was used for the purpose of identification of *Candida* and their subspecies *Candida* colonies were prepared during a twenty-four hour incubation period on Sabouraud dextrose agar plates for the purpose of ensuring colony purity. The yeast suspension was modified for API *Candida* using sterile saline and the turbidity of the suspension was measured using a spectrophotometer at a turbidity equal to 0.3 McFarland standard. The stuck cards for the API *Candida* test have been placed according to the instructions of the company that prepared the test. The tapes were incubated for 18 hours, then the readings were conducted and compared with the test database for the purpose of diagnosing and identifying the types of *Candida* yeast. Materials and reagents filled automatically by the manufacturer. [14].

F. Molecular Detection

1.Primers

Primers were used in the present research from alphadna company *Canada* [15].listed in (Table 1).

Table 1: Shows the primers used in identification of *C.* Spp. isolated from oral thrush.

Genes	Primer Sequences (5'-3')	Size (bp)	Reference
ITS_1 F	5'-TCC GTA GGT GAA CCT GCG G-3'	500-800	Yin *et al*, 2017
ITS_4 R	5'- TCC TCC GCT TAT TGA TAT GC-3'	500-800	Yin *et al*, 2017

2. Polymerase Chain Reaction Protocol

The PCR condition used in this study are listed in table 2.

Table 2: PCR condition for amplification of genes.

Steps	Temperature	Time	No. of cycle
Initial denaturation	95 ℃	10 min.	1 Cycle
Denaturation	95 ℃	45 sec.	30 Cycles
Annealing	55 ℃	45 sec.	
Extension	72 ℃	1 min.	
Final Extension	72 ℃	10 min.	1 Cycle

III. ANTIBIOTIC SUSCEPTIBILITY TEST FOR *CANDIDA.*

According to recommendations made by the Clinical and Laboratory Standard [16]. antibiotic discs were chosen. In 5 ml of sterile normal saline, six pure-isolated colonies of fresh-culture *Candida* were suspended. SDA were next infected by dipping a sterile swab into the inoculum, stretching it over the surface of the medium multiple times, and rotating the plate by 60 degrees to ensure diffusion after each application. The edge of the swab was also pushed. The plates underwent a 3-day incubation period at 37 °C, after which the sizes of the inhibitory zones were determined in millimeters.

IV. STATISTICAL ANALYSIS

The current data were statistically analysis by using Statistical software program SPSS (Statistical Package of Social Science version 26), based in using Non-parametric

and Descriptive Chi-Square for independent, independent variance comparison, and person correlation for identified relationship between parameters at p. value < 0.05.

V. RESULTS AND DISCUSSION

In this study, showed significant difference in prevalence of *Candida* infection according to birth which noted the high *Candida* SPP. in oral infection were *C. albicans* in normal birth (27.87%)
followed by *C. albicans* in caesarean birth (22.95%) this agree with Farked [17]. which showed the results that there were significant differences in the level of birth for children with oral candidiasis while disagree with Jammil and Yehia, [18]. which showed non-significant difference according to birth type.
The infection of the mother a vaginal fungal infection during pregnancy lead to the transmission of the infection to the child during its exit from the vaginal canal at birth. sample t test for means comparison, One Way ANOVA for Table3.The data of age distribution in the study showed that the most affected age group with *Candida* were (1 month to 1 year) with 24% .Taking antibiotics works to eliminate the living organisms in the body ,which leads to providing a suitable environment for the growth of *candida*, However, there is no age group that is completely free of infection this is agreement with Yehia [19]. that found The higher percentage of infection during the study appeared in children less than one year old. While disagree with Rawnuck [20]. which found the most affected age group with cndida were (1 -5 year). Table4.
According to the researcher's opinion, may be the increase in infection in the age groups (1 month-1 years) is due to the immune system at this age is under development and growth and is unable to confront fungi, also the low weight of the child at birth increases the possibility of infection with fungi.

Table 3: Distributed of positive *Candida* Spp. according birth type.

Birth Type / ***Candida* SPP**	**Normal births**	**Caesarean births**	**Total**
	No. & %	No. & %	No. & %
C. albicans	17 (27.87)	14 (22.95)	31 (50.82)
C. dublensis	4 (6.56)	2 (3.28)	6 (9.84)
C. glabrata	2 (3.28)	2 (3.28)	4 (6.56)
C. kefyr	1 (1.64)	0 (0.0)	1 (1.64)
C. krusei	9 (14.75)	2 (3.28)	11 (18.03)
C. tropicals	3 (4.92)	5 (8.20)	8 (13.11)
Total	36 (54.10)	25 (45.90)	61 (100)
CalX^2= 11.902	**TabX^2= 11.07**	**DF= 5**	**P. value 0.036***

Table 4: Effect age of frequency of *Candida* Spp. isolated from oral thrush.

Results / **Age groups**	**Positive**	**Negative**	**Total**
	No. & %	**No. & %**	**No. & %**
Less than 1 month	18 (18)	15 (15)	33 (33)
1 month to < 1 year	24 (24)	19 (19)	43 (43)
1 - < 5 years	13 (13)	2 (2)	15 (15)
5 – 12 years	6 (6)	3 (3)	9 (9)
Total	61 (61)	39 (39)	100 (100)
CalX^2= 5.339	**TabX^2= 7.81**	**DF= 3**	**P. value 0.149**

Also, the study found the high oral infection were in artificial feeding (33%) , followed in pediatric with breast feeding (13%) of *candida* infection, this mean a significant difference in prevalence of *candida* infection according to type of lactation this agree with [21]. that found higher percentage (24%) was recorded during the study among artificial fed babies than breast feeding (15.8%) which in consistent with other reports that oral thrush occur more frequently in artificial feeding than in breast fed babies while disagree with study Al-Ani [22]. Table (5).

Table5: Pediatric oral infection according to type lactation

Lactation / **Results**	**Breast feeding**	**Artificial feeding**	**Non-feeding**	**Total**
	No. & %	**No. & %**	**No. & %**	**No. & %**
Positive	13 (13)	33 (33)	15 (15)	61 (61)
Negative	15 (15)	21 (21)	3 (3)	39 (39)
Total	28 (28)	54 (54)	18 (18)	100 (100)
CalX^2= 6.27	**TabX^2= 5.99**	**DF= 2**	**P. value 0.043***	

After culturing sample on chrome agar *candida* medium Through the results obtained in the chromogen diagnostic medium, which leads to the emergence of colonies of different colors according to the type of *Candida*, the type *Candida albicans* appeared in a light green color that distinguishes it from the rest of the other species [23]. While *C. krusei* appears in a purple color, *C. glabrata* Cream color, *C. tropicalis* Blue with purple, *C.*

kefyr Cream to white with slight purple center, *C. dubliensis* pale green. A chromogenic agar medium is used for the differentiation of candida spp. The resulting color is based on the interaction between the isolates and medium. In this study, showed that *Candida albicans* had higher percentage (31%) followed by *C. krusei* (11%), followed by *C. tropicalis* (8%), this agree with Khairi, [24]. Figure (1) which mean there is a significant difference in Candida species by CHROMagar medium.This is in agreement with Raafat [25]. found that *C. albicans* (37%) was the most common species.

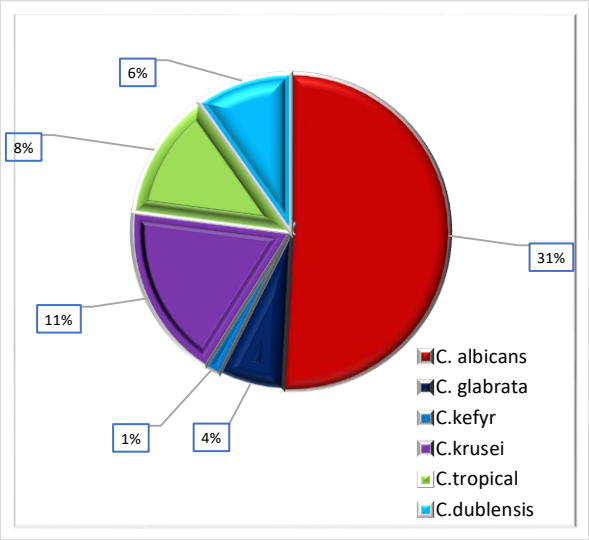

Fig.1 Identification of Candida Spp. isolated from pediatric with oral infection

The results of current study documented that there are differences between chrome, KB006 Hi *Candida* Identification Kit techniques while found similarity between chromagar and molecular diagnosis in three types of *Candida*. All *Candida* spp. diagnosed by chrome agar technique not corresponding with the same species when diagnosed using KB006 Hi *Candida* technique except *C. albicans* and *C. kefyr* was found in the both techniques.Table 6.

Table 6: Confirm of *Candida* Spp. isolation using KB006 HiCandidaTM Identification Kit and molecular diagnosis

Dignosis by CHROM agar	Dignosis by KB006 Hi *Candida*	Dignosis b (PCR)
C. krusei	*C. catenulat*	*C. albicans*
C. tropical	*C. albicans*	*C. tropical*
C. glabrata	*C. parasilosis*	*C. tropical*
C. albicans	*C. albicans*	*C. species*
C. dubliensis	*C. tropicals*	*C. dubliensis*
C. kefyr	*C. kefyr*	*C .kefyr*

The sequencing results found that all isolated species using ITS_1 and ITS_4 primer pairs were confirmed to be *C. albicans* with a similarity percentage 92.21%, *C. tropicalis* with a similarity percentage 97.73%, *C. dubliniensis* 94.76% , C. *kefyr* 100% and *Candida* spp 95% based on the NCB1 database. Table7

The identification of yeast based on ITS sequence differed from the identification based on morphological characteristics (such as CHROMagar). In other words, the sequencing results showed that 2 isolate (22%) of *C. tropical*, 1 isolate (11%) of *C. albicans*, 1 isolate (11%) of *C. kefyr*, 1 isolate (11%) of *C. dubliniensis*, 1 isolate (11%) of *Candida* Spp. Figure (2)

Table 7: Number of SNPS that detected between isolated *Candida* Spp. and NCBI registered *Candida* Spp.

Isolated Organism	NCBI-Blast Homology Sequence Identity %			
	Isolate-AN	NCBI-AN	No. of SNPS	Identity%
C. tropicalis	LC752256.1	MK809258.1	**664/675 (11)**	97.73
C. tropicalis	LC752257.1	MK809258.1	567/614 (47)	92.35
C. dubliniensis	LC752258.1	ON875327.1	470/496 (26)	94.76
C. kefyr or K. cesmarxianus	LC752259.1	OP764004.1	Non-SNPs (0)	100
C. albicans	LC752259.1	MH813940.1	438/475 (37)	92.21
Candida Spp	LC752259.1	JF909923.1	389/406 (17)	95.81

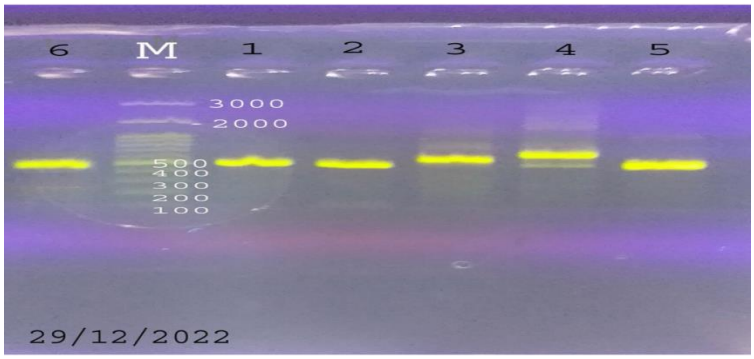

Fig2: DNA bands of SIX strains isolated from Oral swab in pediatric with Oral infection :(M: 1bp DNA Ladder; 1: *C. tropicalis*; 2: *C. glabrata*; 3: *C. dubliensis*; 4; *C. kefyr*; 5: *C. krusei*; 6: *C. albicans*).

Six antifungals were tested against 6 *Candida* spp. isolated from oral swabs samples. In our study, in-vitro susceptibility for yeast strains *Candida* was performed using the disc diffusion method and found that the most effective antifungals were Itraconazole, Clotrimazole, Nystatin. The resistance was most commonly seen against Fluconazole, Ketoconazole , and Amphotericin B. These results agree with those of Khan [26]. where it was found that Amphotericin B is resistant and Itraconazole is sensitive. These results disagree with those of Ahmed [27]. reported that the most effective drugs for their isolates were Nystatin and amphotericin-B, the resistance was most commonly seen against Itraconazole followed by fluconazole and with Sheneef [28]. reported that yeast showed the sensitive to Nystatin and Amphotericin B. while resistance to Itraconazole table (8).

Table8: Summarise effective of different antifungals required for inhibiting fungal growth by six Candida spp. on Sabouroud Dextrose agar medium at 37oC.

Fungal spp	Ketoconazole	Fluconazole	Itraconazole	Nystatin	Clotrimazol	Amphotericin B
C. krusei	R	R	+++	++	R	R
C. albicans	R	R	++	R	+	R
C. glabrata	R	R	+++	++	+++	+++
C. tropicalis	R	R	+++	R	R	R
C.dubliensis	R	R	+++	R	R	R
C. kefyr	+++	R	+++	+++	+++	+++

Key: R= Resistance; +++= Senstive (10mm); ++=(5mm); +=(2mm)

VI. CONCLUSION

Candida species were the most common fungal pathogens in the child department. and molecular assay and sequencing have been important tools in the identification and profiling of candida species and best antifungal for *candida* was Itraconazole

ACKNOWLEDGMENT

We would like to express our gratitude to the lab staff in Nasiriyah city for their assistance in organizing the data and collecting the samples.

ETHICAL CONSIDERATION

To conduct the ethical research, permission was obtained from the hospital, and all participants in this work, pediatric infected and non-infected, The patient selection was an accomplishment

CONFLICT OF INTERST

The authors declare no conflicts of interest

REFERENCES

[1] T. Vila, A.S. Sultan, D. Montelongo-Jauregui, & M.A. Jabra-Rizk, "Oral candidiasis: a disease of opportunity", *Journal of Fungi*, vol. *6*(1), pp.1-28, 2020.

[2] C. Chamorro-Petronacci, A. García- García, A.I. Lorenzo-Pouso & F.J. Gómez-García**,** "tools for oral candidiasis: Current and new antifungal drugs Guillermo", Med Oral Patol Oral y Cir Bucal, vol.24(2),pp. 80–172, 2019.

[3] J. Riordan, K & Wambach, (Eds.). ‘‘Breastfeeding and human lactation‘‘. *Jones & Bartlett Learning*, 2010.
Journal of Medicine, vol.120(10), pp.871-879, 2007.

[4] B. Bottcher, C. Pollath, P. Staib, B. Hube & S. Brunke**,** "*Candida* species rewired hyphae developmental programs for chlamydospore formation". *Journal Frontiers in Microbiol*ogy, vol. 7, pp.1-17, ID 1697, 2016.

[5] R.A. Hajjeh, A.N. Sofair, "Incidence of bloodstream infections due to *Candida* species and *in vitro* susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program." *Journal of Clinical Microbiology*, vol. 42(4),pp. 1519-1527, 2004

[6] M.D. Richardson & D.W. Warnock, ’’Fungal Infection: Diagnosis and Management’’, 3rd Edition. Blackwell Publishing**,** 2003**.**

[7] D. Ellis, D. Stephen, A. Helen, H. Rosemary & B. Roben, **’’**Description Of Medical Fungi. Second Edition. Morphogenesis in *Candida*.’’ MSC. Theses, University of Adelaide Australia, Collage of Science, 2007

[8] M. Whiteway, C. Bachewich**,** "Morphogenesis in *Candida albicans*." *Annual Review Microbiol*, vol.61(2) , pp.529-553, 2007

[9] D.J. Diekema, S.A. Messer, "Epidemiology of candidemia: 3- year results from the emerging infections and the epidemiology of Iowa organisms study", *Journal of Clinical Microbiology*, vol.40(4), pp. 1298-1302, 2002.

[10] D.M. Kuhun, T. George**,** "Antifungal susceptibility of *Candida* biofilms: unique efficacy of amphotericin B lipid formulations and echinocandins. Antimicrobial Agents and Chemotherapy", *Journal American Society for Microbiology*, vol. 46(6), pp. 1773-1780, 2002.

[11] D. Jabar & D. Aljaza, "Conventional and molecular Identification of *Candida* spp. and antifungal Susceptibility test in pregnant women". University of Thi-Qar *Journal of Science*, vol.9(2), pp.13-20, 2022.

[12] P.S. Bhavan, R. Rajkumar, S. Radhakrishnan, C. Seenivasan & S. Kannan**,** "Culture and Identification of *Candida albicans* from Vaginal Ulcer and Separation of Enolase on SDS-PAGE", *International Journal of Biology*, vol.2(1), pp. 84-93, 2010.

[13] D.W. Williams M. & Lewis, "Isolation and identification of candida from the oral cavity. Oral Diseases", *Journal Wiley Online Library*, vol.6(1), pp. 3–11, 2002
https://doi.org/10.1111/j.1601-0825.2000.tb00314.x

[14] H.K. Mattaa, Khalil Hannab, C. Serge Chiron, "Clinical Characteristics of Patients with Acute Pulmonary Embolism: Data from PIOPED". *The American Journal of Medicine*, vol.120(10), pp.871-879, 2007

[15] S. Farahyar, Z.G. Mobaser, E. *Journal of Microbiology*, vol.13(8), pp.1-7. 2020.
Razmjou, M. Roudbary, M. Rahimi, "Molecular investigation of etiologic agents causing candidiasis", *Jundishapur Journal of Microbiology*, vol.13(8), pp.1-7. 2020.

[16] V. Krcmery, and A. J. Barnes **,** "Non-albicans *Candida* spp. Causing fungaemia: pathogenicity and antifungal resistance." *Journal of Hospital Infection*, vol.50(4),pp. 243-260, 2020.

[17] P.M. Glee, J.E. Cutler, "Inhibition of Hydrophobic Protein- Mediated *Candida albicans* Attachment to Endothelial Cells during Physiologic Shear Flow. Infection and immunity", *American Society for Microbiology*, vol.69(5),pp. 2815-2820, 2001.

[18] N.N. Jammil, &M.M. Yehia, "Oral yeasts infection in children", *Iraqi Journal of Pharmacy*, vol. *14*(1),pp. 7-15, 2019.

[19] M.M. Yehia, H.Y.M., Ali, M.A. Al- Jammas, "Oral candidiasis (Thrush) in debilitated children in north Iraq", *Journal Bahrain Medical Society*, vol.16(1),pp. 28-3, 2004.

[20] T. Rawnuck, M.S. Reza, R. Ahmed, M.F.U., Islam, A.L. Hossain, N. Sultana &S. Monwar, "Prevalence of Oral Candidiasis among children caused by different *Candida* Spp." *Journal of medicine today*, vol.34(1), pp.57-60, 2022.

[21] U.C. Nneka, O.M. Ebele**,** "The occurrence of oral thrush yeasts among school children in Onitsha Urban,.Anambra State", Nigeria. *Journal Medical Sci*ences, vol. 5(3),pp.177-80, 2005.

[22] D.K. Al-Ani, F.H. Musa &H.K. Buniya**,** "Isolation and identification of *candida albicans* from children patient with candidiasis from Ramadi city", Iraq. *Human immunodeficiency virus Nursing*, vol.23(2),pp. 441-449, 2023.

[23] K. Tintelnot, G. Hasse, M. Seibold, F. Bregmann, M. Steammler, T. Franz& D. Naumann, "Evalution of phenotypic markers for selection and identification of *Candida* dubliniensis". *Journal Clinical Microbiology*, vol.38(4), pp.1599-1608, 2000.

[24] F.H. Musa, H.K. Buniya, D.K.J., AL-Ani, "Isolation and identification of *candida albicans* from children patient with candidiasis from Ramadi city", Iraq, *Human immunodeficiency virus Nursing*, vol.23(2),pp. 441-

[25] L. Raafat, R. Mhand, F. Mellouki & N Rhallabi, "Contamination of the surfaces of a health care environment by multidrug- resistant (MDR) bacteria", *International Journal of microbiology*, pp. 1-7, ID 3236526, 2020

[26] P.A. Khan, N. Fatima, S. Nabeela, H.M. Khan &A. Malik, "Antifungal susceptibility pattern of *Candida* isolates from a tertiary care hospital in north India: a five-year study", *International Journal of Current Microbiology. and Applied Science*, vol.(1), pp.177-181, 2015.

[27] M.M. Ahmed, H. Hanan, K.S. Sohair, M. Maha, M. Doaa & Mohammed, "Identification and *in vitro* susceptibility pattern of fungal pathogens in immunocomprimised patients with pulmonary fungal infections", *The Medical Journal of Cairo University*, vol.86(8), pp.4307-4316, 2018.

[28] A. Sheneef, H. Hassan, K.A. Ali, M. Saad-Eldin &A. Esmail**,** "Identification and in vitro Susceptibility Pattern of Fungal Infection Isolated from Patients with Otomycosis",The Egyptian *Journal of Medical*. Microbiology, vol.26(3), pp.53-59, 2017**.**