The Medical Journal of Tikrit University (2022) 28 (2): 85-97
DOI: http://dx.doi.org/10.25130/mjotu.28.2.8

IRAQI Academic Scientific Journals

ISSN: 1813-1638

The Medical Journal of Tikrit University

Available online at:www.mjotu.com

MJTU

The Medical Journal of Tikrit University

Candida Albicans Detection and Antifungal Sensitivity Among Children Less Than 2 Years Old in Diyala Province/ Iraq

Omar D. Salman [(1)]; Luma T. Ahmed [(2)]

[(1)] Bilad Alrafidain University College, Diyala, Iraq; [(2)] College of medicine, University of Diyala, Diyala, Iraq

***Keywords*:**

Candida albicans, fungal infection, children and antifungal sensitivity.

ARTICLE INFO

Article history:

Received 22 Jun 2022
Accepted 17 Jul 2022
Available online 31 Sep 2022

Citation: Omar D. Salman; Luma T. Ahmed. Candida Albicans Detection and Antifungal Sensitivity Among Children Less Than 2 Years Old in Diyala Province/ Iraq. The Medical Journal of Tikrit University (2022) 28 (2): 85-97

DOI: http://dx.doi.org/10.25130/mjotu.28.2.8

ABSTRACT

Background: Genus *Candida* is the major yeast infecting humans. *Candida albicans (C. albicans)* have a role in the most opportunistic yeast infections in candidiasis, yet remains as the most common between genus species. About (50-90) % of human candidiasis occurs due to such yeast.
Objectives: This research aims to detect, isolate and identify *C. albicans,* also examining the *C. albicans* susceptibility to some antifungal agents.
Methods: A total of 200 dermal and oral children's samples have been collected, their age was not more than 2 years., the diagnosis was made via conducting a confirmative and routine process through direct microscopic examination. Then, they have been cultured on Sabouraud Dextrose Agar (SDA) as well as microscopic examination related to colonies and sub-cultured on the CHROM agar which has been a selective media used for identifying the species of *candida. C. albicans* were identified via the use of a germ tube test.
Results: From those 200, a total of 91 have been diagnosed as *Candida spp.* infections. In terms of GIT, *C. non albicans* was maximum (20%) and *C. albicans* for diaper rash was (20%). In a total of 60 isolates *Candida spp.*, the results of anti-fungal susceptibility were equivalent for nystatin and amphotericin B. where, 56,7% of isolates were sensitive, while 41,7% and 1,7% of the isolates are resistant and intermediate, respectively. whereas for ketoconazole, 13,3% of isolates were sensitive, also 85,0% and 1,7% were resistant and intermediate to ketoconazole, respectively, lastly, 23,3%, 25,0% and 51,7% of isolates were intermediate, sensitive and resistant to polymyxin, respectively.
Conclusions: An increase in fungal infections and resistance to antifungal medications due to the increased usage of some immunosuppressive medications and extreme chemotherapy.

Corresponding author E mail: omardawood83@gmail.com

Introduction:

Fungi are prevalent and might develop in intestinal tract, mucous membranes and on skin. They are existing in trees, seeds, soil and many vegetations. Even though that not all fungi are considered to be pathogenic; however, a few might result in dangerous illnesses and posing a main risk to the public health [1].

Genus *Candida* is the major yeast infecting humans. *C. albicans*, have a role in the main opportunistic yeast infections in candidiasis, yet remains as the most common between genus species. The yeast is accountable for between 50 and 90% of human candidiasis [2, 3, 4], and an increase in prevalence of yeast infections which are induced via *non Albicans* that include *Candida* like *C. krusei, C. parapsilosis*, *C. tropicalis* and *C. glabrata,* has been reported in others [5, 6, 7]. *C. albicans* is specified as a diploid and one of the opportunistic polymorphic pathogens that is part of almost all normal microbial flora of humans. It was indicated that is borne by (30-60) % of microflora of healthy humans [8, 9]. *C. albicans* were identified in many locations such as vaginal mucosa, oral mucosa, skin and gut [10]. Urinogenic tract, gastrointestinal tract and oral cavity were colonized by a least 70% if population [11, 12]. Yet, within a few conditions, such mucosa, like vulvovaginal candidiasis (VVC) or oropharyngeal candidiasis (OPC), might result in superficial infections. In addition, the human oral cavity involves various microorganisms such as bacteria and yeasts [13]. There were many yeast species identified among the mouth's normal microbial flora; yet a lot of isolates are belonging to species Candida [14]. The fungal genus of *Candida* has been specified as one of the major pathogenic opportunistic yeasts like organisms in the human pathogens. The broad genus of *Candida* comprises over two-hundred species which have been identified as medically vital pathogenic yeasts by a few of them, such as *C. albicans* [15, 16].

Methods and Materials: -

Patients and Samples Collection

The presented work was conducted in the city of Baqubah, the center of Diyala province /Iraq. The study subjects were children with age of two years or less from the two genders, referred to Al-Batool Teaching Hospital, experiencing dermal and oral candidiasis. For such purpose, a personal information survey has been prepared, such survey included questions such as the patients' data, gender, age, body weight, address, rural and urban, water supply, feeding history and so on. Nearly took samples from mouth with the use of a sterile cotton swab.

The sample collection from patients was conducted from Oct-2021 to Feb-2022, after that, all samples have been cultured on SDA. The medium is left to cool to a temperature of 45 Celsius, after that, chloramphenicol antibiotic (250mg/liter) that solvent in distill water has been added, while the mixture is poured into sterile Petri plates [17]. Following an incubation period of 18 hrs., direct slide studied within light microscope. In addition, the microscopic features involving the slides have been subjected to staining via the use of Lacto phenol cotton blue [18]. The samples were sub-cultured on chromogenic agar medi, such media has been a selective media utilized for differentiating the Candida species (parapsilosis, albicans, glabrata and cruzi) [19].

Anti-fungal sensitivity test for *C. albicans*

In this work; the *C. albicans* isolates have been put to test via Kirby Bauer disc diffusion method (DDM) on agar plates of Mueller-Hinton [20] for anti-fungal sensitivity test. The anti-fungal agents: Ketoconazole, amphotericin B, Nystatin and Polymyxin (Bioanalyze/ France) were typically applied for treating dermal and oral candidiasis are utilized in the presented work for sensitivity, after that Muller-Hinton-Bromothymol blue agar (MHB) has been prepared to culture the samples of C. albicans for sensitivity test. Thus, the medium is prepared based

on the instructions of the manufacturer (HiMedia, India);

Results and Discussion

Mother Information

In terms of mixed candida were, UTI (46%), GIT (16%), monilia (34%), followed by fungal breast which was (4%), whereas negative results with no fungal infection was (46%) succeeded by the C. albicans was (34%). The negative results of UTI reach (14%) similarly monilia negative results was (14%), whereas the fungal breast *C. non-albicans* had (4%). This is indicating that there were no relations between the mother information and the results of examination.

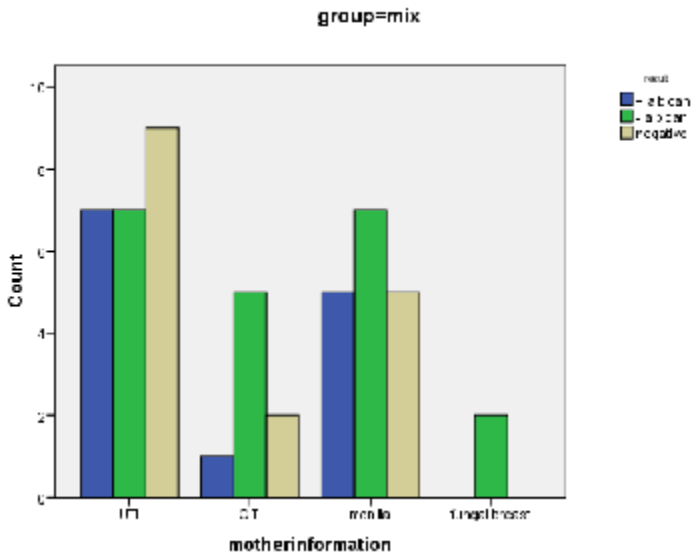


Figure (1) shows result with Mother Information for mixed infection

Baby information

Mixed C. albicans percentages were 32% and 42% for negative result specimens. In case of *C. non albicans* GIT infection was (18%) and In terms of GIT, *C. non albicans* has been maximum (18%) and for the oral thrush negative has (16%), also showing that UTI percentage in mix samples is (12%) and GIT (44%), while the diaper rash is (44%). The

percentage of albicans is (32%) succeeded via negative (42%). This work indicates that the *C. albicans* percentage of UTI is (6%) as can be seen in the figure (2). In terms of GIT, *C. non-albicans* has been maximum by (20%) and for diaper rash, the -albicans has (20%), reporting no relations between baby information and the results of examination.

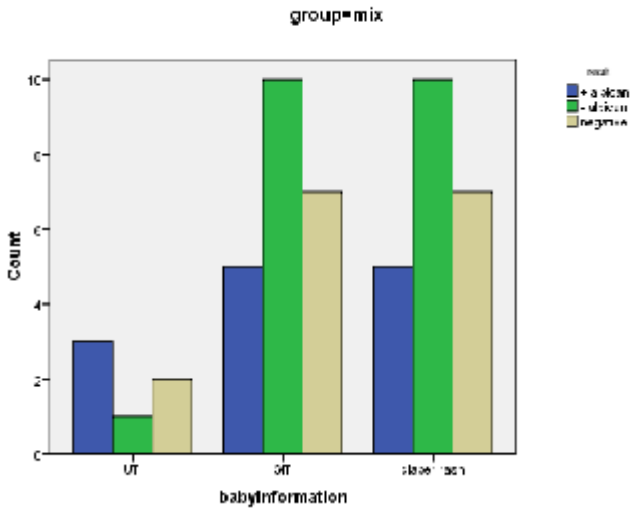


Figure (2) shows result with baby information for mixed infection

Drugs

Figure (3) shows that the percentage of negative of used has been maximum (22%). In terms of not-used, the *C. albican*s and negative were maximum (18%). The results exhibited in Figure (3) shows that the percentages of the drug that has been utilized in mixture has been (64%) and not-used percentage is (36%), whereas percentage of *C. non-albicans* is maximum at (42%) succeeded via negative (32%), it is reported that the percentage of *C. non-albicans* of used is maximum (26%). In terms of not-used, *C. non-albicans* has been maximum (16%) indicating no relation between the drug usage and the results of examination. *Candida spp.* majorly existing in human beings, might be separated in about 50% of the healthy people with no clinical infection signs. [21].

Typically, the infections of *Candida* happen because of the destruction regarding the mission of the epithelial barrier happening in every age group, particularly in elderly and newborns [17]. Also, fungal infections are still considered as one of the main health problems globally and particularly affecting children. Thus, many researches were carried out on different control, economic and therapeutic epidemiological properties of such infection [22].

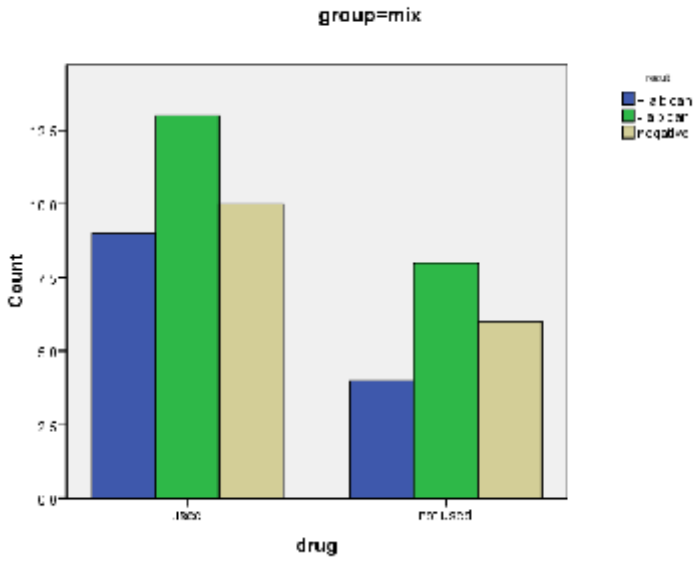


Figure (3) shows result with drug for mixed infection

Sensitivity of Candida spp. to anti-fungal drugs.

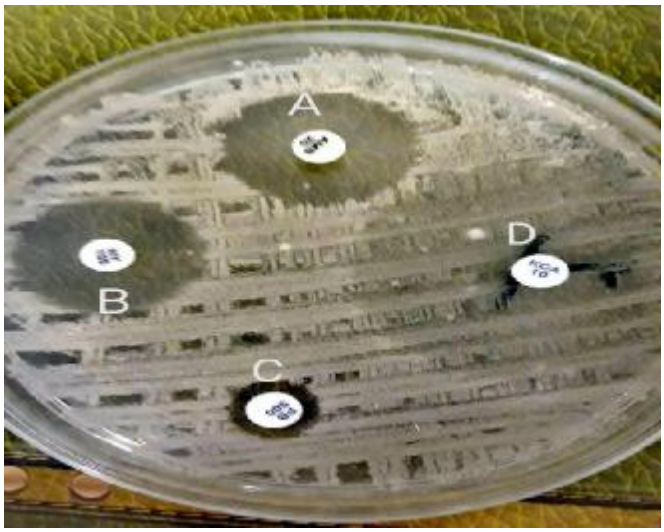
Table (1) is showing the sensitivity of Candida spp. to anti-fungal drugs (nystatin, amphotericin B, polymyxin and ketoconazole) through DDM. In this test, the results have been interpreted based on the guidelines of CLSI (2002, 2011, 2018).

Table 1: Anti-fungal susceptibility test of Candida spp. via DDM.

			Oral		dermal		Total
			C.albican	*C.non albican*	*C.non albican*	*C.albican*	
Nystatin	Sensitive	Count	14	7	5	8	34
		%	70.0%	70.0%	50.0%	40.0%	56.7%
	Resistance	Count	6	3	5	11	25
		%	30.0%	30.0%	50.0%	55.0%	41.7%
	intermediate	Count	0	0	0	1	1
		%	.0%	.0%	.0%	5.0%	1.7%
Total (n) %			(20)100	(10) 100	(10) 100	(20) 100	(60) 100
Chi-Square test		6					
P – value		0.423					
Amphotericin- B	Sensitive	Count	12	7	5	10	34
		%	%60.0	%70.0	%50.0	%50.0	%56.7
	Resistance	Count	7	3	5	10	25
		%	%35.0	%30.0	%50.0	%50.0	%41.7
	intermediate	Count	1	0	0	0	1
		%	%5.0	%0.	%0.	%0.	%1.7
Total (n) %			(20) 100	(10) 100	(10) 100	(20) 100	(60)100
Chi-Square test		3.628					
P – value		0.727					
Ketoconazole	Sensitive	Count	1	2	2	3	8
		%	%5.0	%20.0	%20.0	%15.0	%13.3
	Resistance	Count	19	7	8	17	51
		%	%95.0	%70.0	%80.0	%85.0	%85.0
	intermediate	Count	0	1	0	0	1
		%	%0.	%10.0	%0.	%0.	%1.7
Total (n) %			(20) 100	(10) 100	(10) 100	(20) 100	(60)100

Concerting a total of 60 Candida spp. isolates, 56,7% were sensitive to nystatin, whereas 41,7% and 1,7% were resistance and intermediate to nystatin, respectively. In terms of amphotericin B, an overall 56,7% of isolates have been sensitive, 41,7% and 1.7% were resistance and intermediate to amphotericin B, and 13.30% of isolates have been with sensitivity to ketoconazole, also 85,0% and 1.70% of isolates have

been resistant and intermediate to ketoconazole, respectively, lastly, 23,3%, 25,0% and 51.70% of isolates have been intermediate, sensitive and resistant to polymyxin, respectively.



The increase in fungal infections' occurrence is one of the main concerns for health-care professionals due to the fact that they were growing rapidly over the last decades. Due to the use of some immunosuppressive medications and extreme chemotherapy, such increase in fungal infections has been associated directly to the increase in the immunocompromised individuals [23]. Based on DDM, the acquired results have been recorded based on the appearance regarding the inhibition zone around antifungal disc. Furthermore, the isolates are categorized into 3 groups: moderate, resistant and sensitive based on the inhibition zone diameter.

The presented work shows that with regard to 60 *Candida spp.* isolates, 56.70% of them had sensitivity to the nystatin, 41.70% and 1.70% have been resistant and intermediate to nystatin, respectively, in terms of amphotericin B, 56.70% of isolates have been sensitive, 41,7% and 1.7% were resistance and intermediate to amphotericin B, whereas 13,3% of isolates are sensitive to ketoconazole, 85,0% and 1,7% were resistance and intermediate to ketoconazole, respectively, lastly 23,3%, 25,0% and 51,7% of isolates were

intermediate, sensitive and resistant to polymyxin, respectively, as can be seen in figure 1.

These results are in accordance with the results of Mohammed (2017) indicating that all the isolates of *C. albicans* have susceptibility to Amphotericin B succeeded via Ketoconazole, Fluconazole and Clotrimazole, respectively [24]. Also, they reported high-resistance to Ketoconazole succeeded via Fluconazole then Clotrimazole, on the other hand; they have been less-resistant to the isolates of Amphotericin B. C. albicans showing high susceptibility to Clotrimazole in moderate range succeeded via Fluconazole and Amphotericin B, while they have shown lower susceptibility to the Ketoconazole.

Those results have been in agreement with Ashor *et al.* (2015), that all of the isolates of Candida had susceptibility to the amphotericin B, ketoconazole, voriconazole and econazole [25]. Kaur *et al.* (2014) stated: Out of 103 isolates of Candida, 6.70% have shown the resistance to the amphotericin B, whereas 60 (58.25%) have shown the resistance to the fluconazole (unlike 22.70% & 2.30% for Fluconazole and Amphotericin B, respectively in this research) [26]. Typically, the majority of the C. albicans have sensitivity to the Amphotericin B. moreover, this medication has shown good in vitro activities towards the C. albicans biofilm [27]. Mohammad (2012) had concluded that the Candida had sensitivity towards the anti-fungals (Clotrimazole, Ketoconazole, Nystatin and Metronidazole) in the concentration levels that are higher than 50 µg/ml, and Clotrimazole has been the optimal anti-fungal against the candidiasis, followed by Ketoconazole and nystatin that agreed with the results that have been presented in this paper [28].

Van den Bossche *et al.* (1978) have stated that the azole that includes the ketoconazole has 2 effects on the living cells, first: the impact on the cytochrome P-450 inhibiting the creation of the ergosterol through

removing 14α methyl sterol in the membrane of the plasma, whereas the second impact by the anti-fungal direct interference with the lipid membrane that resulted in destroying the membrane [29]. Typically, the resistance arises due to the mutation in sterol bio-synthesis path-way. Similarly, Fluconazole and Clotrimazole, belonging to the azoles. The polyene anti-fungal agents include the nystatin, pimaricin and amphotericin B [30]. Amphotericin B action occurs by binding to the ergosterol, one of the major components of fungal cell membranes. When existing in required concentration values, it forms the pores in membrane, leading to K+ leakage and fungus death. Ergosterol is rather unique to the fungi, which is why drug has no such catastrophic impacts on the animals [31]. The frequent utilization of the antifungal agents for the prophylactic in the studied patients became the main colonization cause of the Candida species and the cumulative resistance to the anti-fungals [32].

Funding: "This research received no external funding"

Informed Consent Statement: "Informed consent was obtained from all subjects involved in the study."

Conflicts of Interest: Declare conflicts of interest or state "The authors declare no conflict of interest."

Institutional Review Board Statement: "The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee)

References

1. Pfaller, M.A, Diekema, D.J, Procop, G.W, Rinaldi ,M.G.(2007).Multicenter comparison of the VITEK 2 antifungal susceptibility test with the CLSI broth microdilution reference method for testing amphotericin B, flucytosine, and voriconazole against Candida spp. J Clin Microbiol. ;45(11):3522-8.
2. Vázquez-González ,D, Perusquía-Ortiz ,A.M, Hundeiker, M, Bonifaz ,A. (2013).Opportunistic yeast infections: candidiasis,

cryptococcosis, trichosporonosis and geotrichosis. J. Ger. Soc. Dermatology. 2013 May 27;11:381-94.

3. Wächtler B, Citiulo F, Jablonowski N, Förster S, Dalle F, Schaller M, et al. (2012).Candida albicans-epithelial interactions: dissecting the roles of active penetration, induced endocytosis and host factors on the infection process. PLoS One . 2013 May 27;7:1-10.

4. Brunke S, Hube B. Two unlike cousins: C andida albicans and C. glabrata infection strategies. Cellular microbiology. 2013 May;15(5):701-8.

5. Pfaller MA, Diekema D. Epidemiology of invasive candidiasis: a persistent public health problem. Clinical microbiology reviews. 2007 Jan;20(1):133-63.

6. Miceli MH, Díaz JA, Lee SA. Emerging opportunistic yeast infections. The Lancet infectious diseases. 2011 Feb 1;11(2):142-51.

7. Akinjogunla O, Asamudo N, Divine-Anthony O. Susceptibility of Drug Resistant Candida Isolates to Aqueous Leaf Extracts of Phyllanthus amarus, Senna alata AND Nymphaea lotus. World. 2019;11(2):157-67.

8. Ruhnke M, Maschmeyer G. Management of mycoses in patients with hematologic disease and cancer--review of the literature. European journal of medical research. 2002 May 1;7(5):227-35.

9. Taheri Sarvtin M, Shokohi T, Hajheydari Z, Yazdani J, Hedayati MT. Evaluation of candidal colonization and specific humoral responses against Candida albicans in patients with psoriasis. International Journal of Dermatology. 2014 Dec;53(12):e555-60.

10. Bougnoux ME, Diogo D, François N, Sendid B, Veirmeire S, Colombel JF, Bouchier C, Van Kruiningen H, d'Enfert C, Poulain D. Multilocus sequence typing reveals intrafamilial transmission and microevolutions of Candida albicans isolates from the human digestive tract. Journal of clinical microbiology. 2006 May;44(5):1810-20.

11. Mavor AL, Thewes S, Hube B. Systemic fungal infections caused by Candida species: epidemiology, infection process and virulence

attributes. Current drug targets. 2005 Dec 1;6(8):863-74.

12. Ruhnke M. Skin and mucous membrane infections. Candida and candidiasis. ASM Press, Washington, DC. 2002:307-25.

13. Behzadi ,P, Behzadi ,E. Microbiology of Prokaryotes. 1st ed. Tehran: Islamic Azad University, Eslamshahr branch and Shar-e-Qods branch. 2006.

14. Behzadi P, Behzadi E. Medical mycology and the methods of laboratory diagnosis of pathogenic dermatophyte fungi. Kamal-e-Danesh, Tehran. 2003:17-8.

15. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. Journal of oral microbiology. 2011 Jan 1;3(1):5771.

16. Behzadi P, Behzadi E. Evaluation of UVB light efficacy for inducing apoptosis in Candida albicans cultures. Roum Arch Microbiol Immunol. 2012 Jan 1;71(1):39-42.

17. Horvath LL, Hospenthal DR, Murray CK, Dooley DP. Direct isolation of Candida spp. from blood cultures on the chromogenic medium CHROMagar Candida. Journal of clinical microbiology. 2003 Jun;41(6):2629-32.

18. Ellis D, Gosai J, Emrick C, Heintz R, Romans L, Gordon D, Lu SE, Austin F, Smith L. Occidiofungin's chemical stability and in vitro potency against Candida species. Antimicrobial agents and chemotherapy. 2012 Feb;56(2):765-9.

19. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest. 2000 Jul 1;118(1):146-55.

20. Vandepitte J, Verhaegen J, Engbaek K, Piot P, Heuck CC, Rohner P, Heuck CC. Basic laboratory procedures in clinical bacteriology. World Health Organization; 2003 Dec 31.

21. Davis SL, Vazquez JA, McKinnon PS. Epidemiology, risk factors, and outcomes of Candida albicans versus non-albicans candidemia in nonneutropenic patients. Annals of Pharmacotherapy. 2007 Apr;41(4):568-73.

22. Seebacher C, Bouchara JP, Mignon B. Updates on the epidemiology of dermatophyte infections. Mycopathologia. 2008 Nov; 166(5):335-52.

23. Kumar A, Thakur VC, Thakur S, Kumar A, Patil S. Phenotypic characterization and in vitro examination of potential virulence factors of Candida species isolated from blood stream infection. World Journal of Science and Technology. 2011;1(10):38-42.

24. Mohammed NA, Abdulbaqi NJ, Ajah HA. Epidemiological Study of Candida Species among Vaginal and Oral Candidiasis from different clinical states. International Journal of ChemTech Research. 2017;10(5):844.

25. Ashour SM, Kheiralla ZM, Maklad SS, Ameen MR, Zaki SS. Relationship between virulence factors of Candida species with candiduria and myeloperoxidase concentrations. Int J Curr Microbiol App Sci. 2015;4(1):108-23.

26. Kaur R, Goyal R, Dhakad MS, Bhalla P, Kumar R. Epidemiology and virulence determinants including biofilm profile of Candida infections in an ICU in a tertiary hospital in India. Journal of Mycology. 2014 Jan 12;2014.

27. Mahmoudabadi AZ, Zarrin M, Kiasat N. Biofilm formation and susceptibility to amphotericin B and fluconazole in Candida albicans. Jundishapur journal of microbiology. 2014 Jul;7(7).

28. Mohammed NA. *Detection of Candida spp. and other pathogens responsible for vulvovaginitis in women with contraceptive methods* (Doctoral dissertation, MSc thesis. College of Science, University of Baghdad, Iraq).

29. van den Bossche H, Willemsens G, Cools W, Lauwers WF, Le Jeune L. Biochemical effects of miconazole on fungi. II. Inhibition of ergosterol biosynthesis in Candida albicans. *Chem Biol Interact.* 1978; 21(1):59-78. doi:10.1016/0009-2797(78)90068-6

30. Chang YL, Yu SJ, Heitman J, Wellington M, Chen YL. New facets of antifungal therapy. *Virulence.* 2017; 8(2):222-236. doi:10.1080/21505594.2016.1257457

31. Ralston SH, Penman ID, Strachan MW, Hobson R, editors. Davidson's Principles and Practice of Medicine E-Book. Elsevier Health Sciences; 2018 Feb 2.

32. Kothavade RJ, Kura MM, Valand AG, Panthaki MH. Candida tropicalis: its prevalence, pathogenicity and increasing resistance to fluconazole. Journal of medical microbiology. 2010 Aug 1;59(8):873-80.