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PHENOTYPIC AND GENOTYPIC DIAGNOSIS OF *CANDIDA ALBICANS* ISOLATED FROM DIFFERENT SOURCES

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Abstract

The present study was conducted to isolation and identification Candida species isolated from different clinical cases patients by different methods including direct examination, laboratory culture, finally, identification by PCR and determine. During the period from September 2018 to January 2019, a total of 150 clinical isolated different clinical cases patients were collected from candidiasis patients with attending to center in AL-Sader Medical City Hospital in AL-Najaf Governorate, the samples were collected as following:- 100(66.6%) oral swab samples, 30(20%) samples of urine and 20(13.4%) vagina samples. Screening survey was conducted to study, the resistance of *Candida albicans* isolates for 4 antibiotics using hole and disk diffusion Methods. The result showed Significant difference at P 0.05 to different antibiotics such as Griseoflavin, Trbinafine, Amphotercin B and Fluconazole.

***Keywords*:** Candidiasis, Fluconazole Amphotercin B.

Introduction

Candidiasis is an acute or chronic infection produced by the Candida that is normally present in the mucous membranes of oral healthy individuals, upper respiratory tracts, gastrointestinal tracts and genital tracts of women, can produce serious systemic disease and it is developed to infection usually in vagina, mouth, on the skin that causes itching, red patches or white patches and irritation (Dabas, 2013).

There are three main types of Candidiasis which can cause occasional symptoms in healthy people, the first one is known as Mouth Candidiasis, the second one is known as Urinary candidiasis, and the third one is known as Vulvovaginitis *Candida*. Therefore, in the following paragraphs, a brief description of each type was highlighted. *Candida species* are considered as one of the most important causes of human infections. Candidacies are a common infection caused by yeast-like fungus (Lohse *et al*., 2018).

Candida is not harmful in healthy hosts, but may cause opportunistic infections in immune-compromised hosts, such as patients suffering from AIDS, leukemia and diabetes (Batool *et al.,* 2011).

Candida albicans varied in their susceptibility to the most commonly used antifungal agents, and the intrinsic resistance to antifungal therapy seen in *Candida albicans*, along with the development of acquired resistance then seen in other species, that could be form a major problem in the management of *Candida* infection. A better understanding of the mechanisms and clinical impact of antifungal drug resistance is required for efficient treatment of patients that infected with *Candida* and for improving treatment outcomes (Alenzi, 2016).

Material and methods

Specimen's collection

The patients who complain of oral swab, urine and vagina samples, in Medical AL-Sader city Hospital (150) were collected from patients. The samples were then transported to the laboratory. Four plates of Sabouraud's dextrose agar with the addition of 0.05 g/L chloramphenicol were inoculated: two plates were incubated at 25 ºC for 48h (Sayyada *et al*., 2010).

Then, the colony from Sabouraud's dextrose agar was inoculated on CHROM agar. Finally, identification by PCR using universal primer is *(CTS*). 5'-TCG CAT CGA TGA AGA ACG CAG C-3**'** 5'-TCT TTT CCT CCG CTC ATT GAT ATG C-3'. The program for this primer is the initial denaturation of template DNA for all primers, the reaction program included one cycle at 94 °C for 3 min. Then, 30 cycles of amplification were done; each cycle included 1 min at 94 °C for template denaturation; 30sec at 60 °C for annealing of *CTS* primers ,then 1min at 72 °C for extension of *CTS* primers. Finally, the final extension for 10min at 72 °C.

Antifungal test: according to (CLSI, 2014).

Results and Discussion

Collection of samples

The present study included a collection of 150 samples from the randomly recruited patients with different clinical cases, these samples were obtained from immuno-compromised patients whose attended to AL-Sadder medical city which included 100 (66.6%) oral swab samples were collected from clinical cases, 30(20 %) samples of urine and 20 (13.3 %) vagina samples, as show in (Table 2).

Table 4.1: Numbers and percentages of samples collected from immuno-compromised patients with different clinical cases

Percentage (%)	No. of samples	Type of samples
66.6%	100	Oral
20 %	30	Urine
13.3 %	10	Vagina
100%	150	Total

According to figure and table (2) the result show the prevalence of oral candidiasis is increasing, as it is one of the most common fungal infections these prevalence because of hosting local conditions are: (a) reduce the secretion of saliva, (b) high-carbohydrate diet, (c) teeth wear, (d) local mucosal diseases and epithelial changes, (e) changes in commensal flora (Akpan and Morgan, 2010).

Additional important features are the oral pH and the glycemic control. A study performed by Célia *et al*. (2019) demonstrated that pyruvates and acetates are the major ionic species, generating a quick decrease in pH with *Candida spp*. as found in batch cultures of mixed saliva supplemented with glucose (Célia *et al*., 2019).

The results of table (2) showed that 20 (13.3 %) vagina samples, vulvovaginal *candidiasis* (VVC) remains to be clarified**,** but some investigations propose that the general reduced immune response associated with DM is the main cause of recurrent VVC (Atabek *et al.,* 2013). *C. albicans* is the most common species isolated, followed by C. glabrata in patients both with and without diabetes (Célia *et al*., 2019).

The present study show in figure and table (4-1) that revealed 30(20 %) samples of urine, this result near-link with (Yismaw *et al.,* 2013) who found around 10% to 15% of in-hospital urinary tract infections (UTIs) are related to *Candida spp*. and the prevalence is still increasing the prevalence of candiduria and the type of species implicated differ between institutions and also depend on the underlying predisposing factors among the infecting hosts (Pfaller *et al*., 2014).

Morphological identification

Identification *Candida albicans* on Sabouraud dextrose agar : All collected samples were cultured on Sabouraud dextrose agar (SDA); the colonies of *Candida* spp. were cream colored to yellowish, grow rapidly mature in 24-48 hr., the texture of the colony smooth, glistening or dry depending on the species. These results were agreed with (Bhavan *et al*., 2010) Figure (1).

Fig. 1 : Showing growth of *Candida albicans* on SDA at 37°C for 48 hours

Microscopic identification by Gram stain of *Candida albicans* : Microscopic examination is a preliminary test to diagnose the *Candida ssp*. Microscopic examination has been examined at laboratory of advanced mycology/Faculty of Science/University of Kufa for diagnosis and study. Each sample was stained of Lacto-phenol cotton blue stain and examined microscopically.

All diagnostic yeast species exhibit a positive results when prepared with gram stain, these results were in agreement with (Zahraa, 2016).

Identification of *Candida albicans* on chrom agar medium

Chrom agar is a selective medium for the isolation of yeast that simultaneously provides direct differentiation and identification of several C*andida* spp. (Sayyada *et al.*, 2010).

This study has showed that using chrom agar Candida which is considered a differential agar the colonies appear *C. glabrata* dark pink, and *C. parapsilosis* white pale pink (Hospenthal *et al*., 2002) and *C. albicans* characterized by light green color smooth colonies Figure (3).

Chromogenic media are effective and rapid testing in the diagnosis of *Candida* at the species level of the resulting color after inoculation and incubation compared with other culture traditional methods, change in color produced by reactions of species-specific enzymes with a proprietary chromogenic substrate, the medium greatly facilitates the detection of specimens containing mixtures of yeast species (Iyampillia *et al.*, 2004). All of the yeast isolates tested grew on chrom agar *Candida* after 48 hr. of incubation at 37°C, the majority of yeasts tested had grown well, as specified in the manufacturer's instructions.

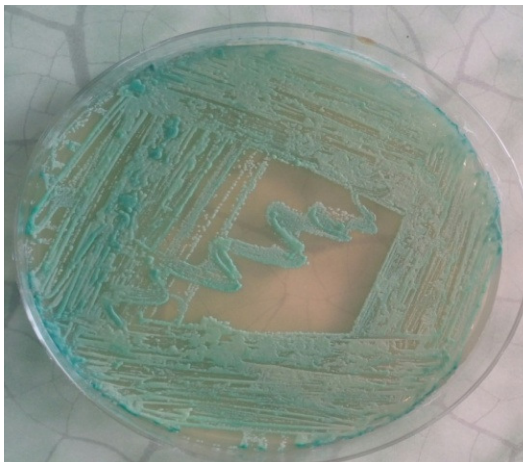


Fig. 3 : The colony of A-*Candida albicans*, on Chrom agar at 37C for 48 hours.

On chrom agar, *Candida* spp. like *C. albicans*, *C. tropicalis* and *C. krusei* can be easily differentiated on the basis of colony morphology and color. *C. albicans* produce leaf-green colored colonies, *C. tropicalis* colonies are dark blue-grey with a purple halo and *C. krusei* forms pink colonies with whitish border. Colonies of other species are entire and smooth and colony color ranges from white to dark pink (Deorukhkar and Shahriar, 2018).

Chrom agar can be reliably used for differentiation of *C. dubliniensis* and *C. albicans*. *C. dubliniensis* produces dark green colored colonies. However, the ability of *C. dubliniensis* to form characteristic dark green colored colony may be lost on storage at -70ºC and after repeated subcultures probably due phenotypic switching (Neppelenbroek *et al*., 2014).

Frequency of *Candida species*

The predominance of *Candida albicans* compared with other *Candida* species was a notable as shown in figure (4-6) which indicate that the majority of the isolates were *C. albicans* 56.4%, because of Candidiasis caused by opportunistic overgrowth of *C. albicans*, followed by *Candida parapsilosis* 20.5%, *Candida galabrata* 15.4% then *Candida tropicalis* 7.7%.

Candida albicans is generally considered as most pathogenic member of the genus and most common cause of different types of candidiasis (Deorukhkar *et al.,* 2014a).

C. albicans is the most virulent among the Candida species, and can cause several forms of candidiasis in immuncompromised. Several factors were described as virulent for pathogenesis of *C. albicans*. The secreted aspartyl proteinases, phospholipases, germ tube formation, adherence to host tissues and phenotypic switching (Da Costa *et al*., 2009).

NAC is a heterogeneous group of *Candida* species with approximately 19 species implicated in human infections. *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis* are most commonly reported NAC spp. (Krcmery *et al.,* 2002).

Molecular identification

PCR assay : The results showed that our different molecular sizes of *CTS* Primer of *Candida spp* (this primer considered as universal primer for detection *Candida spp*). In addition, it offered PCR products of these isolates in Figure (5).

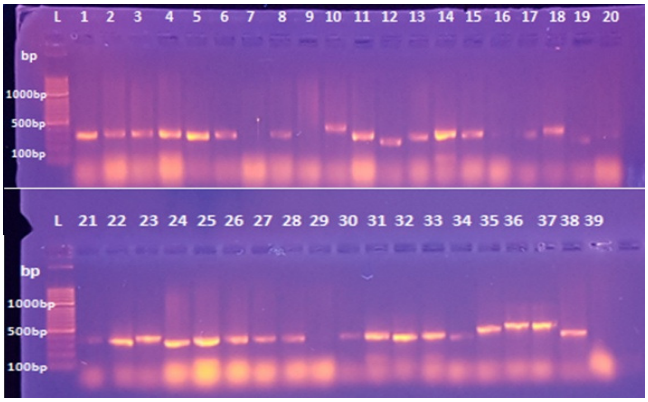


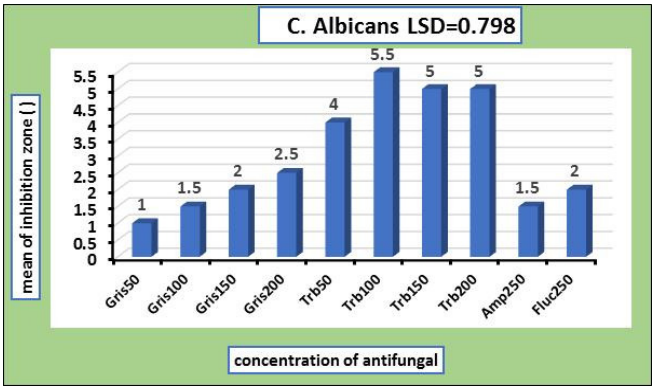
Fig. 5 : Agarose gel electrophoresis of PCR products for *Candida* spp isolates amplified by CTS Primer (1.3gagarose gel 80 volts for1 hour).

Molecular techniques are targeted to detect Candida species in a short period of time, with a high sensitivity and specificity. For this purpose several PCR methods have been developed, such as nested PCR, multiplex PCR**,** Taq-man PCR, Light-Cycler PCR and fluorescent PCR (Innings *et al*., 2007).

The use of PCR-based tests to detect *Candida* DNA has produced encouraging results (Khan and Mustafa, 2001). However, detection of *Candida* species by PCR lacks sensitivity when the test is performed with blood or serum specimens (Martin *et al*., 2000). DNA amplification with universal fungal primers followed by detection using species-specific probes greatly improved the sensitivity of *Candida* detection (Posteraro *et al.,* 2000).

Antifungal susceptibility of *Candida albicans*

The result of this study for the antifungal activity for (Griseoflavin, Trbinafine, Amphotercin B and Fluconazole), explaining that Griseoflavin expressed maximum inhibitory zone at concentration 200 mg/ml which was 2.50 + 0.29 mm against *C. albicans*, but in low concentration 50 mg/ml was 1.00 + 0.29 mm against *C. albicans*. While the inhibition zone diameter reach to 5.50+0.29 mm against *C. albicans* in 100 concentration of Trbinafine. The low concentration of Trbinafine recorded 4+0.29 mm for *C. albicans*. This result show the significant difference at P 0.05 between different antifungal and different concentration, as shown in Figure (4-11 and 4-12)**.**



Significant difference at P 0.05

Fig. 4-11 : Effects of some Antibiotics against growth of *C. albicans*.

The limited availability of antifungals is a major impediment for the effective treatment of fungal infections (Vandeputte *et al*. 2012). This is further compounded by the fact that the generation of newer antifungals has lagged behind when compared to the pace of emergence of fungal infections. The components of the fungal CW such as mannans, glucans and chitins; and a few of the enzymes of the ergosterol biosynthetic pathways which are unique to fungal cells are commonly targeted for the development of antifungal agents (Munro *et al*., 2001).

In our study Fluconazole sensitivity had shown Significant difference at P▪ 0.05 against *C. albicans*, which is quite comparable with study of (Babin *et al*., 2013), while study done by (Ajitha *et al.,* 2014) and (Emam *et al*., 2012) showed higher sensitivity rate of 93.3% and 96.7% respectively.

There have been continuous efforts to develop new antifungal agents or to improve the efficacy of conventional antifungal methods (Nami *et al*., 2019). However, current intervention strategies often have limited efficiency in treating fungi, especially those pathogens resistant to drugs or fungicides (Beardsley *et al.,* 2018). The use of high-throughput screenings/bioassays to develop new antifungal agents and/or define cellular targets of newly-identified antifungal agents is still a developing field. This is especially true with regard to determining the involvement of specific genes, genetic pathways or previously undetected lipid changes in cellular membranes, cross talks between lipid molecules and mitochondrial dysfunction, cell wall integrity and filamentous fungal growth, etc., which can explain resistance to conventional antifungal agents (Meir and Osherov, 2018).

References

Ajitha, R. and Maimoona, M. (2014). Phenotypic identification of *Candida* species and their susceptibility profile in patients with genitourinary candidiasis International Journal of Advanced Research, 2(12): 76-84.

Akpan, A. and Morgan, R. (2010). Oral candidiasis. Postgraduate Medical Journal, 78: 455-459.

Alenzi F.Q.B. (2016).Virulence factors of Candida species isolated from patients with urinary tract infection and obstructive uropathy. Pak J Med Sci.; 32(1).

Atabek, M.E.; Akyürek, N.; Eklioglu, B.S. (2013). Frequency of Vaginal Candida Colonization and Relationship between Metabolic Parameters in Children with Type 1 Diabetes Mellitus. J. Pediatr. Adolesc. Gynecol., 26: 257–260.

Babin, D.; Kotigadde, S.; Rao, P.S. and Rao, T.V. (2013).Clinico-mycological profile of vaginal candidiasis in a tertiary care hospital in Kerala. International Journal of Research in Biological Sciences; 3(1): 55-59.

Batool, S.N.; Ablollah, R. and Fereshteh, M. (2011). Prevalence of Candida species in the oral cavity of patients with periodentitis. African Journal of Biotechnology, 10(15): 2987- 2990.

Beardsley, J.; Halliday, C.L.; Chen, S.C.-A.; Sorrell, T.C. (2018). Responding to the emergence of antifungal drug resistance: Perspectives from the bench and the bedside. Future Microbiol., 13: 1175–1191.

Bharathi, M. and Usharani A. (2011). Pathogenic fungal isolates in sputum of HIV positive patients. J. AIDS. HIV. Res., 3: 107-113.

Bhavan, P.S.; Rajkumar, R.; Radhakrishnan, S.; Seenivasan, C. and Kannan, S. (2010). Culture and identification of *Candida albicans* from vaginal ulcer and separation of enolase on SDS-PAGE. Inter. J. Bio. 2; (1): 84-93.

Célia. F.R.; Maria, E.R.; Mariana, H. (2019). Candida sp. Infections in Patients with Diabetes Mellitus. J. Clin. Med., 8: 76.

Clinical and Laboratory Standards Institute (CLSI) (2014). Performance Standards for Antimicrobial Susceptibility Testing; 22ed. Informational Supplement.32(3).PA, USA.

Da Costa, K.R.C.; Ferreira, J.C.; Komesu, M.C. and Candido, R.C. (2009). *Candida albicans* and *Candida tropicalis* in oral candidosis: quantitative analysis, exoenzyme activity, and antifungal drug sensitivity. Mycopathologia. 167: 73-9.

Dabas, P.S. (2013). An approach to etiology, diagnosis and management of different types of candidiasis. Academic Journals. 4(6): 63-74.

Deorukhkar, S.; Saini, S.; Mathew, S. (2014). Non-albicans Candida Infection: An Emerging Threat. Interdiscip Perspect Infect Dis.; 2014: 615958.

Deorukhkar, S.C. and Shahriar, R. (2018). Identification of Candida Species: Conventional Methods in the Era of Molecular Diagnosis. Remedy Publications LLC. Annals of Microbiology and Immunology 1(1): Article 1002.

Emam, S.M.; Abeer, A.; Abo, E. and Walid, A. (2012). Exo-enzymes Production and Antifungal Susceptibility of Candida Species Isolated from Pregnant Women with Vulvovaginitis. Journal of American Science, 8(12).

Hospenthal, D.R.; Beckius, M.L.; Floyd, K.L.; Horvath, L.L. and Murray, C.K. (2002). Presumptive identification of *Candida* species other than *C. albicans, C. krusei* and *C. tropicals* with the Chromogenic medium Chrom agar *Candida*. Annclin microbial Antimicrob. 5: 1-5.

Innings, A.; Ullberg, M.; Johansson, A.; Rubin, C.J.; Noreus, N.; Isaksson, H.B. (2007). Multiplex real-time PCR targeting the RNase P RNA gene for detection and identification of *Candida* species in blood. J. Clin. Microbiol., 45: 874-880.

Iyampillia, T.; Micheal, J.S.; Mathai, E. and Mathews, M.S. (2004). Use of CHROM agar medium in the differentiation of *Candida* species: is it cost effective in developing countries, Ann. Trop. Med. Parasitol., 98: 279-282.

Khan, Z.U. and Mustafa, A.S. (2001). Detection of *Candida* species by polymerase chain reaction (PCR) in blood samples of experimentally infected mice and patients with suspected candidemia. Microbiol. Res. 156: 95–102.

Krcmery, V. and Barnes, A.J. (2002). Non-albicans *Candida* spp causing fungaemia: pathogenicity and antifungal resistance. J Hos Infect. 50(4): 171-177.

Lohse, M.B.; Gulati, M.; Johnson, A.D. and Nobile, C.J. (2018). Development and regulation of single-and multi-species *Candida albicans* biofilms. Nature Reviews Microbiology, 16(1): 19.

Martin, C.; Roberts, D.; van Der Weide, M.; Rossau, R.; Jannes, G.; Smith, T. and Maher, M. (2000). Development of a PCR-based line probe assay for identification of fungal pathogens. J. Clin. Microbiol. 38: 3735–3737.

McGinnis, M.R. (1980). Laboratory hand book of medical mycology, Sabouraudia 17: 145-154.

Meir, Z. and Osherov, N. (2018). Vitamin Biosynthesis as an Antifungal Target. J. Fungi, 4: 72.

Munro, C.A.; Winter, K.; Buchan, A.; Henry, K.; Becker, J.M.; Brown, A.J. *et al.* (2001). Chs1 of *Candida albicans* is an essential chitin synthase required for synthesis of the septum and for cell integrity. Mol Microbiol., 39: 1414–1426.

Nami, S.; Aghebati-Maleki, A.; Morovati, H. and Aghebati-Maleki, L. (2019). Current antifungal drugs and immunotherapeutic approaches as promising strategies to treatment of fungal diseases. Biomed. Pharmacother. 110: 857–868

Neppelenbroek, K.; Seo, R.; Urban, V.; Silva, S.; Dovigo, L.; Jorge, J.H. *et al*., (2014). Identification of *Candida* species in the clinical laboratory: a review of conventional, commercial, and molecular techniques. Oral Dis.; 20(4): 329-44.

Pfaller, M.A.; Andes, D.R.; Diekema, D.J.; Horn, D.L.; Reboli, A.C.; Rotstein, C.; Franks, B. and Azie, N.E. (2014). Epidemiology and outcomes of invasive candidiasis due to non- albicans species of *Candida* in 2,496 patients: Data from the Prospective Antifungal Therapy (PATH) registry 2004–2008. PLoS One, 9, e101510.

Posteraro, B.; Sanguinetti, M.; Masucci, L.; Romano, L.; Morace, G. and Fadda, G. (2000). Reverse cross blot hybridization assay for rapid detection of PCR-amplified DNA from *Candida* species, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* in clinical samples. J. Clin. Microbiol. 38: 1609–1614.

Sayyada G.N.; Shazia, T.H. and Shahana, U.K. (2010). Use of Chrom agar *Candida* for the presumptive identification of *Candida species* directly from clinical specimens in resource-limited settings Libyan J Med., 5: 2144.

Vandeputte, P.; Ferrari, S. and Coste, A.T. (2012). Antifungal resistance and new strategies to control fungal infections. Int. J. Microbiol., 2012: 713687.

Yismaw, G.; Asrat, D.; Woldeamanuel, Y.; Unakal, C. (2013). Prevalence of candiduria in diabetic patients attending Gondar University Hospital, Gondar, Ethiopia. Iran. J. Kidney Dis., 7: 102–107.

Zahraa, M.W. (2016). Phenotypic and molecular characterization of Candida species isolated from hospital acquired infections in Hilla city. MSc. College of Science for Women/ University of Babylon.