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 Governate, 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 Grisoflavin, Trbinatine, 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 °C 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

<table>
<thead>
<tr>
<th>Percentage (%)</th>
<th>No. of samples</th>
<th>Type of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>66.6%</td>
<td>100</td>
<td>Oral</td>
</tr>
<tr>
<td>20 %</td>
<td>30</td>
<td>Urine</td>
</tr>
<tr>
<td>13,3 %</td>
<td>10</td>
<td>Vagina</td>
</tr>
<tr>
<td>100%</td>
<td>150</td>
<td>Total</td>
</tr>
</tbody>
</table>

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 spp. 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 Candida 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 -70°C and after repeated subcultures probably due phenotypic switching (Neppeleenbroek 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 glabrata 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).
PCR, Light-Cycler PCR and fluorescent PCR (Innings detection (Posteraro developed, such as nested PCR, multiplex PCR species in a short period of time, with a high sensitivity and specificity. For this purpose several PCR methods have been tropicalis produced encouraging results (Khan and Mustafa, 2001).

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 (1.3%agarose 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, Amphoterin 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).

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 laggèd 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**


