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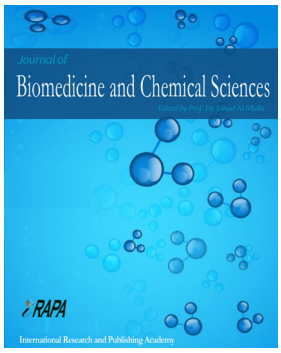

Fungal Survey of Different Clinical Samples Collected from Patients

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ABSTRACT

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide. Candida spp. are eukaryotic representatives of the fungi kingdom. The genus Candida includes about 150 species, but many species are endosymbionts of humans, causing infections mainly in immunosuppressed hosts. The frequency of Candida isolates as a cause of hospital infections has risen in recent years, leading to high rates of morbidity and mortality. Knowledge of the epidemiology of those hospital-acquired fungal infections is essential to implement an adequate antifungal therapy. Fungal survey of different clinical samples collected from patients, to determine the effect of antifungal on C. albicans isolates from different body sites and lesions (blood, wounds swabs, ear swabs, bronchoalveolar lavage, high vaginal swabs, sputum, throat swabs, and urine). Swab sticks were collected from different sites of patients' bodies (blood, wounds swabs, ear swabs, bronchoalveolar lavage, high vaginal swabs, sputum, throat swabs, and urine) then streaked directly on labelled Sabouraud's dextrose agar (SDA) plates and incubated at 25°C for (2-7) days. The growing colonies of Candida spp. isolates were initially diagnosed on (SDA) agar, then confirmed by Chrome Candida Agar, the plates were incubated under aerobic conditions for 24 hr. at 37°C. Thereafter, an antifungal sensitivity test was done. Only 209 of 1596 samples taken from the patients and cultured on Sabouraud's dextrose agar were found to be positive. From 1596, just 59 isolates were Candida albicans after subculture on CROM Candida Agar, API Candida and germ tube formation. Antifungal susceptibility tests were performed on 59 C. albicans isolates. Regarding antifungal resistance by C.albicans it was found 8 (14%) of isolates were resistant isolates resistant to fluconazole, 11 (19%) were resistant to miconazole as well as 11 (19%) were resistant to econazole, while 50 (85%) of isolates were sensitive to Nystatin, Clotrimazole (56, 95%), and to Ketoconazole (54, 92%). The study shows a higher antifungal resistance in the clinical samples, which proves the risk in the C. albicans management program.

INTRODUCTION

The history of the discovery and naming of Candida extends from the ancient Greeks to modern day researchers (Singh, &Raksha, 2013). Among fungal species, the Candida genus has generated interest both as a threat to human health and as an asset to industrial manufacturing. Candida pathogens cause a range of infections from common superficial mucosal infections to life-threatening invasive infections, especially amongst immunocompromised individuals (Uthayakumar, et al., 2021). The incidence and prevalence of invasive fungal infections have increased since the 1980s, especially in the large population of immunocompromised patients and/or those hospitalized with serious underlying diseases

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(Sardi, et al., 2013). Around 80% of infections are caused by Candida albicans, although Candida non-albicans infections C.glabrata, C.tropicalis, C.krusei, C.dubliniensis are becoming more frequent (Ciurea, et al., 2020). C.albicansis the most prevalent among Candida spp., which causes both superficial and systemic infections (Chandra, et al., 2015).

Pathogenesis of candidiasis depends on the expression of virulence factors like germ tube formation, adhesions, phenotypic switching, biofilm formation, and the production of hydrolytic enzymes(Marak&Dhanashree, 2018). The frequency of Candida isolates as a cause of hospital infections has risen in recent years, leading to high rates of morbidity and mortality. The knowledge of the epidemiology of those hospital acquired fungal infections is essential to implement an adequate antifungal therapy (Jacobsen &Hube, 2017). Current medical knowledge and research on patients' management are still evolving, and several protocols on minimizing risk of infection by Candida spp. (Wang, et al., 2020) C. albicanscells initially adhere to host cells in the yeast budding form, which can grow normally on the mucosal and skin surface of the host without causing immune response (Du, et al., 2020). It is largely related to its polymorphic nature in response to environmental factors such as temperature, pH, and media composition (Williams, & Lorenz, 2020). The shift from commensalism to pathogenesis among Candida spp. in various body sites is attributed to many virulence determinants among which are host tissue adhesion, response to environmental stresses, secretion of hydrolases, biofilm production (She, et al., 2008), and secretory proteinases, as Candida virulence factors, can improve the potential of fungal organisms to colonize and penetrate the host tissue and disrupt the immune system (Morgan, 2016).

Biofilm formation can enhance the ability of C.albicans to resist immunity and antifungal drugs, resulting in repeated infections. Therefore, biofilm formation is another important virulence factor of C.albicans (Agarwal, 2015). Biofilms are genetically resistant to antifungal agents including amphotericin B (AMB) and fluconazole (Sadeghi, et al., 2018). Unlike antibacterial drugs, the array of available antifungals is somewhat scarcer. Azoles, polyenes, and echinocandins are the three main antifungal classes, being the last considered first-line therapy in many hospitals for the treatment of invasive candidiasis (Gleiznys, et al., 2015). With the increase of clinical and/or microbiological antifungal resistance or tolerance, susceptibility tests play an ever-increasing role in the selection of antifungal drugs. Notably, correlation between in vitro susceptibility and treatment success is not always straightforward (Bihari, et al., 2014). This study aims to determine the effect of antifungal on C.albicansisolates from different body sites and lesions.

MATERIALS & METHODS

Samples Collection

This study comprises a total of 1596 samples collected from different body sites and lesions (blood, wounds swabs, ear swabs, branchoalveolar lavage, high vaginal swabs, sputum, throat swabs, and urine) of in and outpatients with different illness from both sexes who attended and admitted AL-Imammain AL-Kadhimain medical city during the period from January-2021 to October-2021.

Isolation and Identification of Fungi

Culture on Sabouraud's Dextrose Agar

After collection of samples its transport immediately to laboratory to streak directly on the well labeled Sabouraud's dextrose agar (SDA) plates and incubated at 25°C for (2-7) days. The growth was identified based on their morphological and cultural characteristics and microscopic examination which was done using lactophenol cotton blue staining technique(Koneman, et al., 1997).

Culture on ChromeCandida Agar

Chrome Candida Agar was a selective and differential medium for the isolation of candida spp. producing different colors on the medium, the colonies of C. albicans weregreen. The colonies of Candida grown on (SDA) were sub-cultured on Chrome Candida Agar and incubated at 35 °C (± 2 °C) for 24 hours (Holmes et al., 1994).

Germ Tube Formation

The production of yeast germ tube isolates were tested by inoculation a small portion of an isolated

colony on 0.5ml of human serum. The suspension was incubated at 37°C for 3hrs. Then a drop of this suspension was put on a clean glass slide covered by cover-slip and examined under light microscope. Germ tube was appeared as short lateral hyphal filament. The serum used in this test was prepared by aspirating blood then centrifuged at 3000 rpm for 15 minutes (Zehm, et al., 2012).

Biochemical Assay

Api Candida was a standardized system for the identification of yeasts within (18-24) hours notably those most frequently encountered in clinical samples. The Api Candida strip consists of 10 tubes containing dehydrated substrates, which enable the performance of 12 identification tests (sugar acidification or enzymatic reactions). The reactions produced during incubation are revealed by spontaneous color changes. The reactions were read visually according to the reading table and identification was obtained by consulting the list of profiles supplied in the package(Gunasekera, et al., 2015).

Statistical analysis

Data of this study samples were analyzed using statistical package for social sciences (SPSS) version 16. Descriptive statistics were presented as frequencies, percentage.

RESULTS & DISSCUSION

This study employed 1596 samples from patients with different complains, separated into 10 age groups per decade, the samples included blood, ear swabs, high vaginal swabs, bronchoalveolar lavage, wound swabs, sputum, throat swabs, and urine samples. The primary risk factors for Candida infections are colonization of the skin and mucous membranes, change or disruption of natural host barriers, such as wounds, surgery, and the installation of indwelling intravascular catheters (Costa-de-Oliveira, et al., 2020). Is significant difference between the age groups of patients in this Study (table 1). The highest number of cases were between the ages of (41-50), which was 278 (17.4%), and the fewest were between the ages of (91-100), with only 2 (0.1%). This finding is consistent with (Alshehriet al., 2022) who discovered that the highest number of infections of Candida spp. were recorded in the age group (40-49) 178 (26.9%), and the lowest infection was seen seen in the age group above 80 years, 17 (2%).

The fact that samples of patients obtained from hospitals were either inpatients or outpatients suggests that sampling bias may be to blame. As a result, there was a noticeable variation in the infection rates of males and females. More than half of the samples were obtained from 910(57%) females, while only 686(43%) were obtained from males in different conditions. This result agrees with (Losteret al., 2016),when found samples collected from infected females (613, 66%) were more than those collected from infected males (307, 33.4%). Factors that play role in these differences between males and females are the immune response and awareness of the fungal disease, work environment, the type of work, the housing environment, and whether or not patients have taken medications (Kraševec, 2022). With the impacts of sex chromosomal differences acting as a background, human sex differences result in a complicated interaction between sex hormones, genetic variation, and the environment (Cui, et al., 2022).

Table 1

Patients distribution according to the sex and age group

Age Groups	Male	Female	Total	Percentage %
0-10	103	111	214	13.4%
11-20	77	114	191	12.0%
21-30	96	171	267	16.7%
31-40	82	170	252	15.8%
41-50	112	166	278	17.4%
51-60	91	89	180	11.3%
61-70	81	50	131	8.2%
71-80	34	33	67	4.2%
81-90	8	6	14	0.9%
91-100	2	0	2	0.1%
Total	686	910	1596	100%

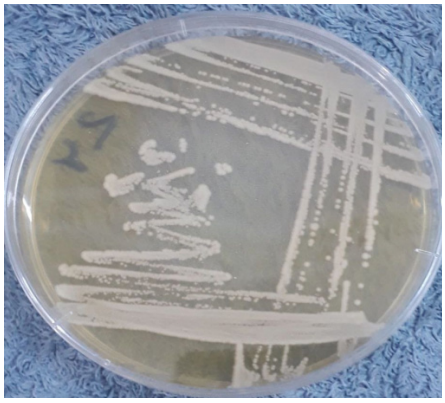
More half of samples were obtained from females 910(57%) while males were 686(43%), as shown in table (2):

Table 2

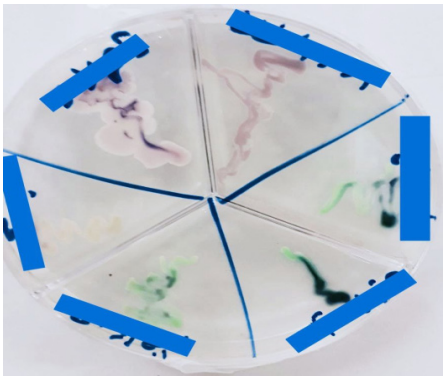
Number and percentages of samples according to the sex factor

Samples	Male	Female	Total	Percentage
Blood	137	91	228	14.20%
Ear swab	21	13	34	2.10%
High vaginal swab	0	119	119	7.50%
Bronchoalveolar lavage	77	43	120	7.60%
wound swab	40	24	64	4.10%
Sputum	66	48	114	7.10%
Throat swab	16	4	20	1.30%
Urine	329	568	897	56.20%
Total	686	910	1596	100%

Only 209 of 1596 samples that were taken from the patients and cultured on Sabouraud's dextrose agar were found to be positive (figure 1). The results were 202 (96%) isolates of Candida; 3 (2%) isolates of Aspergillus; 2 (1%) isolates of Mucor; and only 2 (1%) isolates of Cryptococcus. Following that, Candida isolates were cultured on Chrom Candida Agar the results revealed that 59 of the isolates were Candida albicans, while the remaining 143 were not, as shown in figure (2).Among Candida species, C. albicans was the most dominant species of Candida (28%), similar findings (Kmeid,et al., 2020), have been reported in other studies that Candida species were the most prevalent fungal pathogens, especially in critically ill people. Further, (Murray, et al., 2005), reported that C. albicans was responsible for (63%) of all candidiasis infections in their study. Yeast can be identified with certainty based on its appearance, cultivation method, and spore arrangement. Lactophenol cotton blue stain, a mounting medium staining agent, is utilized to produce transparencies for research on microscopic fungi (Panizo, et al., 2022; Matare, et al., 2017). After incubation with human serum, positive samples produced germ tubes with parallel walls and no constriction at the site of origin, which is the blastospore mother cell. It has been hypothesized that it contributes to the pathogenesis of C. albicans as a virulence factor (Aryal, 2015; Campbell, et al., 1999).



(A): Fungal culture on Sabouraud's Agar medium with chloramphenicol (50µg/ml)



(B): Candida spp. on Chrom Candida Agar, shows different colors of colonies

Fig. 1. Fungal isolates detected by culture methods.

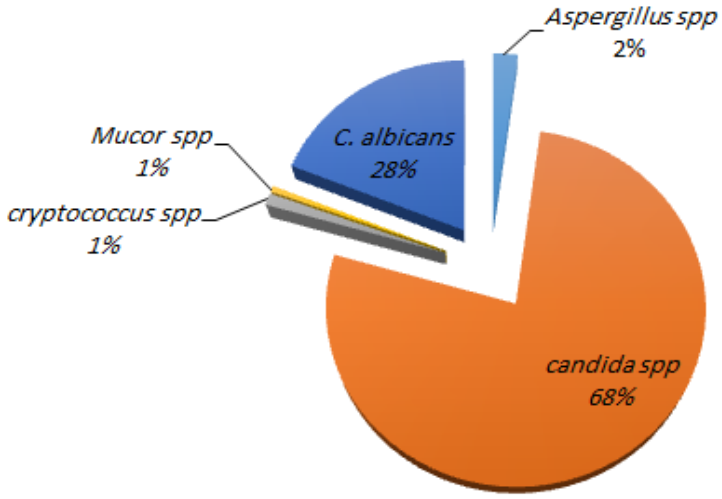


Fig. 2. Frequency of fungal isolates detected by culture methods

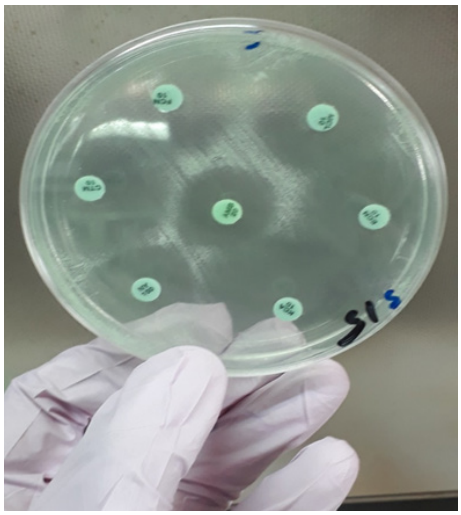
Antifungal susceptibility tests were performed on 59 C. albicans isolates. The isolates were classified as sensitive (S), intermediate (I), and resistant (R) based on their antifungal susceptibility. Seven antifungal medications including: Miconazole (10 mg), Fluconazole (10 mg), Ketoconazole (10 µg), Clotrimazole (10 mg), Nystatin (100 U), Econazole (10 mg) and Amphotericin-B (20 µg) were used. Regarding antifungal resistance byC.albicans it was found 8 (14%) isolates were resistant to fluconazole, miconazole(11, 19%), and oeconazole (11, 19%), while 50 (85%) of isolates were sensitive to Clotrimazole, and Ketoconazole (54, 92%). The susceptibility of C. albicans to antifungal agents was determined by the ability of cells to grow or not in the presence of the indicated drug. Table (3), which summarizes the results of the disc diffusion method used to assess C. albicans resistance, reveals that the isolates were resistant to Amphotercin B as 4(7%). The polyene class, including Amphotericin B and Nystatin, has been utilized extensively in therapy (Lakhani, et al., 2019).

This study a little bit disagree with (Terças, et al., 2017) who found C.albicans was sensitive to Amphotercin B as 29(100%), and completely agree with other study (Badiee and Alborzi, 2011) found isolated C.albicans resistant to Amphotercin B as 20 (7%). Isolated C. albicanswere resistant to Nystatin as 5(8%), and sensitive ones to Nystatin as 50 (85%), this result disagree with (Khan,et al., 2018) who proved sensitivity to Nystatin as 12 (26.6%) of C. albicans isolates, intermediate by 8 (17.7%) C. albicans isolates and 25 (55.5%) resistant ones of C. albicans isolates. In the current study, C. albicans resistance, reveals that the isolates of this fungus resistant to fluconazole were 8(14%), this result disagree with other study (Zaidi,et al., 2018) who found C. albicans was resistant to fluconazole as 81(56.5%). this was due to overexpression/mutations of the target enzyme, lanosterol 14a-demethylase; overexpression of efflux pumps (CDR and MDR genes) (Monroy-Pérez, et al., 2016).C. albicans was resist to Clotrimazole as 1 (2%), and sensitive as 56 (95%), this result agree with (Khadka,et al., 2017) who found the percentage of isolated C. albicansto Clotrimazole as 4 (7.2%) and sensitive as 44 (78.6%).

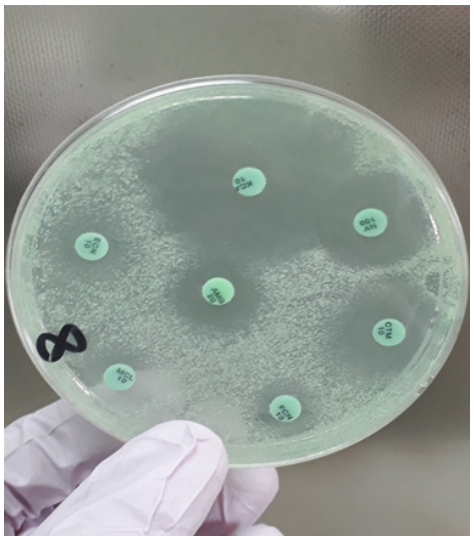
Fungistatic rather than fungicidal action of azole drugs leads to the frequent emergence of azole-resistant(Bitew&Abebaw, 2018). The current isolates of C. albicans resist to Econazole were 11 (19%) and sensitive were 45 (76%), this result disagree with other study (Carvalhinho,et al., 2012) who found only one isolate was resistant to Econazole (2.5%) from 40 isolated C. albicans. Regarding resistance to Miconazole the isolated resistant C. albicans were 11 (19%) and sensitive as 41 (69%), this result disagree with previous study (Al-mamari,et al., 2014) found isolated C. albicans were less resistant to Miconazole (1, 1.1%)while the sensitive one was 89(96%). Azole resistant candidiasis appears to be on the increase, and the reasons for resistance may include incomplete therapy, overgrowth of resistant strains, and induction of drug resistance in the particular species, colonization and subsequent infection with a resistant organism(Deravi, etal.,2021).

Table 3
Antifungal susceptibility test for C.albicans

Antifungal	Resistance%	Intermediate%	Sensitive%	Total%
Amphotercin B 20 mg	(4)7%	(7)12%	(48)81%	(59)100 %
Clotrimazole 10 mg	(1)2%	(2)3%	(56)95%	(59)100 %
Econazole 10 mg	(11)19%	(3)5%	(45)76%	(59)100 %
Fluconazole 10 mg	(8)14%	(2)3%	(49)83%	(59)100 %
Ketoconazole 10 mg	(3)5%	(2)3%	(54)92%	(59)100 %
Miconazole 10 mg	(11)19%	(7)12%	(41)69%	(59)100 %
Nystatin 100 units	(5)8%	(4)7%	(50)85%	(59)100 %



(A): C.albicans isolate sensitive to all antifungal disc



(B): C.albicans resist to Miconazole Fluconazole, andEconazole

Fig. 3. Antifungal susceptibility test for C. albicans

CONCLUSIONS

- More half of samples were obtained from females 910(57%) while males were 686(43%) collected from different body sites.
- Only 209 of 1596 samples that were taken from the patients and cultured on Sabouraud's dextrose agar were found to be positive.
- Candida isolates were cultured on Chrom Candida Agar the results revealed that just 59 of the isolates were Candida albicans.
- Candida albicans highest resist to Econazole, and Miconazole and lowest resist to Clotrimazole, and Ketoconazole, respectively.
- The study shows a higher antifungal resistance in the clinical samples which proves the risk in C. albicans management program.

Competing Interest

The authors had no competing interests.

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