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Isolation and Identification of Some Pathogenic Yeasts in Clinical Samples Obtained from Hospitalized Patients in Najaf Province

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Abstract
Yeasts are unicellular eukaryotic fungi. This study aims to identify yeast species from different clinical samples. A total of the 160 clinical specimens were collected, which included vaginal, mouth, and diabetic foot swaps, also sputum, stool, and urine specimen. All the clinical samples were diagnosed and identified by morphological and biochemical methods. The results of swabs cultures from the total 160 clinical samples 94 (58.75%) samples were positive for yeast growth. The Antifungal Susceptibility Test results showed that the susceptibility pattern for Fluconazole was 12 (12.76%) resistant and 82(87.23%) susceptible with no intermediate isolate while the susceptibility pattern for Ketoconazole was 6 (6.38%) resistant, 14 (14.89%) intermediate and 74 (78.72%) susceptible, which differed slightly from that of Miconazole which was 4 (4.25%) resistant, 14(14.89%) intermediate and 76 (80.85%) susceptible. The susceptibility pattern of Amphotericin-B was 16 (17.02%) intermediate and 78(82.97%) susceptible with no resistant isolates. While for Nystatin all the 94 (100%) isolates were susceptible. This study concluded the incidence of yeast infections was high especially among the vaginal samples and the most predominant species among the clinical isolates was *C. albicans.*

Keywords: Isolation, Pathogenic Yeasts

Introduction
Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom, whether ascomycetes or basidiomycetes. Most yeasts reproduce asexually by mitosis, and many do so by the asymmetric division process known as budding [1]. In last 30 years there has been a significant increase in the incidence of fungal infections in humans. Such infections may either be superficial, affecting the skin, hair, nails, and mucosal membranes, or systemic, involving major body organs. The members of the genus Candida are the most frequently recovered from human fungal infection, Candida genus contains over 150 heterogeneous species [2]. *Candida albicans* represents the most implicated species, however, other non albicans species such as *Candida tropicalis*, *Candida parapsilosis* and *Candida glabrata*, are increasingly being reported as agents of mycoses. Other yeasts of the genus: *Cryptococcus*, *Rhodotorula*, *Pichia*, *Trichosporon*, *Malassezia*, and may exceptionally be the cause of superficial mycosis, as they can be responsible for deep systemic infections in immunocompromised patients [3].

Material and Methods
A total of the 160 clinical specimens were collected in the present study, which included the 46 vaginal swaps, 58 mouth swaps 3 diabetic foot swaps, 35 sputum specimens, 6 stool specimen and 12 urine specimen who attended the Euphrates Cancer Hospital, AL-Sadder Medical City, AL-Hakem Hospital, and private clinics in Najaf Province, which included 71(44.37 %) males, 79(49.37 %) females and 10(6.25%) children.

Identification of Yeast Spp.
Culture Examination: The yeasts grew faster than molds on the Sabourauds' Dextrose agar with (Amoxicillin, Tetracycline and Gentamicin) antibiotics and the petri dishes were incubated at 37C° and 25C° separately. The pathological types were considered to have those values on growth at both temperatures. Colonies can be distinguished after 24-48 hours and differ in color, size, and luster [4] Then, a microscopic examination of the yeasts was conducted.

Microscopic Examination: A small portion from colony of the culture was placed on a slide with lactophenol cotton blue. Covering with a cover slip and tested under the light microscope [5, 6].

Biochemical Tests: included HiCrome™ Candida Differential Agar and Tobacco agar plates, these tests were performed by inoculating the plates which prepared previously with isolated colony taken from Yeast isolates culture grown on SDA for 24 hours, and then incubated at 37C° for 24-48 hours and Tobacco agar plates which were streaked with a small amount of inoculums from the isolated colonies. The culture plates were incubated at 28C° and observed daily up to 96 hours for colony characteristics, such as surface topography (rough or smooth), formation of hyphal fringes at the periphery and color [7].

Results and Discussion

From the total 160 clinical samples included in the present study 94 (58.75%) samples were positive for yeast growth according to the colony morphology appeared on the fungal selective cultural media supplemented with antibacterial agents, and to the microscopical examination [8].

The highest incidence of yeast infections was among the vaginal samples which appeared in 35 out of 46 (76.08%) followed by mouth samples 40 out 58 (68.96%), 2 from 3(66.66%) Diabetic foot, sputum samples 15 out of 35(42.85%), 1from the 6(17.66%) stool specimen and 1from the 12(8.33%) urine specimen. This agreed with [9] (Table 1).

The highest rate of infection was among the vaginal samples because they included the vulvovaginal candidiasis (VVC) which is a widespread vaginal infection primarily caused by *Candida albicans*. VVC affects up to 75% of women of childbearing age once in their life, and up to 9% of women in different populations experience more than three episodes per year, which is defined as recurrent vulvovaginal candidiasis (RVVC).

Table 1: Distribution of pathogenic yeast isolates according to the samples type

Type of samples	No. of samples	Positive yeast sample	Percentage
Mouth swap	58	40	68.96%
Vaginal swap	46	35	76.08%
Sputum	35	15	42.85%
Urine	12	1	8.33%
Stool	6	1	17.66%
Diabetic foot	3	2	66.66%
Total	160	94	58.75%

Cultural Characteristics

According to the colony morphology appeared on Sabouraud dextrose agar (SDA) plates inoculated with the clinical samples and incubated at 37°C for 48 hr, the identification was done as follows: Yeast colonies look similar to bacterial colonies. Generally, 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. *Pichia* spp. colonies are raised, smooth, and white to cream colored. The colonies of *Cryptococcus* spp. were shiny white to tan or translucent colonies with a mucous consistency and the colonies of *Rhudotrula* spp. were distinctive orange/red colonies [10]. These results were agreed with [11] (Fig 1).

Fig 1: *Candida spp.*, *Rhodoturla*, *Cryptococcus* and *Pichia* grawing on SDA agar

Microscopic Characteristics

The microscopic examination is a preliminary test to diagnose the pathogenic yeast. The microscopic examination of slides prepared from the isolates colony and stained with Lacto-phenol cotton blue stain, Gram stain or Indian ink and examined microscopically (100x). *C. albicans* and *C. dubliensis* produced chlamydospores, blastospores, pseudohyphae and hyphae. *C. tropicalis* produced blastospores, pseudohyphae and hyphae without chlamydospores. *C. parapsilosis* produced blastospores and pseudohyphae while *C. glabrata* produced only blastospores. None of the *Candida* spp. isolates produced arthroconidia. *Cryptococcus* produce cells appeared as spherical to oval, surrounded by a transparent halo due to its possession of the polysaccharide capsule, which is the most important diagnostic feature of this yeast, as all the isolates under study showed possession of the capsule and the size varied from one type to another. The shape of Pichia cells can be oval or ellipsoidal to elongate [12]. Rhodotorula species isolates were screened based on the texture and typical color exhibited by their colonies on SDA, as well as by their micromorphology after culturing each isolate that were able to produce carotenoid pigments conferring a salmon-pink to coral-red color to the colonies and presenting only spheroidal to oval budding cells and pseudohyphae are rarely present, a faint capsule is sometimes formed [13] (Fig 2).

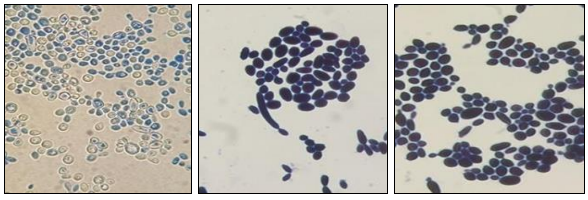

Fig 2: Microscopic feature of yeast spp. isolates pigmented with Lactophenol cotton blue and Gram stain and india ink under power magnification 100X

The results of HiCrome™ Candida Differential Agar test was *C. albicans/ C. dubliensis* was green, *C. tropicalis* was blue, *C. parapsilosis* was white to purple, and *C. glabrata* was cream to white. And it was agreed with the results [14]. While *Pichia kudriavzevii* produce light pink colonies agreed with the results of [15].

The HiCrome™ Candida Differential Agar test are effective and rapid testing in the diagnosis of *Candida* at the species level and some other yeast genera like *Pichia* spp. 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, all of the yeast isolates tested grew on HiCrome™ *Candida* after 48 hr. of incubation at 37C°, the majority of yeasts tested had grown well, as specified in the manufacturer's instructions [16] Colonies of other species are entire and smooth and colony color ranges from white to dark pink [17] (Fig 3).

Fig 3: Yeast isolates growth on HiCrome™ Candida Differential Agar shows different colony colors: C. *albicans* / *C. dubliensis* = green, *C.tropicalis* = blue, *C. parapsilosis* = white to purple, and *C. glabrata* = cream to white. While the species of *Pichia kudriavzevii* produce light pink colonies

The result of tobacco agar were the isolates of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and other species produced colonies appeared as smooth with creamy white color without filamentous extensions around the colony.

While *C. dubliniensis* isolates produced rough colonies of yellow-brown color with filamentous extensions. Although the remaining isolates were incubated for up to 10 days, the characteristics of the colonies did not change. These results were agreed with [18, 19] (Fig 4).

Fig 4: Yeast Isolates growth on tobacco agar plates. A: growth showed in *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and other species colonies with no hyphal fringes. B: showed typical Candida colonies with hyphal fringes from *C. dubliniensis*. C: Zoomed hyphal fringes from *C. dubliniensis*

Distribution of Yeast Isolates According to Species

The results of the morphological, biochemical and molecular identification conducted during this study showed that the most predominant species among the clinical isolates was *C. albicans* at 42(44.68 %) out of 94 clinical isolates, followed by *C. dubliensis 14(*14.89 %), *C. tropicalis 12(*12.76 %), *Pishea kudriavzevii (Krussi*) 7 (7.44 %), *C. glabrata* 6 (6.38 %), *C. parapsilosis* 6 (6.38 %), *Cryptococcus* 4(4.25 %), *Rhodotrula* 2(2.12 %) and *Magnusiomyces capitatus* 1(1.06%) (Table 2).

Table 2: Distribution of clinical isolate according to yeast species

Yeasts species	No. of Isolates	Percentage
C. albicans	42	44.68 %
C. glabrata	6	6.38 %
C. dubliensis	14	14.89 %
C. parapsilosis	6	6.38 %
C. tropicalis	12	12.76 %
Pichia kudriavzevii (Formerly referred to as Candida krusei)	7	8.51 %
Cryptococcus neoformans	4	4.25 %
Rhodotrula roseus	2	2.12 %
Magnusiomyces capitatus	1	1.06 %
Total	94	100 %

Conclusions

The incidence of yeast infections was high especially among the vaginal samples which was 76.08%. and The most predominant species among the clinical isolates was *C. albicans.*

References

1. Hoffman CS, Wood V, Fantes PA. An Ancient Yeast for Young Geneticists: A Primer on the Schizosaccharomyces pombe Model System. Genetics. 2015; 201(2):403-423.
2. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: Biology, epidemiology, pathogenicity and antifungal resistance. FEMS microbiology reviews. 2012; 36(2):288-305.
3. Adadi S, Ben-saghroune H. Yeast Infections: Epidemiological and Mycological Profile of Different Yeasts Isolated at the Hassan II University Hospital of Fez. Afro-Egyptian Journal of Infectious and Endemic Diseases. 2022; 12(3):245-251.
4. Ellis DH. Clinical mycology: The human opportunistic mycosis. Gillingham. Printers pty. Ltd. Australia, 1994, p166.
5. Morello JA, Granato PA, Mizer HE. Antimicrobial agent susceptibility testing and resistance. Laboratory manual and workbook, Ed. McGraw- Hill, 2003, 95-105.
6. Kayser FH, Bienz KA, Eckert J, Zinkernagel RM. Medical Microbiology, 2005.
7. Khan ZU, Ahmad S, Mokaddas E, Chandy R. Tobacco agar, a new medium for differentiating Candida dubliniensis from Candida albicans. Journal of Clinical Microbiology. 2004; 42(10):4796-4798.
8. Lima LA, Faria MAS, de Paula Menezes R, Penatti MPA, dos Santos Pedroso R. Phenotypic Characteristics of Yeasts the Genus Candida and Cryptococcus in Differential Culture Media. Int J Curr Microbiol App Sci. 2018; 7(8):1912-1921.
9. Abdulla NQF, Ismael HM. The Efficacy of Antifungal Medications and Plant Extracts against Candida albicans Isolated from Vulvovaginitis Women. Iraqi Journal of Science, 2023, 560-572.
10. Zhao Y, Zhao Y, Fu R, Zhang T, Li J, Zhang J. Transcriptomic and metabolomic profiling of a

Rhodotorula color mutant to improve its lipid productivity in fed-batch fermentation. World Journal of Microbiology and Biotechnology. 2021; 37:1-10.
11. Abadi AK, Mejbel FAH. Study of the effect of antifungal on candida albicans isolated from different cases. Plant archives. 2020; 20(1):2711-2716.
12. Oberoi HS, Babbar N, Sandhu SK, Dhaliwal SS, Kaur U, Chadha BS, Bhargav VK. Ethanol production from alkali-treated rice straw via simultaneous saccharification and fermentation using newly isolated thermotolerant Pichia kudriavzevii HOP-1. Journal of Industrial Microbiology and Biotechnology. 2012; 39(4):557-566.
13. Statzell-Tallman A, Fell JW. Rhodotorula FC Harrison. In The Yeasts. Elsevier, 1998, 800-827.
14. Baradkar VP, Mathur M, Kumar S. Hichrom candida agar for identification of Candida species. Indian Journal of Pathology and Microbiology. 2010; 53(1): p93.
15. Sayyada GN, Shazia TH, Shahana UK. Use of CHROMagar Candida for the presumptive identification of Candida species directly from clinical specimens in resource-limited settings. Libyan J Med. 2010; 5:p2144.
16. Iyampillai T, Michael JS, Mathai E, Mathews MS. Use of CHROMagar medium in the differentiation of Candida species: Is it cost-effective in developing countries. Annals of Tropical Medicine & Parasitology. 2004; 98(3):279-282.
17. Deorukhkar SC, Roushani S. Identification of Candida species: conventional methods in the era of molecular diagnosis. Ann Microbiol Immunol. 2018; 1(1):p1002.
18. Khan Z, Ahmad S, Joseph L, Chandy R. Candida dubliniensis: An appraisal of its clinical significance as a bloodstream pathogen. PLoS ONE. 2012; 7:e32952.
19. Kumar CG, Menon T. Tobacco agar: A new medium for chlamydosporulation in Candida albicans and Candida dubliniensis. Medical Mycology. 2005; 43(5):473-475.