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ORIGINAL RESEARCH

Identification and Usage of Antifungal Agents Against the Opportunistic Yeast Causing Oral Candidiasis in Immunosuppressed Patients

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ABSTRACT

Background: Candida species are among the most frequent opportunistic fungal pathogens in immunocompromised patients, often causing localized or systemic infections. Rising antifungal resistance has increased the need for regional studies on Candida prevalence and drug susceptibility.

Objectives: This study aimed to identify Candida species isolated from immunocompromised patients in Sulaimani, Kurdistan Region, Iraq, and to evaluate their susceptibility to commonly used antifungal agents.

Methodology: A total of 100 clinical samples were collected from patients with diabetes, renal failure, gastroenteritis, jaundice, and malnutrition. Samples were examined microscopically, cultured on Sabouraud Dextrose Agar and CHROMagar, and further tested using the germ tube method for species identification.

Results: Antifungal susceptibility testing was performed using the disk diffusion method against fluconazole, clotrimazole, and nystatin following CLSI guidelines. Five Candida species were identified. C. albicans was the most prevalent (42%), followed by C. glabrata (23%), C. krusei (15%), C. tropicalis (10%), and C. guilliermondii (10%). Antifungal susceptibility testing revealed universal resistance to nystatin. C. albicans was sensitive to fluconazole, while non-albicans species showed resistance. Clotrimazole exhibited broad sensitivity across all species tested, indicating its effectiveness as a primary therapeutic option. The predominance of C. albicans aligns with global data, but the emergence of resistant non-albicans Candida species highlights the need for routine species identification and antifungal susceptibility testing.

Conclusion: Clotrimazole demonstrated the highest efficacy and may serve as a preferred treatment option for oral candidiasis in immunocompromised patients. Continuous monitoring and rational antifungal use are essential to address the growing challenge of antifungal resistance.

Keywords: Candida species, antifungal resistance, immunocompromised patients, clotrimazole, fluconazole, nystatin.

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INTRODUCTION

Candidiasis is the most commonly observed fungal infection in humans, caused by yeasts of the genus Candida. Among Candida species, at least 15 Candida species can lead to infection [1].

Opportunistic fungal infections pose a high threat to immunocompromised patients [2]. Many factors, like low birth weight, cancer, diabetes, AIDS, burns, and organ transplantation, can affect the immune system [3]. In addition, both radiation therapy and chemotherapy can lead to irritation and injury to the oral mucosa (mucositis), resulting in hyposalivation and xerostomia, and an increase in oral yeast amplification and growth [4]. The Candida genus can cause pathologies of varying degrees depending on the pathogen and the host's immune condition. The colonization of the mucous membranes can occur by a change in the microbial population of the microbiota with a preponderant growth of Candida, which can then develop into a disseminated form, in synergy with other oral diseases. The most frequently encountered forms are superficial infections affecting the mucous membranes of the oral cavity (thrush) [5].

Candida albicans exhibits characteristics such as phenotypic switching, dimorphism, adhesive properties, enzyme production, and biofilm formation, which are implicated in the development of oral squamous cell carcinoma through interactions with epithelial cells [6]. This interaction promotes cytokine and matrix metalloproteinase production, fostering a pro-invasive epithelial phenotype, particularly in biofilm states. Traditionally, the genus name Candida referred to yeast species unable to produce sexual spores, but recent analyses show they form a polyphyletic group within Saccharomycotina. C. albicans is the most common infectious species, although other species like C. glabrata and C. parapsilosis are also noted. Diagnosis typically relies on phenotypic methods, deemed the standard for identifying yeast species in clinical settings through macroscopic and microscopic analyses of culture samples [7].

Clinical culture, though commonly used with biochemical methods using chromogenic media for microbial identification, has disadvantages such as extended result times. For treating oral candidiasis, effective antifungal medications include Fluconazole (oral), Nystatin (topical mouthwash or lozenge), and Clotrimazole (topical troche or tablet). Antifungal Susceptibility Testing (AFST) assesses a fungal isolate's susceptibility to antifungal drugs, aiding treatment decisions [8].

AIMS OF THE STUDY

This study aims to identify Candida species from the Oral cavity of immunocompromised patients by using CHROMagar and germ tube methods, and also evaluates the efficacy of antifungal agents using the Disk Diffusion Method to determine the most effective antifungal treatment for Candida species in different clinical samples.

METHODOLOGY

- **Study design:**

  A cross-sectional study design is chosen because it is suitable for determining prevalence and identification of different yeasts.

- **Study setting and duration:**

  The study was performed in Sulaimani, Kurdistan, Iraq, where the data were collected in Hiwa Hospital, Dr. Jamal Ahmed Kids Hospital, Share Hospital, and Teaching Gastroenterology and Hepatology Hospital from 1st of October 2024 to 30th of April 2025 in Sulaymaniyah city.

- **Inclusion Criteria:**

  All recorded cases had signs and symptoms with trush candidasis (n=100). Among them, cases with complete information, such as (type of disease, age, gender), were included.

- **Exclusion Criteria:**

Out of 100 cases, some of them were excluded because some patients before sampling used antifungal, like nystatin, some had missing data, and some had incomplete demographic information.

- **Data collection and Classifications:**

The study was conducted at Hiwa Hospital, Dr. Jamal Ahmed Kids Hospital, Share Hospital, and Teaching Gastroenterology and Hepatology Hospital, with a 100-sample of clinical patients for the identification of Candida species. The samples were taken from a patient group that had conditional problems such as Diabetes (n=40), Jaundice (n=30), Gastroenteritis (n=12), renal failure (n=11), and Malnutrition (n=7). The samples included males (n=45) and females (n=55). Their ages ranged from 3 months to 87 years. Samples were taken using sterile cotton swabs that had been moistened with sterile saline, and all samples were delivered to the microbiology laboratory within 25-30 minutes of collection. Before collecting the samples, ethical approval was obtained from the Research Protocol Ethics Committee of the College of Health and Medical Technology/ Sulaimani Polytechnic University, and informed consent was obtained from all study participants' parents.

- **Direct microscopic examination:**

Microscopic Examination: Examine the prepared slide under a microscope. Use the 40X or 100X objective lens (oil immersion if needed) for better visualization. Look for characteristic fungal structures such as: Oval or budding yeast cells (3–8 µm). Pseudohyphae (elongated chains of yeast cells with constrictions at septa). Hyphae (long, tube-like structures in invasive cases). The morphology of Candida albicans: budding yeast cells are round or oval. Pseudohyphae appear as chains with constrictions [9].

- **Culturing and isolating Candida species**

For isolation of Candida spp. Oral swabs were cultured on Sabouraud-dextrose agar (SDA) medium [Mumbai, India] with Chloramphenicol, then incubated for 48 h at 37 °C. All of the yeast isolates were examined using a wet mount. As primary identification, fresh colonies were cultured on HiCrome™ Candida Differential agar [Mumbai, India], which is a special medium used for the identification of Candida spp that are listed in Table 1. depend on The colony color after 48 to 72 hours of incubation at 37ºC [9].

Table (1): Identification of Candida species on Hichrom agar

N	*Candida species*	Color on Hichrom agar
1	C. albicans	Light green
2	C. krusei	Pink. purple, fuzzy
3	C. glabrata	cream to white
4	C. tropicals	Blue
5	C. ***guillermondii***	Purple

- **Serum germ tube technique:**

Principle of Germ Tube Test: Formation of germ tube is associated with increased synthesis of protein and ribonucleic acid. Germ Tube solutions contain tryptic soy broth and fetal bovine serum, essential nutrients for protein synthesis. It is lyophilized for stability. The germ tube is one of the virulence factors of Candida albicans. This is a rapid test for the presumptive identification of C. albicans. The procedure lightly touches a yeast colony with a wooden applicator stick. Suspend the yeast cells in an appropriately labeled tube of fetal bovine serum (make a light suspension). Incubate the tube for 2-3 hours in a 35 – 37°C incubator [10]. Place a drop of the suspension on a slide using a Pasteur pipette. Place a coverslip over the suspension. Examine the wet mount microscopically (at 40X) for the presence or absence of germ tubes.

- **Antifungal sensitivity test**

The antifungal activity test was performed using agar disk diffusion. SDA Agar was used for this test, along with Glucose and ND Peptone Agar as a supplement. The inocula were generated during 24-hour Candida plate cultures [11]. The colonies were placed in 5ml of 0.85% saline, and the turbidity was regulated and modified to an optical density (OD600).

The range received was between 0.11 and 0.14 by spectrophotometer at 600nm, which is equal to 0.5 McFarland standards, to produce a yeast suspension of 1x106 to5x106 cells/m [12, 13]. Two sterile swabs were rolled separately on the surface of two sets of plates containing Sabouraud dextrose agar SDA after being immersed in the suspension. The inoculated plates were allowed to dry for 10 minutes at room temperature in a laminar hood. Three antifungal paper disks were laid on each plate using forceps, and the plates were then incubated aerobically at 37°C for 24 hours. Nystatin (50 µg), Fluconazole (10 µg), and Clotrimazole (10µg), [Mumbai, India] were used. After 24 hrs., each antifungal disk's zone diameter was physically measured with a ruler. The interpretation criteria for the disks Fluconazole, Nystatin, and Clotrimazole were listed in Table 2 according to Clinical and Laboratory Standards Institute CLSI [14].

Table (2): Interpretive criteria of Resistance and Susceptibility of the used Antifungal disk (mm) according to CLSI. (14)

Antifungal agent	Sensitive	Dose dependent	Resistance
Fluconazole	≥19	15-18	≤14
Clotrimazole	≥20	12-19	≤11
Nystatin	≥25	17-24	≤16

Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Results statistics, including frequencies, percentages, tables, and graphs, were used to present the distribution of Candida species and antifungal susceptibility patterns. Continuous data, such as inhibition zone diameters, were expressed as the mean.

RESULTS

- **The rate of Candida species among different types of immunocompromised patients**

Figure 1 presents a pie chart illustrating the distribution of various Candida species. Candida albicans constitutes the highest proportion, accounting for 42% of the total isolates, indicating its predominance in clinical or environmental samples. Candida glabrata follows with 23%, suggesting a significant but lesser role. Candida krusei comprises 15%, while both Candida tropicalis and Candida guilliermondii are equally represented at 10% each. The data reflect the relative frequency of these opportunistic fungal pathogens, with C. albicans remaining the most commonly encountered species, which is consistent with its well-known virulence and adaptability in human hosts. Understanding this distribution is crucial for clinical diagnosis and antifungal treatment strategies.

The figure (2) illustrates the budding morphology of Candida species cultured on Sabouraud Dextrose Agar (SDA). The sample was incubated at 37 °C for 48 hours to promote optimal fungal growth. Microscopic examination was performed at 40× magnification, revealing characteristic budding yeast cells.

- **Isolation and Identification of Candida species from oral candidiasis by culturing:**

All isolates were identified on SDA and Candida-differentiated agar (HiCrome agar). Candida species were identified based on their colors and morphological characteristics after incubation for 48 hours at 37°C on SDA and 48 hours at 35°C on Hichrom agar, as shown in Figure 3. The colors in Hichrom Agar, HiCrome Candida differential agar, are a chromogenic medium used to differentiate various Candida species based on the colors of their colonies. The medium contains chromogenic substrates that react with enzymes produced by different Candida species, resulting in distinct colour changes. This allows for the presumptive identification of Candida species directly from the culture plate.

Candida albicans recorded the highest rate (42%); it looked like a smooth, light green colour on HiCrome agar, Candida Galabrata rate (23%), and showed glistening and cream to white on HiCrome candida differential agar. Candida Krusei rate (15%), the colony was purple, fuzzy. Another type of

Candida is Candida Tropicalis rate (10%) showed blue colors and may have slightly wrinkled edges. The final type was Candida Guillermondii typically forms a small, flat or smooth colony on SDA, with colony pointed purple colony on HiCrome agar, as shown in Figure 3.

- **Germ tube test**

This test is a rapid method for identifying Candida albicans by its capability to produce short, slender, tube-like structures called germ tubes when it is incubated in human blood serum at 37°C for 2-3 hours, as shown in Figure 4.

- **Antifungal Susceptibility**

Overview of Antifungal Susceptibility: The susceptibility of oral Candida isolates to fluconazole, clotrimazole, and nystatin was evaluated using the disc diffusion method. This method involves placing discs containing different antifungal agents on an inoculated agar plate. The diameter of the zone of inhibition around each disc is measured to determine the susceptibility of the fungus to the antifungal agent. In a total of 100 Candida species, the most isolated species, C. albicans, was sensitive to fluconazole and clotrimazole, with a zone of diameter nearly 30mm by CLSI, but resistant to nystatin, which has no zone of inhibition. As shown in Table 3. The other species include C. tropicale and C. guilliermondii, which are just sensitive to clotrimazole; the zone of diameter is nearly 28mm by CLSI guidelines, but resistant to both fluconazole and nystatin. Both (C. krusi and C. glabrata) are like other species sensitive to clotrimazole, and the diameter of the zone is nearly 25 mm by the same guideline (CLSI), but resistant to both fluconazole and nystatin, as shown in all species in Figure 4. So, this result in 66 samples indicates that clotrimazole is sensitive to all Candida types and fluconazole just responds to albicans, but nystatin is resistant to all Candida species.

DISCUSSION:

The study confirmed the presence of five commonly encountered Candida species. However, Candida albicans remained the most frequently isolated yeast, followed by C. glabrata, C. tropicalis, C. krusei, and C. guillermondii.In the present study, different types of Candida were observed among four types of cancer patients in Hiwa hospital, Sulaymaniyah, Kurdistan Region, Iraq, including Candida albicans (70 percent), C. glabrata (12 percent), C. kefyr (6.7 percent), C. tropicalis (5.3 percent), C. krusei (3.3 percent), and C.dubliniensis (2.7 percent) [15]. A prevalence of yeast strains was identified in Southern European areas, such as Croatia, through microbiological assessments. Among the isolated Candida species, C. albicans remained the most predominant (61.9%), followed by C. krusei (14.3%), C. valida (9.5%), and C. glabrata, C. tropicalis, and C. intermedia (each constituting 4.8%) [16]. A study conducted in Argentina revealed that C. albicans colonized approximately half of the patients (42.9%), whereas non-albicans species, such as C. tropicalis, C. krusei, and C. lusitanae, represented 33% of the colonization cases [17, 18].

Our findings on the prevalence of Candida species closely align with those reported in studies from Colombia. The predominance rate of Candida albicans in our study was lower than that reported in a study from Northern Kenya (82.3%) but higher than findings from Southern Sudan [19]. Schelenz et al., in a United Kingdom-based study, reported the distribution of Candida species in 400 patients with hematologic malignancies, head and neck cancers, and solid tumors. C. albicans was implicated in 74% of cases, followed by C. glabrata (11.5%), C. tropicalis (2.6%), C. krusei (2.6%), and C. parapsilosis (1.9%) [20]. Another study showed that around 85% (n=50) of cancer patients tested positive for Candida species cultures from oral mucosa samples, with C. albicans being the most prevalent, followed by C. glabrata in 14.5% of cases. Similar observations were made in the present study, where C. glabrata was the second most commonly isolated species in the patient group. Previous studies conducted in Pakistani and Jordanian populations

also identified C. albicans as the most frequently isolated species from the oral cavities of cancer patients [21]. In our study on oral Candida isolates from different immunocompromised patients, Nystatin showed complete resistance across all species. These results could be attributed to C. albicans having point mutations, insertions, and deletions in the genes encoding target proteins, which are frequently linked to antifungal drug resistance. Additionally, gene overexpression is frequently linked to both antifungal resistance and a rise in the activity of proteins that prevent oxidative damage. Fluconazole was effective only against Candida albicans, with resistance observed in non-alþicans species. Clotrimazole demonstrated sensitivity across all isolated species, making it the most effective agent in our sample. Jopical antifungals, such as nystatin oral suspension or clotrimazole, lozenges, are typically prescribed for mild to moderate infections. These agents act locally to inhibit fungal growth and are favored for their limited systemic side effects. However, their effectiveness depends on prolonged contact with the mucosa and patient compliance [22]. Systemic antifungals, particularly fluconazole, are used for more severe or recurrent cases, especially in immunocompromised patients. Fluconazole is effective against Candida alþicans, but resistance among non-albicans Candida species has been increasingly reported. Long-term or repeated use of fluconazole may also contribute to resistance development. In contrast, recent studies from 2023 have highlighted a concerning rise in antifungal resistance, particularly to nystatin and fluconazole. This has led to the exploration of novel antifungal agents such as ibrexafungerp and rezafungin, which offer broader antifungal coverage and improved activity against resistant Candida strains [23]. Our study confirms growing resistance to commonly used antifungals such as nystatin and fluconazole, while newer research underscores the potential of modern antifungals, non-pharmacological therapies, and enhanced delivery systems to improve treatment outcomes. This aligns with the need to update oral candidiasis management protocols, especially in vulnerable cancer patients.

Germ tube test, which is an elementary, quick, and dependable technique for distinguishing Candida albicans, the most common pathogenic yeast causing candidiasis. When germ tubes of yeast are produced, it is the result of incubation of Candida albicans in human serum at 37°C for 2-3 hours [24]. These germ tubes exhibit filamentous outgrowths marking the onset of hyphae development, and they are short, slender, and tube-like projections from yeast cells. Positive Result (Germ Tube Formation Observed): Interpretation: A positive test indicates the presence of Candida albicans. Other Candida species (e.g., C. glabrata, C. krusei, C. tropicalis) do not form germ tubes. These may require further biochemical or molecular testing for precise identification. Clinical Relevance: Non-albicans Candida species can also cause infections, especially in immunocompromised patients, and some may be less susceptible to antifungals [25].

According to the data, cancer was the most common type among immunocompromised patients in Sulaimani, accounting for 40% of cases. Diabetes ranked second, representing 25% of patients, followed by breast cancer at 20.8%. Out of a total of 100 patients, 10 were diagnosed with Gastroenteritis, making up 10% of the cases. The remaining 10% were diagnosed with renal failure, Jaundice 10%, and Malnutrition %5. Candida infections occur more frequently in cancer patients than in individuals with normal immune function. These patients are more susceptible to both localized and systemic infections, including oral infections, due to their illness and the side effects of chemotherapy, which weaken the immune system. Immunodeficiency in cancer patients—caused by cytotoxic chemotherapy, radiation therapy, or the cancer itself—prevents the immune system from effectively controlling Candida-related oral infections. This condition is further exacerbated by the use of chemotherapeutic drugs,

radiation, and high doses of both oral and systemic corticosteroids, all of which impair immune function and contribute to the increased risk of infection.

CONCLUSIONS:

This study highlights the predominance of Candida albicans among immunocompromised patients in Sulaimani, Kurdistan Region, Iraq, followed by C. glabrata, C. krusei, C. tropicalis, and C. guilliermondii. The findings reaffirm that C. albicans remains the most virulent and frequently encountered species, consistent with global epidemiological trends. Antifungal testing revealed universal resistance to nystatin, limited fluconazole effectiveness, and strong sensitivity to clotrimazole, suggesting its use as a first-line treatment. The findings highlight rising antifungal resistance, especially in non-albicans species, stressing the importance of susceptibility-based therapy, routine screening, and rapid diagnostics. Overall, the research provides key regional data, emphasizing continuous surveillance and the need for new antifungal strategies.

RECOMMENDATIONS:

The study recommend that the current Folic Acid recommendations for the prevention of Nural tube defects are reviewed and communicated effectively by healthcare professionals and public health agencies.

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TABLES:

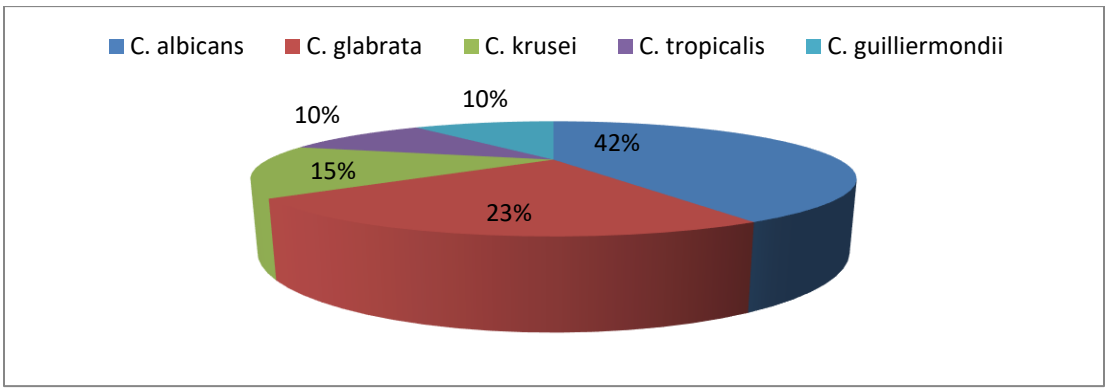

Figure (1): The percentage of Candida species (C.albican 42%, C.glabrata 23%, C.krusei 15%, C.tropical 10%, and C.guillermondii 10%)

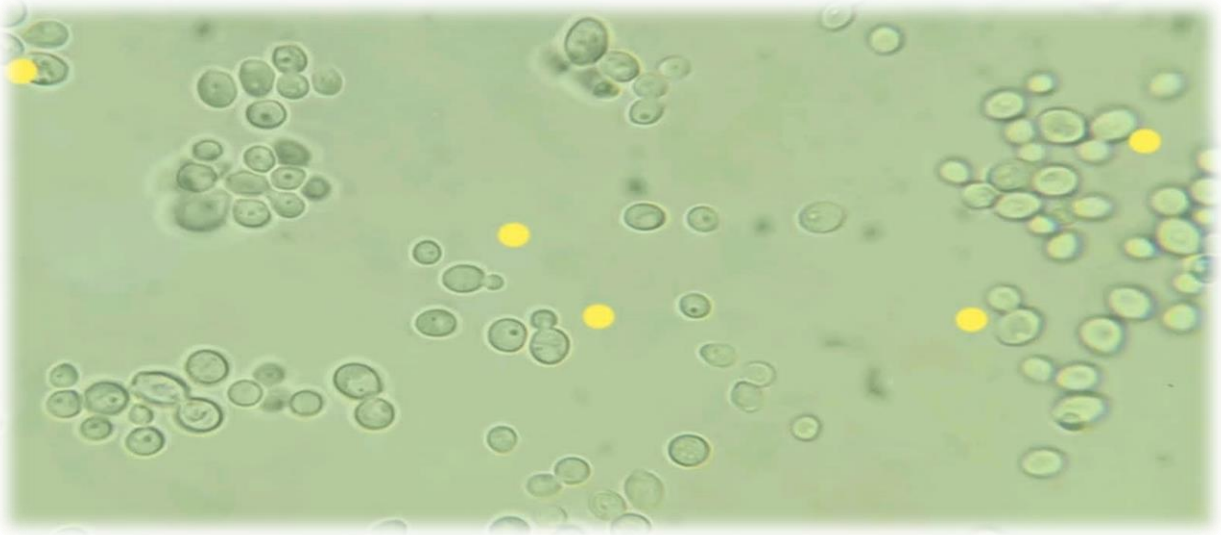

Figure (2): Budding of Candida species on (SDA), incubated at 37c° for 48 hours, was examined at 40X.

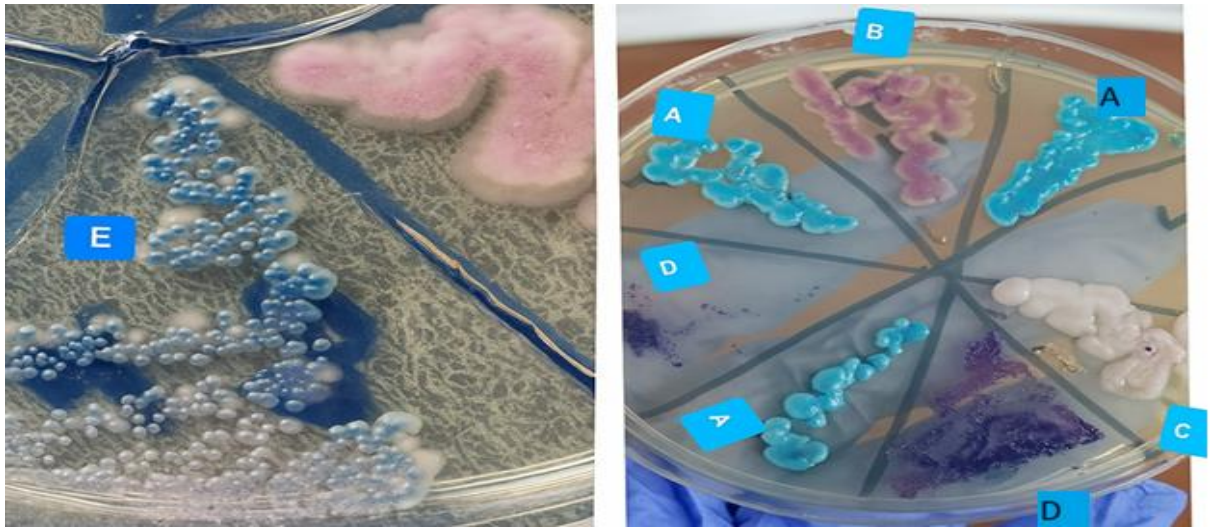

Figure (3): A/C.albicans B/C.krusei C/C.glabrata D/C.guillermondii E/C.tropical colony grown on HiCHrome agar (Candida differential agar) after 48h at 35C°.

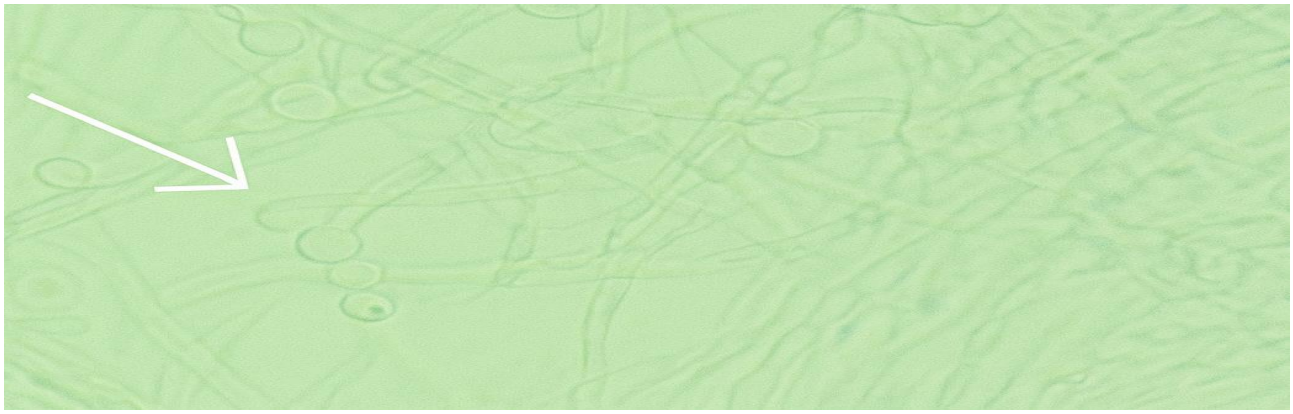

Figure (4): germ tube test formation by C. albicans, the yeast has straight walls, without a septum and constriction at the junction between the cells, light microscope 40X.

Table (3): Interpretive antifungal disk (mm) Resistance (R) and Susceptibility(S).

Antifungal	C.albican	C. glabrata	C.krusei	C.tropical	C.guillermondii
Fluconazol	30 (S)	R	R	R	R
Clotrimazole	30 (S)	25 (S)	25 (S)	28 (S)	28 (S)
Nystatin	R	R	R	R	R

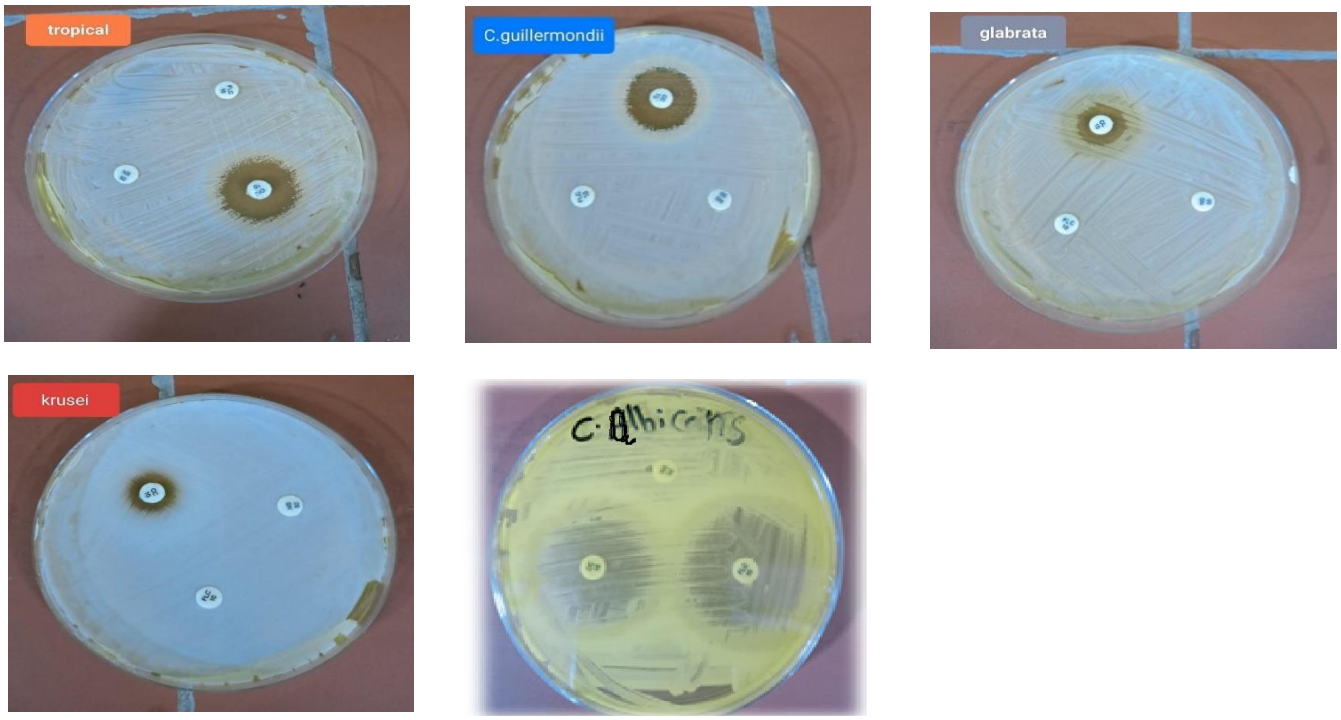

Figure (5): Antifungal susceptibility of different types of Candida: C. tropicalis, C.gullermondii, C.glabrata, C.krusei, and C.albicans.