

STUDY SOME VIRULENCE FACTORS AND ANTIBIOTIC SUSCEPTIBILITY OF SOME CANDIDA SPP. ISOLATED FROM DIFFERENT CLINICAL SITES

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Abstract: The current study aimed to study some virulence factors and test the antibiotic susceptibility of some fungi isolated from different clinical sites. This study was conducted in the Postgraduate Studies Laboratory / College of Science - University of Kirkuk. (220) samples were collected from chronic wound infections for patients hospitalized in Kirkuk General Hospital and Azadi Teaching Hospital in the city of Kirkuk. Each group included (69) samples from bed sore patients and (62) Samples from surgical infections (58) samples from diabetic foot patients and (31) samples from burn patients in the period between September to November 2023. Three types of yeast were isolated and diagnosed: *Candida albicans*, *Candida tropicalis*, and *Candida krusei*, and the most frequent ones were It is the yeast *C. albicans*, with 28(50%), *Candida tropicalis*, 18(32%), and *C. krusei*, 10(18%). Regarding virulence factors, *C. albicans* yeast showed the ability to form germ tubes and chlamydial spores. Chlamydospore formation While *C. krusei* and *C. tropicalis* were able to form surface growth on SDB liquid medium, the types of yeast showed different colors by growing them on chromo agar, as *C. albicans* was light green and *C. krusei* was pink. Light while *C. tropicalis* yeast has a metallic blue color. Regarding the drug sensitivity test, the results showed that all types of yeast were sensitive to the antibiotic Nystatin, reaching the diameters of inhibition. As for Amphotericin, all types of yeast were sensitive to it, and it was the most effective antibiotic among all types, with a rate of 100%. On the other hand, it was observed that the types of yeast were 100% sensitive, with the exception of two isolates of the type *C. krusei*, which were resistant to the antibiotic Clotrimazole, and to the antibiotic Ketoconazole. All types showed sensitivity to this antibiotic, except for one isolate, the yeast *C. krusei*, which was resistant to this antibiotic.

Key words: *C. albicans*; antibiotic susceptibility; wounds; virulence factors.

Introduction

Chronic wound infections are defined as wounds that do not heal as quickly as expected over a period longer than 6 weeks, often lacking recovery of normal function even after 3 months [1]. Compared with acute wounds, chronic wounds have the characteristics of delayed or even non-healing. The prevalence of chronic wounds is estimated to range from 1% to 4% [2]. Tumors, inadequate nutrition, and chronic mechanical stress have also been confirmed as major factors causing poor wound healing [3]. Chronic wounds are thought to be colonized by multiorganismal communities containing bacteria and fungi. Multiorganism interactions during wound infections contribute to persistent inflammation and delayed healing [4-6]. According to an analysis of historical data for the Medicare program, the cost of treating non-healing wounds is estimated to be between \$28.1 and \$96.8 billion [7]. The value of wound treatment and care is approximately \$18.22 billion and is expected to reach \$26.24 billion globally in 2023 according to the latest wound care market report. More than 38 million chronic wound infections occur as a result of treatment failure to heal wounds and are associated with a poor prognosis [8]. The wound microbiome that forms as a result of the colonization of bacteria and fungi is thought to hinder the healing process and cause the development of chronic wounds through community-based microbiota processes [9]. Fungal species were reported to be present in 23% of chronic wounds received for a study of 915 cases, including diabetic foot ulcers, pressure ulcers, non-healing surgical wounds, and venous ulcers. Although *Candida* spp yeast species have the highest prevalence [10]. Data indicate that the prevalence of fungal-infected diabetic wounds ranges between 9%-40.1%, and the major fungal species include *C.*

albicans, *C. tropicalis*, *Candida parapsilosis*, and *Candida guilliermondii*, followed by *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium* spp [11]. Analysis of the prevalence of fungi in 152 ulcers in the lower extremities and surrounding skin showed that 6% of ulcer samples and 27.6% of skin samples were positive for the presence of three fungal species, namely *Candida albicans*, *Candida parapsilosis*, and *Candida kefyr* [12]. Therefore, the current study aimed to study some virulence factors and test the antibiotic susceptibility of some fungi isolated from different clinical sites.

Materials and Methods

Sample collection

The current study was conducted in Kirkuk Governorate for the period from September to November 2023. 220 samples were collected from patients with chronic wound infections lying in Kirkuk General Hospital, Azadi Teaching Hospital, and the Burns Center in Azadi Teaching Hospital. Samples began to be collected from patients after wearing personal protective equipment and using... Swabs containing preservative medium Amies media. The sample was taken from the festering infection areas and the samples were transferred to the fungi laboratory in the College of Science. The initial examination was conducted for the presence of fungi and yeasts by microscopic examination using (10%) KOH. At the same time, the samples were planted using cotton swab on media containing the medium. Sabouraud Dextrose Agar The dishes were incubated at 37°C for 72 hours.

Yeast diagnostic tests

Morphological and Microscopic diagnosis

Phenotypic characteristics of colonies growing on SDA medium are identified by observing colony color, shape, texture, odor, and height. Then, microscopic examination is done by preparing a glass slide of the colonies growing on SDA medium, where a part of the colony was taken using a vector and placed on the slide, dyed with lactophenol blue dye and covered with the cover of the slide, then examined with an optical microscope at X40 power to observe the pseudohyphae and giant spores, then a glass slide was taken. Others were sterilized, stained with gram dye, fixed over a flame, and examined under a microscope to observe sprouting [13].

Germ tube formation test

This test is used to distinguish *Candida albicans* from other types of yeast. The test was performed by placing 0.5 ml of human blood serum in sterile test tubes, then the tubes were inoculated with a small portion of the growing colonies of *Candida*, then the tubes were incubated at a temperature of 37°C for three days. Hours, then we take a drop of the suspension and put it on a clean glass slide, cover it with the cover of the slide and examine it under a microscope (X40 power), which revealed the presence of small tube-like cells [14].

Urease test

Transfer the yeast *Candida* spp to test tubes containing urea agar medium, and incubate at 37 degrees for 48 hours. This test was conducted to detect the ability of the yeasts to produce the enzyme urease, and this is indicated by the color change of the medium [15].

Antifungal Susceptibility Test

A susceptibility test to yeasts was conducted, selecting five types of antifungals: Nystatin, Ketoconazole, BClotrimazole, Amphotericin, and Fluconazol. 54 pathogenic isolates were used, 27 of which were *C. albicans* Calbicans, 18 *Ctropicalis* *Candida*, and 9 *C.krusei* yeast. Medium was used. Muller Hinton Agar. Then the antibiotic tablets were transferred using sterile forceps from the tablets' tubes to the surface of the aforementioned medium, covered with a plate cover, and covered with parafilm to protect them from contamination. They were incubated at a temperature of 37°C for a period of (24-48) hours while monitoring growth. Then they were taken out of the incubator, and it was noted that there was growth of different diameters around the antibiotic tablets. The results were read using a ruler to measure the diameters of the inhibition zone around the antifungal tablets [16].

Results & Discussion

Sample collection

Direct laboratory examination of the samples was carried out using 10% KOH during the collection process, and laboratory examination after culturing the samples on SDA medium, Subroid Dextrose Agar, where the results of direct microscopic examination showed 87 positive isolates, with a positive rate of 39.54%, while the number of negative samples was 133 samples, with a rate of 60.45%. The laboratory culture results showed that there were 101 positive samples, at a rate of 45.9%, while the number of negative samples was 119, at a rate of 54.1%. No culture growth appeared, as shown in Table (1), which shows the results of the laboratory microscopic and cultural examination of the study samples.

Table (1) Number and percentages of laboratory examination and microscopic examination of samples

Testing type	Total number	Positive +ve	Percentages	Negative -ve	percentages
Microscope	220	87	39.54	133	60.46
Culture	220	101	45.90	119	54.10

Characteristics of *Candida* spp. on SDA

Candida spp. Colonies appeared white to cream, spherical, convex, smooth to wrinkled, and had a typical yeast odor on the SDA plate. They developed rapidly within 24 hours and matured in three days (Figure 1). SDA medium is commonly used to isolate different types of

yeasts because it is a typical medium for *Candida* isolation, enabling it to grow while inhibiting the growth of many types of bacteria due to the low pH of the medium and the addition of antibacterial. It increases the selectivity of the medium [17].

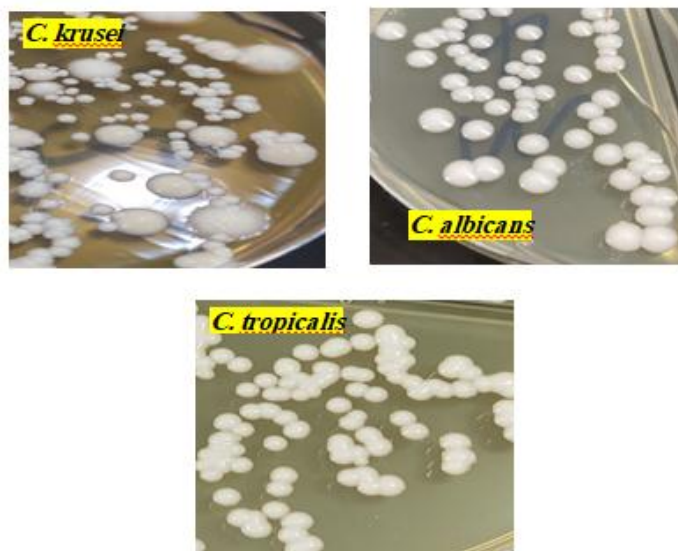


Figure (1): *Candida* spp. colonies on SDA medium at 37°C for a period of (24-48)

Germ tube production

Germ tube testing is frequently used to obtain a prior diagnosis of *C. albicans* [18]. The ideal standard for differentiating *Candida albicans* from other *Candida* species is the germ tube test, which is the fastest, simplest, and inexpensive test available [17]. In this study, all 28 *C. albicans* isolates formed a short, one-piece germ tube after 2 h of incubation following inoculation of 2 ml of serum with a

portion of the colony, which distinguishes them from non-white species; There was no restriction of the germ tube at the point of contact with the yeast cells (Figure 2). Tubular extensions indicate a step in the evolution of true hyphae. *C. albicans* germ tubes developed within three hours of incubation, distinguishing them from other fungi. Other yeasts do not usually form germ tubes in this period of time, and this is consistent with the findings of [19].

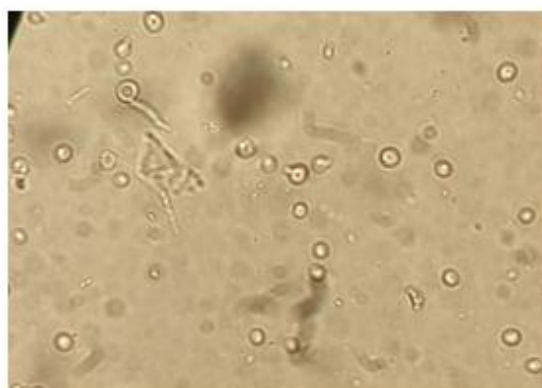


Figure (2): Germination tube formation in *C. albicans* (under 40X power)

Urease test

The results show that all *Candida* isolates are unable to degrade urea, due to their inability to produce the urease enzyme. These are consistent with the results of previous studies conducted by Ellis et al., [20]. The test is used as a diagnostic feature to differentiate between yeast species.

Table (2): urease test of *Candida* isolates

Yeast type	Urease test
<i>C. albicans</i>	-
<i>C. tropicalis</i>	-
<i>C. krusei</i>	-

Antibiotic susceptibility

Four types of antifungals were used: Amphotericin, Nystatin, Ketoconazole, Clotrimazole, and *Candida* spp., with 56 yeast isolates. The current study showed that all species of *Candida* spp yeast were sensitive to the antifungal Nystatin, as the diameters of inhibition ranged between 18-25 mm, 16-21 mm, and 15-20 mm for the yeasts *C. albicans*, *C. tropicalis*, and *C. krusei*, respectively. Table (5) and Figure (2). These results are consistent with AL-Maliki & AL-Ani, [21] who stated that the yeasts of *C. albicans*, *C. tropicalis*, and *C. krusei* were sensitive to Nystatin, while they did not agree with what It was concluded by Al-Janabi, [22], who indicated that the antibiotic Nystatin did not have any inhibitory

effect on resistant *Candida* species. It also did not agree with Salehei et al., [23] and Mahmoudabadi et al., [24] who found that all types of *C. krusei*, *C. tropicalis*, and *C. albicans* are resistant to Nystatin. Espinel-Ingroff, [25] stated that the reason for the effect of Nystatin on *Candida* yeast may be due to its combination with the components of sterols present in the cell membrane, which leads to the exudation of important cellular components and thus cell death. Random and repeated use of this antibiotic leads to the emergence of resistant types of *Candida* spp. yeast, so it is natural that It varies from one type to another, and also varies depending on the source from which this type is taken [26].

Table (3): The effect of Nystatin on some types of *Candida* spp.

Yeast type	Inhibition diameter in millimeters
<i>C. albicans</i>	18-25
<i>C. tropicalis</i>	16-21
<i>C. krusei</i>	15-20

As for the antifungal Clotrimazole, it caused allergic reactions in all types, as the diameters of inhibition ranged between 20-24 mm, 17-22 mm, and 15-17 mm for the yeasts *C. albicans*, *C. tropicalis*, and *C. krusei*, respectively, according to Table No. (4) and Figure (3). These results are consistent with (AL-Maliki & AL-Ani, [21] and Hussein et al., [27]. The

mechanism of action of the azole antifungal is to inhibit cytochrome P450 oxidase. Inhibiting the action of this enzyme leads to preventing the formation of ergosterol from the fungal cell wall, as it causes many holes. It also works to disrupt oxidation enzymes associated with the plasma membrane, in addition to the accumulation of phospholipids. within the cell and thus its death [28].

Table (4): The effect of Clotrimazole on some types of *Candida* spp.

Yeast type	Inhibition diameter in millimeters
<i>C. albicans</i>	20-24
<i>C. tropicalis</i>	17-22
<i>C. krusei</i>	15-17

As for the antifungal Ketoconazole, all species showed sensitivity to this antibiotic, with the exception of two isolates of the yeast *C. krusei* that were resistant to this antibiotic, as the diameters of inhibition ranged between 22-32 mm, 19-28 mm, and 9-21 mm for the yeasts *C. albicans*, *C. tropicalis* and *C. krusei*, respectively, according to Table (5) and Figure (4). These results are consistent with Awari,

[29] and Rajeevan et al., [30]. They found that there were types of *C. krusei* that were resistant to this antibiotic. It did not agree with Salehei et al., [23]. Which stated that all types of *C. krusei* were 100% sensitive to the antibiotic, according to Table No. (5). This antibiotic has high inhibitory activity against yeasts, but it has harmful effects in the liver [31].

Table (5): The effect of Ketoconazole on some types of *Candida* spp.

Yeast type	Inhibition diameter in millimeters
<i>C. albicans</i>	22-32
<i>C. tropicalis</i>	19-28
<i>C. krusei</i>	9-21

As for the antibiotic Amphotercin-B, all the studied *Candida* spp yeast species were sensitive to it, as the diameters of inhibition ranged between 16-21 mm, 15-19 mm, and 20-25 mm, for *C. krusei*, *C. tropicalis*, and *C. albicans* yeasts, respectively. Table (6) and Figure (5) agree with the findings of Lima et al., [32]. When they studied *Candida* spp yeast species, they were sensitive to this antibiotic, and it did not agree with Padmapriya et al., [33] who found that some types of *C. tropicalis* and *C. albicans* yeasts were resistant to this antibiotic. According to table (6). The effect of this antibiotic is on steroid compounds, as it

leads to a defect in the permeability of plasma membranes [22]. Fungal susceptibility varies from one species to another, depending on the location where samples are collected and depends on the concentration of the antibiotic. Also, excessive use of azole antibiotics indiscriminately increases the resistance of some types of yeast to these antibiotics [34]. Azole antagonists have two effects, the first on cytochrome (450P-), which works to inhibit the arcsterol resulting from the removal and deletion of α . Methstrol. As for the second effect, it results from the direct interaction of antifungals with membrane lipids, leading to the breakdown of the membrane [22].

Table (6): The effect of Amphotercin-B on some types of *Candida* spp.

Yeast type	Inhibition diameter in millimeters
<i>C. albicans</i>	16-21
<i>C. tropicalis</i>	15-19
<i>C. krusei</i>	20-25

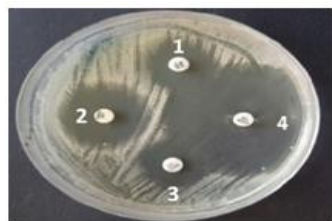


Figure (3): The effect of antifungals on *C. albicans*

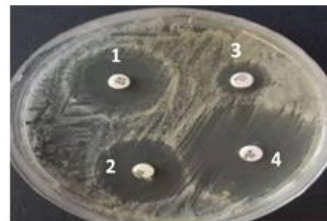


Figure (4): The effect of antifungals on *C. tropicalis*

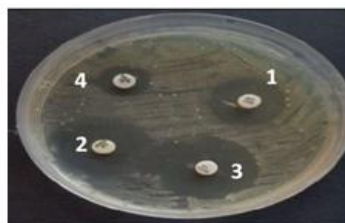


Figure (5): The effect of antifungals on *C. krusei*

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