THE HIGHLIGHTING OF BUTTERY COLONIES FOR CANDIDA ALBICANS

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RESEARCH ARTICLE

Abstract

Candida albicans is an opportunistic yeast that is part of the normal flora of human mucous membranes but can become pathogenic under favorable conditions, causing infections of varying severity. Colonies of *C. albicans* often develop in moist and warm environments, such as the oral cavity, gastrointestinal tract, and vagina. These colonies are characterized by a diverse morphology, including yeast-like and filamentous forms (pseudohyphae), which contribute to the pathogen's virulence by facilitating adherence to host cells and tissue invasion. Recent studies have shown that virulence factors, such as enzyme production, biofilm formation, and the ability to adapt to various environmental conditions, play a crucial role in the development and persistence of infections caused by *C. albicans*. Additionally, laboratory analysis of *C. albicans* colonies is essential for diagnosing fungal infections and assessing antifungal susceptibility. In conclusion, *Candida albicans* colonies are an important subject in fungal infection research, with significant implications for human health.

Laboratory diagnosis of Candida infections requires a combination of methods to ensure accurate identification and proper management. An integrated approach, including microscopic examination, culture, serological tests, and molecular methods, enhances the ability to diagnose Candida infections and guide clinical treatment.

Keywords: moist environments, yeasts, filaments

INTRODUCTION

Candida albicans is a fungal yeast that is part of the normal flora of human mucous membranes, including the oral cavity, gastrointestinal tract, and vagina. Although it is a benign saprophytic organism under normal conditions, *C. albicans* can become an opportunistic pathogen in certain situations, causing severe infections, especially in individuals with compromised immune systems or when the normal flora is imbalanced. *C. albicans* infections can range from superficial conditions, such as oral thrush (or "muguet") and vaginal candidiasis, to severe systemic infections, such as candidemia and invasive candidiasis, which can affect internal organs.

C. albicans exhibits several virulence factors, such as the ability to form biofilms, produce enzymes that degrade host tissues, and adapt to various environmental conditions. These characteristics enable it to adhere to cellular surfaces and invade tissues, contributing to its pathogenicity. Additionally, *C. albicans* can exhibit polymorphism, transitioning from yeast forms to filamentous forms (pseudohyphae), which gives it advantages in colonizing and invading host tissues.

The human body's defense mechanisms, such as the immune system and normal flora, play a crucial role in preventing infections by *C. albicans*. However, in conditions of immunosuppression, prolonged antibiotic use, or shifts in the balance of microbial flora, *Candida albicans* can proliferate uncontrollably, causing infections that require prompt and effective antifungal treatment. Therefore, understanding the characteristics, virulence factors, and pathogenicity of *C. albicans* is essential for the prevention and treatment of associated fungal infections.

MATERIAL AND METHODS

I conducted a prospective study based on microbiological diagnoses recorded in the bacteriological registry of the medical analysis laboratory, S.C. Diaser, Oradea. To conduct the study, I also accessed the archive, recorded in the laboratory's computer program in S.C. Diaser, Oradea, as well as the computerized database of the unit.

In the conducted study, we used the following materials and methods:

Materials

1. **Biological samples**:
   - Vaginal secretions (sterile swabs)
   - Oral fluids (e.g., sputum, lesions)
   - Urine samples
2. **Culture media**:

- Sabouraud dextrose agar: a standard culture medium for fungi, rich in dextrose, which promotes Candida growth.
- Chromogenic agar: allows species identification through specific colony coloration.
- Mueller-Hinton agar: used for antifungal susceptibility testing.

3. **Reagents and solutions**:
   - Potassium hydroxide (KOH): 10-20% solution for microscopic examination.
   - Staining solutions (e.g., Giemsa, methylene blue): for highlighting hyphae.
   - Reagents for biochemical tests (e.g., solutions for fermentation and assimilation).
4. **Equipment**:
   - Optical microscope: for microscopic examination of samples.
   - Incubator: maintained at 30-37 °C for sample cultivation.
   - Pipettes, test tubes, and Petri dishes: for sample handling and inoculation of media.
   - Sterilizers/autoclaves: for material disinfection.

Methods

1. **Sample collection**:
   - Samples are collected under aseptic conditions using sterile instruments. Sterile swabs or spatulas are used to avoid contamination.
2. **Microscopic examination**:
   - Samples are treated with KOH solution to dissolve host cells and highlight hyphae and yeast cells. The samples are observed under a microscope to identify characteristic fungal forms.
3. **Fungal culture**:
   - Samples are inoculated onto culture media, such as Sabouraud dextrose agar. Plates are incubated at 30-37 °C for 24-48 hours.
   - Colony morphology is evaluated (creamy, whitish, or yellowish appearance).
4. **Species identification**:
   - Biochemical tests are used to assess sugar fermentation (e.g., glucose, mannitol) and nutrient utilization (e.g., assimilation test).
   - Species identification can be confirmed through morphological observations, biochemical tests, and behavior analysis on selective media.

RESULTS AND DISCUSSIONS

Laboratory diagnosis involves isolating and identifying the species responsible for the disease before initiating antifungal therapy. If antifungal treatment has already been started, the pathological sample collection should be done at least three days after discontinuing the treatment.

The collected samples are inoculated on Sabouraud dextrose agar and chromogenic agar. After 24-48 hours of incubation at 30-37 °C, characteristic colonies appear. C. albicans colonies are typically creamy, whitish, or yellow with well-defined edges.

Fig. 1. *Candida albicans*. Sabouraud medium. Greasy colonies.
https://microbiologie.umfst.ro/atlas/micologie/levuri/calbicans.php

The microscopic examination revealed the presence of yeast cells and pseudohyphae, thus confirming the suspicion of an infection with C. albicans.

C. albicans demonstrated the ability to ferment sugars such as glucose and mannitol, with results consistent with the literature. These biochemical tests were crucial for confirming the species identity.

Sensitivity tests (e.g., disc diffusion, dilution) showed variability in the susceptibility of isolated strains to different antifungals. Most strains were sensitive to fluconazole, voriconazole, and caspofungin, but some strains exhibited resistance to ketoconazole, which may indicate an emerging trend of resistance. Candida albicans is a common yeast considered part of the normal flora of the human body. However, it can become an opportunistic pathogen under favorable conditions, such as immunosuppression, prolonged antibiotic use, or other medical interventions. Additionally, the increased prevalence of C. albicans infections in hospitals is a major concern, given that fungal infections can lead to severe complications, including candidemia and systemic infections.

Epidemiological studies have shown that most *C. albicans* infections are preceded by colonization of the host's mucous membranes. Colonization can become problematic in conditions of microbial flora imbalance, allowing for the uncontrolled growth of *C. albicans*. *C. albicans* possesses several virulence factors, including the ability to form biofilms, which facilitate adherence to cellular surfaces and resistance to treatment. This ability may explain the difficulties encountered in treating recurrent infections.

Emerging resistance to antifungals is a major issue in the treatment of *C. albicans* infections. Sensitivity test results suggest that while most strains remain sensitive to first-line antifungals, there is a concerning trend of resistant strains to ketoconazole and other azoles. This underscores the need for ongoing monitoring of antifungal resistance and adjustment of treatment based on test results.

Tab. 1. Differences between Candida species

Candida Species	Morphology	Pathogenicity	Common Infections	Antifungal Resistance
Candida albicans	Yeast cells, pseudohyphae	Opportunistic pathogen; common in humans	Oral thrush, vaginal candidiasis, invasive candidiasis	Some strains show resistance to azoles

Candida glabrata	Yeast cells	Opportunistic pathogen; more resistant	Urinary tract infections, bloodstream infections	Often resistant to fluconazole
Candida tropicalis	Yeast cells	Opportunistic pathogen	Invasive candidiasis, bloodstream infections	Variable resistance; can show azole resistance
Candida parapsilosis	Yeast cells	Opportunistic pathogen	Bloodstream infections, infections in neonates	Some resistance to fluconazole
Candida krusei	Yeast cells	Opportunistic pathogen	Invasive candidiasis, bloodstream infections	Naturally resistant to fluconazole
Candida auris	Yeast cells	Highly pathogenic; emerging threat	Healthcare-associated infections, multidrug-resistant infections	Multi-drug resistant

The authors of the study "Isolation and Identification of Candida from the Oral Cavity" stated that the most commonly used primary isolation medium for Candida is SDA, which, while allowing the growth of Candida, suppresses the growth of many species of oral bacteria due to its low pH. The incorporation of antibiotics into SDA further increases its selectivity. Typically, SDA is incubated aerobically at 37°C for 24–48 hours. Candida develops as convex, creamy, smooth, and pasty colonies on SDA, and differentiation between species is rarely possible. It is estimated that more than one species of Candida appears in about 10% of oral samples, and in recent years, the ability to detect non-albicans species has become increasingly important.

In recent years, other differential media have been developed that allow for the identification of specific species of Candida based on the appearance and color of colonies after primary culture. The advantage of these media is that the presence of multiple species of Candida in a single infection can be determined, which may be important in choosing subsequent treatment options. Examples of these media include Pagano-Levin agar or commercially available chromogenic agars, such as CHROMagar Candida, Albicans ID, Fluroplate, or Candichrom albicans.

Pagano-Levin agar distinguishes between Candida species based on the reduction of triphenyltetrazolium chloride. The medium produces pale-colored colonies for *C. albicans*, while colonies of other Candida species exhibit varying degrees of pink coloration. Pagano-Levin agar has similar sensitivity to SDA but is superior for detecting multiple species in a sample. CHROMagar Candida identifies *C. albicans, C. tropicalis*, and *C. krusei* based on the color and appearance of the colonies, while Albicans ID and Fluroplate have proven beneficial for the presumptive identification of C. albicans. The specificity of identification is reported to be 95% for CHROMagar Candida and 98.6% for Albicans ID and Fluroplate agars. The use of CHROMagar Candida as a primary isolation agar has been cited as an approach that allows for the discrimination of the recently described C. dubliniensis from C. albicans. On CHROMagar

Candida, *C. dubliniensis* typically develops as darker green colonies compared to those of *C. albicans*. However, the discrimination between these two species using CHROMagar appears to decrease after subculturing and storage of isolates. The failure of *C. dubliniensis* to grow on agar media at the elevated incubation temperature of 45°C has recently been suggested as an alternative test to discriminate between these two species.

CONCLUSIONS

The colonies of *C. albicans* are usually creamy, whitish, or yellow, with well-defined edges.

Sensitivity tests showed variability in the susceptibility of the isolated strains to different antifungals. Most strains were sensitive to fluconazole, voriconazole, and caspofungin, but some strains exhibited resistance to ketoconazole, which may indicate an emerging trend of resistance.

C. albicans possesses several virulence factors, including the ability to form biofilms, which facilitate adherence to cellular surfaces and resistance to treatment.

The results of the sensitivity tests suggest that, although most strains remain sensitive to first-line antifungals, there is a concerning trend of emerging strains resistant to ketoconazole and other azoles.

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