

## Experimental Study on Inhibitor Effects of Substances Applied in Differential Dilutions on Albicans Candidate Cultures

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### Abstract.

The study highlights the micolithic in vitro action of 1M acetic acid on *Candida albicans* cultures. It also highlights the lack of action of different concentrations of sodium bicarbonate, sodium chloride and ethyl alcohol or ethanol on the same types of crops.

**Keywords:** *Candida Abicans; Acid Acetic; Sodium Bicarbonate; Sodium Chloride; Ethyl Alcohol.*

### Introduction

Mycosis is a condition commonly found in medical practice. They occur due to contamination with fungal agents in three ways: infection from an ill person, from a sick animal, or the transformation of certain saprophytic fungi in certain environmental conditions. About 100,000 fungi are known, most of them being saprophytes [1].

The genus *Candida* includes about 200 different species, but only a few species are human opportunistic pathogens and cause infections when the host becomes debilitated or immunocompromised. *Candida* infections can be superficial or invasive. Superficial infections often affect the skin or mucous membranes and can be treated successfully with topical antifungal drugs.

However, invasive fungal infections are often life-threatening, probably due to inefficient diagnostic methods and inappropriate initial antifungal therapies [2]. These infections can be superficial and affect the skin or mucous membrane [3]. Deep systemic mycoses are rare mycoses in which the entrance gate is the epidermis, the development of lesions producing dermis and hypoderm, where the parasite can invade the soft tissues and the underlying bones, or can disseminate remotely [4].

The most commonly involved in human pathology is *Candida albicans*. It propagates by sprouting and produces elongated (pseudohife) and, occasionally, hay. The ability to exist both as a hippie and as a lioness is called dimorphism. In its yeast form *Candida* is a commensal germ, and when it forms pseudohife or hife has a pathogenic role [5].

Among the generally favourable factors should be mentioned: diabetes mellitus, congenital or acquired cellular immune deficiency states, broad spectrum antibiotics, antitrychomonasic therapy, iron deficiency and endocrine diseases.

Local factors are involved: rubbing and maceration, contact with hydrocarbon solutions. All the favourable factors are:

- a) the wide spread of the footwear made of synthetic and rubber materials, which promotes perspiration, maintaining a high humidity of the feet;
- b) Use of synthetic fiber stockings and linen, which favour transpiration;
- c) Using detergents instead of soap (detergents appear to have a lower effect on fungal agents);
- d) Frequency of tours, swimming pools, public baths, etc.;
- e) Extending the number of people living part of their life in the community (boarding schools) or frequent joint accommodations (hotels, motels, campsites, etc.);
- f) The prevalence of people who frequently use antibiotics, corticosteroids, cytostatics, etc., factors favouring fungal infections [6].

*Candida albicans* is the major pathogen but also other *Candida* species can cause human diseases: *C. tropicalis*, *C. pseudotropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii*, *C. Glabrata*, etc. [1].



**Fig. 1** Types of Candida [8]

Candidiasis can be located at different levels: cutaneous, oral cavity, gastrointestinal tract, genito-urinary tract. These areas can constitute, under the action of various factors, true Candida reservoirs, thus speeding up the appearance of manifestations.

*Candida albicans* can cause superficial infections of mucous membranes and skin and deep internal organ infections (endocarditis, meningitis, etc.) under immunodepression conditions [5]

There are a series of cutaneo-mucous candidiasis. They can be classified according to their headquarters and clinical appearance: a) Candidiasis of the oral mucosa: the sputum, the candidiasis glossitis, the candidiasis stomatitis, the candidosis. b) Skin candidiasis: candidiasis intertrigo, perionixis and candidiasis onixis. c) Candidiasis of the genital mucosa: candidal balanopostite and candidiasis vulvovaginitis. d) Chronic muco-cutaneous candidiasis: chronic adult muco-cutaneous candidiasis, candidiasis granuloma. e) Systemic candidiasis: pulmonary, intestinal, urogenital and candidal septicemia.

The muguet (the pearl, sister) occurs in the first days of life and the contamination is done on the occasion of expulsion and passing through the mother's genital line. In the jugular mucosa, tongue, laryngeal plaques there appear erythematous plaques that quickly cover up a white-creamy deposit.

Candidiasis glossy occurs in adults and is favoured by prolonged medication, diabetes, or gastritis. In atrophic forms, the lingual mucosa is red-bloated, dry, with atrophied papules. Atrophic forms occur in the elderly, in gastric cancer and in hypochromic anaemia. Candidiasis stomatitis associates a candidal glossitis with lesions of the jugular mucosa, the tonsils and the mucosa of the lips. These regions are small whitish plates that cover a mucous rose.

The candidose pear is characterised by superficial periciformistical cracks, with slightly eroded edges covered by the characteristic white creamy deposit. It occurs in diabetics and in total unstable dentures, or after prolonged antibiotic treatments. Candidiasis is the most common skin type. Loss of folds is due to local factors (friction, maceration, pH changes). It can be in all anatomical or pathological folds. They are in the form of exudative tidal plaques, with possible cracks in the folds, covered by a slightly creamy, slightly fetid deposit. The lesions are pruritic or develop burning sensations.

Levuric perionixis is particularly interesting in the female sex and is more common in people with a history of prolonged immersion of hands in water (confectioners, cooks, housewives, washing-ups). It is presented as an erythematous swelling of the nail replica with a slight desquamation. Candidiasis otixis is frequently accompanied by perionixis and in this case the nail dystrophic disturbances begin at the base of the nail. They may be accompanied by an interdigital plantar inter-trigeminal intervertego, and then the nail disturbing disorders begin at the free edge of the nail.

Candidiasis balanopostite occurs in man and is either a consequence of sexual contamination or a revealing sign of diabetes ("genital diabetes"). The mucosa of the gland and the inner foil of the foreskin are of a red, vivid, exudative, with superficial fissures and oedema, which is a white-cheesy deposit. Candidiasis of vulvovaginitis occurs in young women using birth control pills or gynecopy who have frequently used prolonged antibiotic treatments; sometimes it occurs in the prolongation of vaginal tricomonasis treated with Metronizadol or Fasigyn. It is presented as a creamy, abundant, fetish leucorrhea. The vulvar mucosa is edematous, erythematous. Vulvar pruritus may be rebellious.

The chronic muco-cutaneous candidiasis of the adult associates a nose, mouth, and cutaneous extensor resistant to common theoretical therapy.

Candidiasis granuloma is a variant of chronic muco-cutaneous candidiasis occurring in children. Besides the localizations characteristic of chronic muco-cutaneous candidiasis, there are scalp, face, glabral skin, granulomatous, hyperkeratose and papillomatous lesions. It is commonly associated with cellular immunodepression [7].

Systemic candidiasis occurs in immunosuppressed (AIDS), in the form of a septicemia with *Candida*, with a source of contamination of the digestive tract. It is manifested with altered general condition, persistent fever, positive hemocultures and urocultures. It can affect the kidneys, liver, lungs, eyes, heart. The skin may appear as: macules, papules, follicular pustules, nodules, ulcerations.

Levurids. They are sterile lesions that occur through the hypersensitivity of the organism to *Candida albicans* from a primitive focus, or they may occur following injections of levurin (candidose antigen) for diagnosis (i.d.) or therapeutic purposes.

The Levurids have two main clinical aspects:

- Parakeratosis, with an erythematous sculpting appearance, with centrifugal evolution, at the edges forming a sponge, while the center becomes pale;
- Eczematiform eczematous forms, similar to lesions in eczema, etc. It is located especially in the lower limbs and most of the cases are from rural areas; it is more frequently encountered in men (ratio 2/1), very rare in children, the average age being 40 years [6].

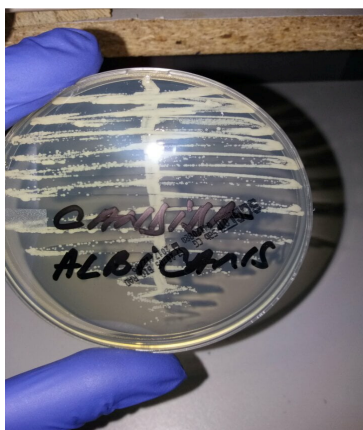
Candidiasis are fairly frequent fungal infections, affecting both men and women. *Candida albicans* is involved in over 70% of Candidiasis cases [1]. If *Candida albicans* enter your bloodstream, they can cause serious infections not only in your blood but in other organs as well [9].

## **Material and Methods**

The study tracked the action of external factors on *Candida albicans* cultures. The place where the experiments took place was the microbiology department of a renowned private laboratory of medical analysis. We used the Sabouraud standard culture medium, in which we seeded *Candida albicans* cultures from a calibrated assortment called ATCC. The plates were thermostated at 37°C. The substances analysed in the study (sodium bicarbonate, sodium chloride, acetic acid and ethyl alcohol) were prepared in various dilutions and applied via absorbent scoops, as in the case of antibiograms or fungigram.

We then analysed the diameter of the myelin colony inhibition and lysis zones at different time intervals (24, 48 and 72 hours).

Finally, I draw the conclusions from this study.



**Fig.2.** Petri plates seeded on Sabouraud medium with a standard ATCC culture of *Candida albicans*

**Fig. 3.** Erlenmeyer and Berzelius glasses with different dilutions of substances used to study the inhibitory effect on *Candida albicans*



We made the dilutions for the substances: sodium bicarbonate, acetic acid, sodium chloride, ethyl alcohol 90°C as follows: - I prepared four dilutions for each substance under study. For sodium bicarbonate ( $\text{NaHCO}_3$ ) and kitchen salt ( $\text{NaCl}$ ), we proceed as follows:

To 1000 ml of sterile distilled water 10 g of sodium bicarbonate and  $\text{NaCl}$  (1%), respectively,

To 500 ml sterile distilled water 1 g sodium bicarbonate and  $\text{NaCl}$  (0.2%)

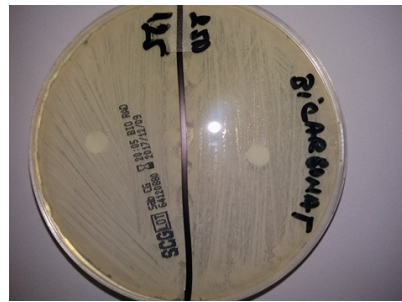
To 250 ml sterile distilled water ... 0.5 g sodium bicarbonate, respectively NaCl (0.1%).

To 125 ml of sterile distilled water ... 0.05 g of sodium bicarbonate and NaCl (0.04%) respectively.



**Figure 4.** Petri plate plated with *Candida albicans*, on which we applied a rondel soaked in sodium bicarbonate solution in two dilutions (1% and 0.2%)

**Figure 5.** Petri plate plated with *Candida Albicans*, to which we applied a rondel soaked in sodium bicarbonate solution in two dilutions (0.1 and 0.04%)



**Figure 6.** The Petri dish seeded with *Candida Albicans*, which was applied in a four-dilution (1%, 0.2%, 0.1%, 0.04%) solution of sodium chloride solution

For 90°C CH<sub>3</sub>-CH<sub>2</sub>-OH ethyl alcohol and 1M acetic acid CH<sub>3</sub>-COOH (glacial acetic acid), the dilutions used were:

To 4 ml of sterile distilled water ..... 3 ml of 90°C alcohol and 1 M acetic acid

To 3 ml of sterile distilled water ..... 2 ml of alcohol at 90°C and 1 M acetic acid

To 2 ml of sterile distilled water ..... 1 ml alcohol at 90°C, 1 M acetic acid

To 1 ml of sterile distilled water ..... 0.5 ml of 90°C alcohol and 1 M acetic acid.



**Figure 7.** Petri plate plated with *Candida Albicans*, on which we applied a rondel soaked in 90°C ethyl alcohol (ethanol) solution in four dilutions (with concentrations: 37.7%, 35%, 28.75% and the same, 28.75%)



**Figure 8.** Petri plate seeded with *Candida Albicans*, which we applied a rondel soaked in glacial acetic acid solution in four dilutions (concentrations: 44%, 41.18%, 34.43% and 34.43%)

## Conclusions

Following these determinations, we found that sodium bicarbonate, ethyl alcohol and sodium chloride did not show the expected inhibitory effect on *Candida albicans* cultures. Only inhibitory effect (at 33.43%, approximately 33.4%) was reported in acetic acid. Applying diluted acetic acid, at a concentration of 33.43% could cause the destruction of *Candida albicans* also in vitro colonies. The study can be extended to many other substances, including herbal extracts.



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