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# Oral *Candida albicans* in Patients in the ICU of a Brazilian Hospital School and *in vitro* Susceptibility of Isolated Yeasts to Extra-virgin Coconut Oil

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors ELR, STF, CCC and CGC designed the study and wrote the first draft of the manuscript. Authors MLB and FLMCC performed the statistical analysis, wrote the protocol, revised first draft of the manuscript and managed the literature searches. Authors GVO, IDAR, ELR and CGC managed the analyses of the study and performed the experiments. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** To verify the presence and growth of *Candida* in oral samples from hospitalized patients in Intensive Care Unit (ICU) the and to detect the *in vitro* susceptibility of isolated yeasts to extra-virgin coconut oil.

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**Study Design:** This is a cross-sectional study used to detect oral Candida among hospitalized patients in Intensive Care Unit in specific time interval.
**Place and Duration of Study:** Sample: ICU of the *Hospital das Clínicas* (HC - hospital school) of the Universidade Federal de Goiás (UFG) (ICU/HC/UFG), between May to October 2015.
**Methodology:** Isolation and the identification from cultures of oral *Candida* derived from patients of the ICU/HC/UFG were performed according to Kreeger van-RIJ. In triplicate, these *C. albicans* yeasts were seeded in Sabouraud dextrose agar (SDA) with chloramphenicol with the addition of commercial use extra-virgin coconut oil, and filtered at concentrations from 5 to 50%. Petri dishes with culture medium and varied concentrations of coconut oil were subjected to 37℃ / 24 h, and those that did not exhibit growth of fungal colonies were considered positive.
**Results:** There was a significant increase in the number of patients colonized with *Candida* yeasts in the first 72 hours after ICU admission, followed by stabilization at 96 hours. The *Candida* species isolated was *Candida albicans*. All cultures of oral *C. albicans* from ICU / HC / UFG patients, regardless of patients' length of stay, were susceptible to the concentration of 30% coconut oil present in the culture medium of SDA plus antibiotic.
**Conclusions:** Keeping patients in the ICU/HC/UFG influenced the number of individuals with *Candida* yeasts in the buccal cavity, particularly in the first 72 hours. *Albicans* is the *Candida* species most commonly detected in the buccal mucosa of patients undergoing hospital intensive care. Extra-virgin coconut oil proved to have a natural antifungal effect in inhibiting oral *C. albicans* isolates.

*Keywords: Candida albicans; intensive care unit; coconut oil.*

## 1. INTRODUCTION

Frequent reports of oral candidiasis in patients hospitalized in intensive care units (ICUs) have stimulated the search for new supplemental therapeutic alternatives, such as plant therapies, which can spur therapeutics, such as new pharmacological resources in mitigating or curing candidiasis.

Coconut oil is a plant substance consisting of fatty acids, among which approximately 90% are saturated acids [1]. It is obtained through pressing the pulp or core of coconut (*Cocos nucifera* L.). It is a natural product commonly used in the cosmetics industry for preparing soaps and creams and also in food preparation in some Asian countries. It acts as a complementary food, with various properties beneficial to health, strengthening the immune system and easing digestion, nutrient absorption and reduction in risk factors for cardiovascular disease [1,2].

An antimicrobial capacity involving an antifungal effect has been associated with coconut oil. Such pharmacological properties are a result of the biochemical composition of coconut oil, which includes lauric acid, myristic acid, and caprylic acid [1-3]. These acids hold medium-chain saturated fatty acids that act together to ensure rapid elimination of fungal infection through destructuring the cell wall [4].

A beneficial association has been observed between topical and oral administration of coconut oil and treatment of candidiasis [5,6]. *Candida* is a yeast-form fungus present in microbiota of the skin, mucous, and folds of the human body as of birth. Clinical manifestation is more common when the patient has low cell immunity, becoming vulnerable to a series of microbiological infections, as occurs in patients hospitalized in intensive care units [7]. White patches covering all internal areas of the oral cavity characterize high yeast-form colonization by *Candida*, which predisposes the ICU patient to the condition of pseudomembranous candidiasis [7,8]. Yeasts of the *Candida* genus are highly opportunistic microorganisms, especially *Candida albicans*, due to the high capacity for manifestation of virulence factors (adherence, morphological dimorphism, phenotypic variability – switching enzymes*:* aspartyl proteinase and phospholipases, and toxins: toxic glycoproteins and canditoxins) when exposed to a pathogenic condition [8-10].

The aim of this study was to detect the presence and growth of Candida isolates in oral samples from patients hospitalized in the ICU of the *Hospital das Clinicas* of the Universidade Federal de Goias (ICU/HC/UFG) and verify the *in vitro* sensitivity of the isolated yeasts to extra-virgin coconut oil.

## 2. METHODOLOGY

### 2.1 Candida Samples

Ninety samples of salivary secretion were collected at time zero, 72 hours, and 96 hours after admission, from 30 patients in the ICU/HC/UFG in the period from May to October 2015. Salivary specimens came from adults subjected to mechanical ventilation, tracheostomies, and non-invasive ventilation, regardless of age, gender, and clinical condition, with or without use of antibiotherapy. A sterilized swab was used to collect each saliva sample from the patient, which was placed in a test tube containing 4 mL of sterilized water for transport to the Laboratory of Medical Microbiological Analysis of the Instituto de Patologia Tropical e Saúde Pública of the Universidade Federal de Goiás (LAMSA/IPTSP/UFG) for fungal isolation and identification.

This study protocol was performed according to the Helsinki declaration and approved by ethics committee of the Clinical Hospital of Federal University of Goiás (Hospital das Clínicas of the Universidade Federal de Goiás-CEPMHA/HC/UFG), protocol number no. 634.432/2014. Written informed consent was obtained from each participant.

### 2.2 Isolation and Identification of Yeasts

The yellowish white colonies that developed up to 15 days in test tubes containing 4 mL of Sabouraud dextrose agar (SDA) medium with chloramphenicol were identified through formation of germination tubes in fetal bovine serum, formation of chlamydospores in corn-meal agar with the addition of tween 80, and carbohydrate assimilation and fermentation test [7,8].

### 2.3 SDA with the Addition of Coconut Oil

Petri dishes of 20 mL volume were prepared with liquefied Sabouraud dextrose agar at a temperature of 30℃ with the addition of chloramphenicol and filtered extra-virgin coconut oil, for commercial use, at different concentrations from 5 to 50% at 5% intervals. These Petri dishes, after preparation of the defined culture medium, were subjected to the sterilization test at 37℃ / 24h [10].

### 2.4 Suspension and Subjection of Candida to SDA with Coconut Oil

*Candida*-test suspensions, in distilled and sterilized water, McFarland scale 3, were carried out and seeded with sterilized swabs in Petri dishes with SDA and chloramphenicol at different concentrations of extra-virgin coconut oil, in triplicate, and kept at 37℃. Petri dishes with the culture medium used that did not exhibit macroscopic growth of *Candida* colonies were considered positive.

### 2.5 Statistics

The chi-square test ($X^2$) was used to compare the analyzed groups. The values obtained were considered statistically significant when $p < 0.05$. The Spearman Correlation Coefficient was used correlate the numbers of samples of *Candida* isolated from salivary secretion of individuals hospitalized in the ICU/HC/UFG at different time intervals (group I – time zero, group II – 72 h, and group III – 96 h) for identification of association between the time period studied and the number of positive cases.

## 3. RESULTS AND DISCUSSION

The occurrence of yeasts of *Candida albicans* observed in 61/90 (67.8%) of the samples of salivary secretion collected from patients hospitalized in the ICU/HC/UFG, without reference to the time the individual remained in the hospital unit was considered in this study. Considering the interval of hospitalization time of the patient in this unit, manifestation of *Candida* isolates was detected in 13/30 (43.3%) of patients' saliva samples at time zero; in 22/30 (73.3%) at 72 h; and in 26/30 (86.7%) at 96 h (Table 1). Statistical test using the Spearman correlation coefficient (*p*=0,976) indicated a positive correlation between length of ICU stay (in hours) and the increase in the number of cases of *C. albicans.*

All the oral samples of *Candida albicans* identified in this study, regardless of the hospitalization time of the patients in the ICU/HC/UFG, showed *in vitro* susceptibility to a concentration greater than or equal to 30% extra-virgin coconut oil present in Petri dishes containing SDA with the addition of chloramphenicol (Table 2).

Yeasts of *Candida albicans* continue to be the yeast-form fungus predominantly detected in the saliva of patients hospitalized in the ICU/HC/UFG. The high rate of fungi in the buccal mucosa of these individuals is due to microorganism carrying capacity as a compositional element of the oral microbiota since birth, the immediate clinical-laboratorial situation of each patient, and the use of therapeutic procedures with or without antibiotics, depending on the disease of the patient [7,10]. A decline in the health of these patients was also observed mycologically; in the interval from zero (Group I) to 72 h (Group II) and then to 96 h (Group III) of hospitalization in the ICU/HC/UFG, the number of patients from whom *Candida* in salivary secretion was isolated doubled; therefore, a statistically significant ($p < 0.05$) increase occurred among the groups considered. This fungal and statistical ($p > 0.05$) reality was not observed in these same patients in the time interval from 72 to 96 h in the ICU. Siqueira et al. [11] evaluated the oral microbiota of patients in Brazilian ICU units and detected continuity of *Candida albicans* as a basic agent of healthcare associated infections (HAIs). In another study conducted in the ICU of a hospital in England, *Candida* colonization was detected in 75% of the patients in one or more anatomical sites, such as the mouth and groin [12]. Likewise, in a hospital in the south of Iran, 63.2% of the patients exhibited colonization of *Candida* in different anatomical locations during hospitalization [13]. Bajwa and Kulshrestha [14] affirmed that with the advances in intensive medicine and introduction of broad spectrum antibiotics, the occurrence of invasive fungal infections in intensive care is rising, especially in patients with immunosuppression.

*Candida albicans* is responsible for up to 78 % of nosocomial candidiasis, however, reports demonstrating infections of Candida non-albicans species have been also documented [15]. For *C. albicans* identification, we used tests of germination tubes formation in fetal bovine serum, formation of chlamydospores in corn-meal agar with the addition of tween 80, and biochemical tests (carbohydrate assimilation and fermentation) [7,8]. However, due to the high degree of phenotypic similarity between Candida species (Candida non-albicans), and due to some mutations in *C. albicans* that may inhibit the formation of germ tubes or alter colony, the phenotypic and biochemical identification problems are imminent. [15,16]. Consequently, it is important to identify the causative organism to the species level correctly by molecular methods.

**Table 1. Occurrence of *Candida albicans* isolates in saliva samples of patients hospitalized at different time intervals (number of patients = 30)**

| Time of hospitalization/Groups (h)/(n) | Positive samples n (%) | Negative samples n (%) |
|---|---|---|
| zero (I) | 13 (43.3)[a,b] | 17 (56.7) |
| 72 (II) | 22 (73.3)[c] | 08 (26.7) |
| 96 (III) | 26 (86.7) | 04 (13.3) |
| Total | 61 (67,8) | 29 (32,2) |

*(a) - Groups I and II – Statistical ratio; $X^2 = 5.54$; $p < 0.05$; (b) - Groups I and III – Statistical ratio; $X^2 = 12.36$; $p < 0.05$; (c) - Groups II and III – Statistical ratio; $X^2 = 1.64$; $p > 0.05$. Spearman correlation coefficient - positive (p=0,976)*

**Table 2. Minimum percentage concentration of extra-virgin coconut oil able to inhibit the proliferation of *Candida albicans***

| SDA with extra-virgin coconut oil concentration (%) | *C. albicans* isolated from saliva of patients of the ICU/HC/UFG at different time intervals, and susceptible in SDA to minimum ercentage concentration of extra-virgin coconut oil Time (h) | | |
|---|---|---|---|
| | Zero n (%) | 72 n (%) | 96 n (%) |
| 5 | - | - | - |
| 10 | 01 (7.7) | 01 (4.5) | 01 (3.9) |
| 15 | 02 (15.4) | 04 (18.2) | 03 (11.5) |
| 20 | - | 03 (13.7) | 01 (3.9) |
| 25 | 10 (76.9) | 02 (9.1) | 03 (11.5) |
| 30 | - | 12 (54.5) | 18 (69.2) |
| > 35 to 50 | - | - | |

Therefore, it is highly recommended that the identification and discrimination of ethological agents is important for early treatment, and preventing the invasion. Therefore, alternative molecular assay with high specificity, reproducibility and sensitivity are necessary [15]. Despite being a future perspective, in our work, we did not carry out molecular identification and therefore did not eliminate the possibility of Candida non-albicans among isolates.

*Candida* and *Aspergillus*, followed by *Cryptococcus* and *Histoplasma*, in endemic areas throughout the world, are the main fungi involved in attacking individuals in intensive therapy. In our study, compatibility was observed regarding the occurrence of colonization of buccal mucosa by Candida yeast-form fungi in patients undergoing intensive therapy. Amiri et al. [17] examined an increase in the number of patients afflicted by *Candida* yeasts in relation to length of stay in the hospital ICU, and they analyzed risk factors leading to hospitalization in intensive therapy in France, such as diabetic individuals and those afflicted by chronic renal disease. They found an increase in the number of patients that were carriers of oropharyngeal colonization by Candida when they were placed in the ICU. The difficulty of adequate oral hygiene, associated with the weakened state of the immunological system of the patient, leading to the dental conditions of periodontitis and gingivitis, among others, seems to contribute to highly increased proliferation of *Candida* isolates in patients in hospital ICUs [7-11]. This fungal condition was observed in the first 72 h of patients in the ICU/HC/UFG. Stabilization in the following 24 h (72 to 96 h) of hospitalization is probably due to the microbiological relation of oral microorganisms among themselves, without considering the immediate situation of the patient and the therapy used [7,9].

Susceptibility of *Candida* yeasts to natural products has been observed, as in the case of the use of coconut oil [1-5]. This antifungal activity presents an alternative for refining natural medications for treatment of infections brought about by fungi. Studies, such as ours, following the model of the agar diffusion method advocated by the Clinical and Laboratory Standards Institute (CLSI) of the Centers for Disease Control and Prevention (CDC) of the United States [11], have shown the gradual sensitivity of oral samples of *Candida albicans* isolates from patients in the ICU/HC/UFG to variation in concentrations from 10 to 30% of extra-virgin coconut oil added to the SDA medium with the addition of antibiotics. The 10% coconut oil susceptible strain was isolated from the same patient at the different collection times. However, at other concentrations it was evident that the susceptibility to coconut oil may suffer interference from the adaptability of the sample to the buccal microbiota and the momentary state of the patient's immune status at the time of collection. Thus, time interferes with susceptibility to the fungus, that is, over time it adapts to the situation of the oral microbiota and becomes more resistant. This was evident at 25% concentration where strains isolated at time 0 were susceptible 10 (76.9%) while most strains isolated at 72 h and 96 h time-point were resistant, requiring a concentration of 30% oil to reach susceptibility in most strains. A study conducted in Ibandan, Nigeria [18] exhibited 52 isolates of the *Candida* species, derived from diverse human biological materials, as having susceptibility *in vitro* to coconut oil by the agar dilution method, and 17/52 (32.7%) cultures of *Candida albicans* identified were completely sensitive to the plant extract used with a minimum inhibitory concentration of 25%. A similar result was observed in our study. The effectiveness of coconut oil has also been shown in experiments [19], in control of oral microbial populations of *Streptococus mutans* and *Candida albicans,* reducing the number of dental cavities and oral infections in humans. Early colonization and a high number of carcinogenic microorganisms present in the oral microbiota of Egyptian children [20], such as yeasts of *Candida albicans*, have encouraged the use of coconut oil as an alternative chemotherapy agent, obtaining laboratory results compatible with the use of azole antifungal drugs, such as ketoconazole. Daily oral hygiene with coconut oil in Nigeria adolescents [21], in the 16-18 age range, reduced the occurrence of gingivitis, showing that the presence of lauric acid stimulates the anti-inflammatory and antimicrobial effect of the plant extract used. Takahashi et al. [22], working with experimental models of mice infected with oral candidiasis, found antifungal activity from the four fatty acids of coconut oil (caproic acid, caprylic acid, capric acid, and lauric acid), inhibiting the morphological dimorphism of Candida yeasts from yeast-form (infectant) to mycelial (parasitic) fungi necessary for establishment of the infectious process. Caprylic acid was the fatty acid with best therapeutic response using 50 µL three times on *Candida* lesions on the tongue of mice. Association of terpineol-4, the main oil of

melaleuca [23], a plant native to Australia and known as the tea tree, with capric acid, a fatty acid of coconut oil, has shown to be a potential natural medication *in vitro* and *in vivo*, with synergistic antimycotic activity on the capacity of pathogenic variability of *Candida* isolates. Nevertheless, Cavalcanti [24] showed that extracts of *Eugenia uniflora* (pitanga tree) leaves, bark from the trunk of *Libidibia ferrea* (*pau ferro* or Brazilian ironwood), and *Psidium guajava* (guava tree) leaves, obtained from the Brazilian *Caatinga* biome, constitute plant therapeutic alternatives due to *in vitro* susceptibility of *Candida* to plant extracts, through the fungistatic effect on standard ATCC yeasts of Candida species, with minimum inhibitory concentration values from 15.62 to 6.25 µg/mL. Santos et al. [25], in a comparative study on alternatives to the use of plant extracts, with the use of an enzymatic oral solution based on lactoperioxidase in patients treated in a hospital ICU, in relation to a control group with use of cetylpyridinium chloride, showed, in clinical evaluation of the simplified oral hygiene index (OHI-S), that there was statistical significance when compared to the study and control groups. The choice of an enzymatic product as an auxiliary method in reduction of oral bacterial plaque arises from the absence of abrasive substances (alcohol, detergent, and stain) in its composition, which hurt even more the already compromised buccal mucosa. It is also important that the enzymatic product contains lactoferrin, which, through its action and interactions on saliva, reduces the occurrence of *Candida albicans* and *Candida krusei* in buccal mucosa. Even so, the antifungal capacity of extra-virgin coconut oil on fungi is broader. In addition to inhibiting proliferation and, consequently, combat the pathogenic action of *Candida* yeasts in infected organic mucosas, it has antimycotic action on isolates of *Cryptococcus neoformans* and filamentous fungi, such as *Aspergilus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Alternaria,* and *Fonsecaea pedrosoi* [26].

## 4. CONCLUSION

This study reveals that patient stay in the ICU in the hospital environment seems to influence the occurrence of *Candida* yeasts in the buccal mucosa, mainly because of the daily difficulty in carrying out adequate oral hygiene. *Albicans* continues to be the *Candida* species most detected in the buccal cavity of patients in the ICU, particularly in the first 72 hours. The use of extra-virgin coconut oil *in vitro*, in a maximum concentration of 30%, added to the SDA medium with the addition of chloramphenicol, could inhibit the proliferation of buccal isolates of *Candida albicans* originating from patients in a hospital ICU. Thus, extra-virgin coconut oil is an alternative plant therapy effective in controlling the proliferation of *Candida* yeasts in buccal mucosa and infection of buccal mucosa by *Candida* yeasts.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Approval for this study was obtained from the Clinical Hospital of Federal University of Goiás (Hospital das Clínicas of the Universidade Federal de Goiás - CEPMHA/HC/UFG), protocol number 634.432/2014.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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