Prevalence of Candida spp. in the oral cavity of patients admitted to the dermatology ward service of a level 3 hospital in Sao Paulo, Brazil

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Research Article

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Abstract

Candida spp. are responsible for most opportunistic fungal infections. The rapid and accurate identification of this type of yeast in hospitalized patients is critical for their effective treatment and prevention of complications arising from resistant strains.

Objective: Evaluate the prevalence, as well as factors that are associated with oral candidiasis in 240 patients who were admitted to the dermatology infirmary of a tertiary hospital in Sao Paulo, Brazil.

Methods: It is a descriptive, observational, and cross-sectional study, in which the clinical condition of each patient was analyzed and a direct mycological examination and culture for fungi were performed. Microorganisms were identified using phenotypic methods and mass spectrometry (MALDI-TOF).

Results: The prevalence of *Candida* spp. was 28.7%. *C. albicans* was the most common species (84%), followed by *C. glabrata* (4.34%), *C. tropicalis* (4.34%), *C. parapsilosis* (4.34%), *C. krusei* (1.4%), and *C. dubliniensis* (1.4%). As discovered by univariate and multivariate logistic regression analysis, such factors as the use of oral prosthesis and immunosuppression increased the risk of candidiasis.

Conclusion: Hospitalized patients showed a significant prevalence of oral candidiasis, related to predisposing local and systemic factors, which affected their microbiota. Although *C. albicans* was the primary microorganism of the infection, other species were identified.

Clinical relevance: The techniques that were used in this study identified the precise causative agent of the infection in a short period, allowing us to modify the therapeutic regimen so that, in turn, direct implications on the patient's health were presented.

Introduction

Candidiasis is an opportunistic disease that can cause local or systemic infections in various areas of the body, manifesting primarily in immunologically compromised individuals, due to prolonged drug therapy, or over long hospital stays [1]. Its evolution depends on predisposing factors in the host, as well as factors that are related to the microorganism, which determine the type of clinical manifestation [2, 3].

The patients in this study were admitted to our dermatological nursing service. Due to their poor health, they were subjected to long periods of hospitalization and administered polypharmaceutical therapies, most of which included broad-spectrum antibiotics and immunosuppressive drugs, the last being the main reason for the patients' selection.

The microbiota of the oral cavity is complex and is usually maintained in a physiological commensal relationship. The presence of unfavorable local or systemic conditions, such as those presented by our patients, can destabilize this flora, increasing the risk of opportunistic infections, such as candidiasis [4].

Candida spp. are a type of yeast normally found in the gastrointestinal tract, as well as oral and genital mucosa, with over 200 identified species. *Candida albicans* is the most common species, but the emergence of other yeasts is being increasingly recognized [5, 6, 7].

An important factor in the identification of types of *Candida* spp. is the lack of sensitivity of certain species to traditional antifungals used in clinical practice. For example, *Candida tropicalis*, *Candida krusei*, and *Candida glabrata* can be four to 32 times less sensitive to fluconazole than *C. albicans* [1, 8], requiring a precise identification of the species by an inpatient service.

The identification of microorganisms by genus and species is important for the correct diagnosis and treatment, as well as its epidemiological implications because this type of information expands the scientific knowledge from a clinicopathological perspective [1, 9]. Microorganisms must be identified quickly and accurately, using traditional and cutting-edge techniques that determine the causative agent of the infection, phenotypic methods, and mass spectrometry (MALDI-TOF).

Thus, the main objective of this study was to evaluate the prevalence of oral candidiasis and discuss the factors related to its presence in 240 patients who were admitted to the dermatology infirmary of a tertiary hospital in Sao Paulo, Brazil.

Patients And Methods

This descriptive and observational study was performed in the Hospital das Clinicas Dermatology Ward Service, Medical School, University of Sao Paulo. The study was approved by the local research ethics committee, under protocol #2.018.627 (4/17/2017). All patients signed an informed consent form after receiving information about the study.

The sample consisted of 240 patients who were admitted to the service between July 2017 and July 2019. There were no exclusion criteria regarding gender or age. Patients with severe systemic conditions and those who used antifungal agents during their hospital stay were excluded. Detailed demographics and clinical information, including gender, age, use of dental prosthesis, and comorbidities were obtained from their medical records.

All selected patients underwent clinical anamnesis of the oral mucosa, a direct mycological examination, and sample collection for culture by a trained dentist.

DIRECT MYCOLOGY

An oral mucosal examination was performed for all patients, followed by the collection of microbiological samples from the surface of the tongue, palate, or labial commissure using a sterile swab. This procedure was performed regardless of the presence of oral lesions due to systemic disease or clinical manifestation of oral candidiasis. The sample was then spread on a glass slide and taken immediately to the FM-USP microbiology department for microscopical analysis.

FUNGAL CULTURE AND IDENTIFICATION

The culture was performed on samples collected from 132 hospitalized patients, 67 with oral lesions and 65 without oral lesions. The patients in the latter group were randomly selected (Figure 1).

On collection, the samples were inoculated into Sabouraud Dextrose Agar (SDA, DIFCO, USA) and incubated at 30 °C for 48 hours. After this time, samples without fungal colony growth were considered negative.

Positive samples were subcultured in CHROMagar *Candida* medium (CHROMagar, Paris, France) and incubated at 30 °C for another 48 hours. A presumptive identification was performed, based on the color of the colonies, according to the manufacturer's instructions. To differentiate between *C. albicans* and *C. dubliniensis*, a thermotolerance test was performed. The samples were grown on Sabouraud agar at 45 °C for 48 hours, and strains that showed colony growth were considered positive for *C. albicans.*

Morphological and biochemical identification was conducted using phenotypic methods by culture in sterile Petri dishes, on which a slide that was covered with Tween 80 corn agar medium was placed. *Candida* spp*.* was then sown in parallel streaks, covered with a sterile coverslip, and incubated for 48 hours at 30 °C, leading to the formation of hyphae, pseudohyphae, blastoconidia, and chlamydoconidia [10].

MALDI-TOF

Strains of *Candida spp. were identified* by mass spectrometry (MALDI-TOF). A 10-µL loop was taken from each sample, which had been sown in SAB for 48 hours and incubated at 30 °C. Then, 300 µL sterile distilled water and 900 µL absolute ethanol were added, followed by centrifugation at 1300 rpm for two minutes. The supernatant was discarded, and the precipitate was left at room temperature to dry.

Next, 50 µL of 70% formic acid and 50 µL of acetonitrile were added to the dry pellet, homogenized in a vortex, and centrifuged for two minutes. One microliter of the supernatant was applied to a specific steel plate in quadruplicate at room temperature, followed by 1 µL of the HCCA matrix (α cyano-4-hydroxycinnamic acid), dissolved in 2.5% TFA matrix solution (trifluoroacetic acid) [11].

The mass spectrometry analysis by MALDI-TOF was performed on a Bruker 3.1, comparing the resulting spectra with a database of reference spectra, expressed in log values from 0 to 3.

ORAL CANDIDIASIS

The diagnosis of oral candidiasis (OC) was based on the presence of oral lesions that were associated with the isolation of *Candida* spp. in culture. The isolation of *Candida* spp*.* in culture without oral lesions was considered colonization [12, 13].

STATISTICAL ANALYSIS

A preliminary analysis of the data was performed in 240 patients using exploratory techniques to check for distribution patterns and trends of the principal variables.

Ultimately, 127 samples were considered for the univariate and multivariate logistic regression analysis: 62 patients with OC and 65 patients without OC. We evaluated the associations between having OC and independent variables: use of dental prosthesis, immunosuppression, age, and gender.

Initially, a univariable analysis was performed to examine the unadjusted association between variables and outcome (OC). The multivariate models had been tested for the multicollinearity of the independent variables using the Variance Inflation Factor (VIF). No multicollinearity was observed between the variables that were integrated into the binary logistic regression model.

To study the multivariate associations between independent variables (age, prosthesis, and immunosuppression) and the prevalence of OC, odds ratios (ORs) were estimated using a hierarchized logistic regression model. The results from all models were expressed as ORs with their corresponding 95% CI for OC The statistical program that we used for this analysis was SPSS (v. 24.0, IBM, New York, NY, USA).

Results

The clinical and demographic data of all 240 patients are summarized in Table 1. The patients' ages ranged from one to 89 years (mean age of 48 years). Over half of the patients were female (136, 57%). Most patients were self-declared Caucasian (176, 73.34%). All patients were admitted to the hospital due to severe systemic conditions that were related to dermatological diseases (inflammatory, autoimmune, infectious, hereditary, and neoplastic). The most prevalent diseases were pemphigus vulgaris (23, 9.6%), erythrodermia (21, 8.7%), atopic dermatitis (20, 8.3%), and psoriasis (12, 5%).

Direct mycological exam, mycological culture, and species identification

DME was performed in all patients. Among patients with oral injury (n = 67), 65 (97.01%) had a positive DME, whereas among patients without oral lesions (n = 173), one of them had yeast.

Candida spp. was detected in 62 patients who had oral lesions, the diagnosis of which was confirmed by culture; the samples from the other five patients were unviable (contaminated). Of the 65 randomized samples, *Candida* spp. was detected in seven (10.77%) and was considered colonization.

The results of the identification of 69 samples that were isolated in culture by phenotyping and MALDI-TOF are shown in Fig. 1. *C. albicans* was the most prevalent species (84.06%) and was observed only in patients with oral lesions. Other species were also detected in this group, including *C. glabrata* (2), *C. tropicalis* (1), and *C. krusei* (1).

C. parapsilosis (3), *C. tropicalis* (2), *C. glabrata* (1), and *C. dubliniensis* (1), isolated from samples of patients without oral lesions, were considered colonization.

Statistical analysis

The anatomical site and the clinical variant of OC had no significant statistical correlation in our cohort.

A total of 62 samples from patients with OC were compared with 65 samples from negative patients. The results of the univariate logistic regression between the presence of OC and prosthesis, immunosuppression, age, gender, and race are listed in Table 2.

By univariate regression analysis, the use of a prosthesis increased the risk of OC by 3.20 times, and patients with immunosuppression had a 3.33-fold higher risk of OC. The one-year increase in age caused a 2.9% rise in the risk of developing OC. Sex and race showed no association with the development of OC.

The model containing prosthesis, immunosuppression, and age in the multivariate logistic regression analysis was a significant predictor of the development of OC for the first two variables: the use of a dental prosthesis increased the risk by 3.49, and immunosuppression increased the risk 3.61 times. In this model, age was not a significant predictor.

The most prevalent site of infection was the palate (50, 74.6%), followed by the tongue (12, 17.4%) and labial commissure (five, 7.3%). The most representative clinical variant was erythematous candidiasis (40, 58%), followed by pseudomembranous (24, 34.8%) and angular cheilitis (five, 7.2%).

There was no significant correlation between the variables and each systemic disease with *Candida* spp. Notably, 13 patients (56.5%) with pemphigus vulgaris presented oral candidiasis.

Figure 2 shows the clinical lesions of candidiasis and its microscopic properties in the direct mycological examination and microculture; it is also possible to observe the growth of *C. albicans* in the CHROMagar medium.

Discussion

Candida spp. is one of the main opportunistic pathogenic fungi, commonly found in the physiological microbiota [14]. The ability to colonize various anatomical sites and its transformation from commensal to pathogen depend on its virulence and the environmental and local conditions that modify the microenvironment in the oral cavity [15], especially in patients with specific conditions, such as those in our research.

Long periods of hospitalization, specific systemic conditions (e.g., pemphigus, pemphigoid, atopic dermatitis, and lichen planus), as well as the prolonged use of corticosteroids and broad-spectrum antibiotics induce cellular immunosuppression, consequently increasing the susceptibility to opportunistic infections [16, 17]. In our study, the prevalence of OC was 28.7%, consistent with other groups, including Stramandinoli et al. [18] and Mahmoudabadi et al. [19].

Mariani et al. [20] reported a lower prevalence (16.3%) in a sample of 141 hospitalized patients; their diagnosis was only clinical, which could underestimate the number of positive cases.

Immunosuppression and the use of oral prosthesis are well-documented systemic and local risk factors for OC [7, 21]. By univariate and multivariate logistic regression analysis, these variables were significant (immunosuppression, univariate analysis = 3.3; multivariate analysis = 3.6; use of prosthesis, univariate analysis = 3.2; multivariate analysis = 3.4), demonstrating the importance of their analysis in hospitalized patients and the inclusion of a dentist in multidisciplinary teams in the hospital environment.

The relatively acidic and anaerobic microenvironment that is created by oral prostheses and the porosity of their acrylic surfaces provide viable conditions for the colonization of bacteria and fungi [21, 22]. Similarly, the physical and emotional tolls of hospitalized patients prevent adequate oral hygiene, which contributes to a microenvironment that favors the establishment of OC.

As in our study, other groups have reported the relationship between the use of prostheses and the presence of OC. Bianchi et al. [23] demonstrated that 83.3% of prosthesis users developed OC, and Mariani et al. [20] observed C*andida* spp. in 56.4% of patients with prostheses.

Fungal infections have increased in number and diversity in recent years, primarily due to the rise in patients who are at risk of invasive infections. An increase in resistance to antifungal agents has also been described, especially in non-*albicans* strains of *Candida* [9].

C. albicans continues to be responsible for more than 50% of human yeast infections. Most oral infections that are caused by this fungus are superficial but present the risk of progressing to a systemic infection if untreated, especially in immunosuppressed patients [24]. In our study, *C. albicans* constituted 84.1% of all species.

Other less common species, such as *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. dubliniensis*, were also observed in our study. Their recognition is significant, given the resistance that they have to certain antifungal agents and their importance in invasive fungal infections [6, 19, 25].

C. glabrata has increased significantly as an infectious agent, mainly in hospital settings [26]. It can become resistant to fluconazole and echinocandins and is more common in older adults [27, 28], as observed in our study, in which all patients were aged over 60 years. Another important aspect is that *C. glabrata* does not generate pseudohyphae or hyphae, which is why only yeasts are observed in direct examinations of patients, making it harder to diagnose.

C. tropicalis is resistant to 5-fluorocytosine and is the second most common pathogen that causes candidemia in adults, especially in patients with malignant pathologies and diabetes mellitus (DM) [29]. In our study, two out of three patients had DM, and none was diagnosed with malignant neoplasms.

C. krusei is resistant to several antifungals, primarily fluconazole, amphotericin B, and 5-fluorocytosine. Its multi-resistance phenotype complicates its treatment, mainly in immunocompromised patients [29].

C. parapsilosis is the most common species in bloodstream infections, primarily in neonates, transplant patients, or patients with central catheter use. In our sample, the three patients were aged under 20 years, none had a report of transplantation, and one was using a central catheter. The clinical isolates of this species are sensitive to amphotericin B and azole derivatives [30].

C. dubliniensis is commonly associated with HIV-positive patients and patients with DM, being able to easily develop resistance to azoles [29, 31, 32]. In our sample, the only patient who was positive for this species did not present any of the pathologies above.

Given the prolonged and recurrent nature of traditional antifungal therapies, strains become resistant in hospitalized patients, such as those in our sample, needing the ability to isolate, identify, and test the drug susceptibility of the causative agents to provide the best therapeutic option.

Recent research has found other *Candida* spp. Mahmoudabadi et al. reported the presence of *C. tropicalis* (15.6%) and *C. glabrata* (6.3%) in hospitalized children [19]. In 2016, Mohammadi et al. evaluated the oral cavity of 106 diabetic patients, noting the presence of *C. albicans* (36.2%), *C. krusei* (10.4%), *C. glabrata* (5.1%), and *C. tropicalis* (3.4%) [25].

More recently, Vieira et al. [33] compared the presence of *Candida* spp. in hospitalized versus outpatient patients and reported colonization by *Candida* spp. in 85.7% of hospitalized patients. *C. albicans* was identified in 60% of the cases, followed by *C. tropicalis* (23.4%) and *C. krusei* (3.3%), whereas in outpatients, only 47% of positive cases had *Candida* spp.

Non-*albicans* species are being increasingly recognized as important agents of infection, with recent studies documenting their rise. Consequently, timely and accurate identification of these species in the hospital setting is essential to provide specific treatments for candidiasis and prevent systemic complications [4, 7].

Mass spectrometry can be valuable in reading and analyzing a broad spectrum of microorganisms rapidly, identifying clinical strains [34, 35, 36]. MALDI-TOF is a highly sensitive diagnostic technology, although its cost, in most cases, renders its routine implementation difficult.

In our study, the new MALDI-TOF system identified the species in a rapid and precise manner, which is significant when defining the therapy for each patient, reducing resistant strains, and implementing effective infection control measures [31, 37, 38].

No previous report has recorded the prevalence of candidiasis or the distribution of particular species in hospitalized patients with dermatological diseases that affect the oral cavity.

Conclusions

The hospitalized patients in our sample had a significant prevalence of oral candidiasis, related to predisposing local and systemic factors, such as the use of oral prosthesis and immunosuppression,

which affected their microbiota. Although *C. albicans* was the predominant microorganism in the infections, other species were identified, guiding the correct and timely choice of therapy.

The rapid and precise recognition of microorganisms by genus and species facilitates a timely diagnosis and the choice of the correct therapy against the offending agent. Further, it provides epidemiological data that expand our knowledge from a clinical-pathological perspective.

Declarations

This manuscript is not being considered for publication in any other journal, and all authors have reviewed and approved the final version.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE – HUMANS

The study was approved by the local research ethics committee, under protocol #2.018.627 (4/17/2017). All patients signed an informed consent form after receiving information about the study.

FUNDING:

No funding was obtained for this study

CONFLICT OF INTERESTS:

We declare that we have no conflict of interest.

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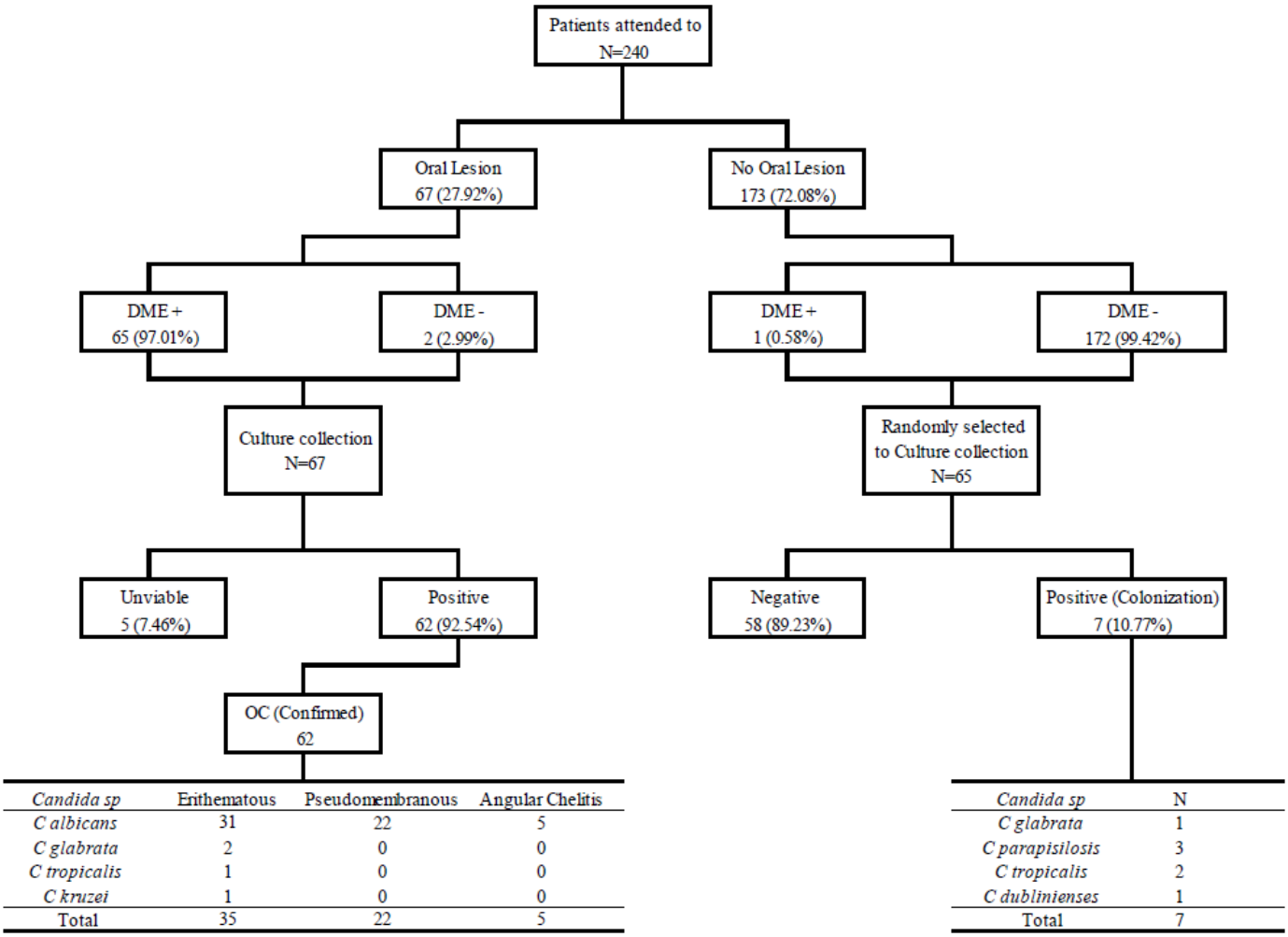
Tables

Tables 1 and 2 are available in the Supplementary Files section

Figures



Candida sp	Erithematous	Pseudomembranous	Angular Chelitis
C albicans	31	22	5
C glabrata	2	0	0
C tropicalis	1	0	0
C kruzei	1	0	0
Total	35	22	5

Candida sp	N
C glabrata	1
C parapisilosis	3
C tropicalis	2
C dublinienses	1
Total	7

Figure 1

Diagram shows overall results of main fungal analyses in this cohort group, evidencing findings of different Candida species. Total positive and negative samples exhibit difference for direct mycological examination and fungal culture techniques, both resulting in albicans and non-albicans strains.

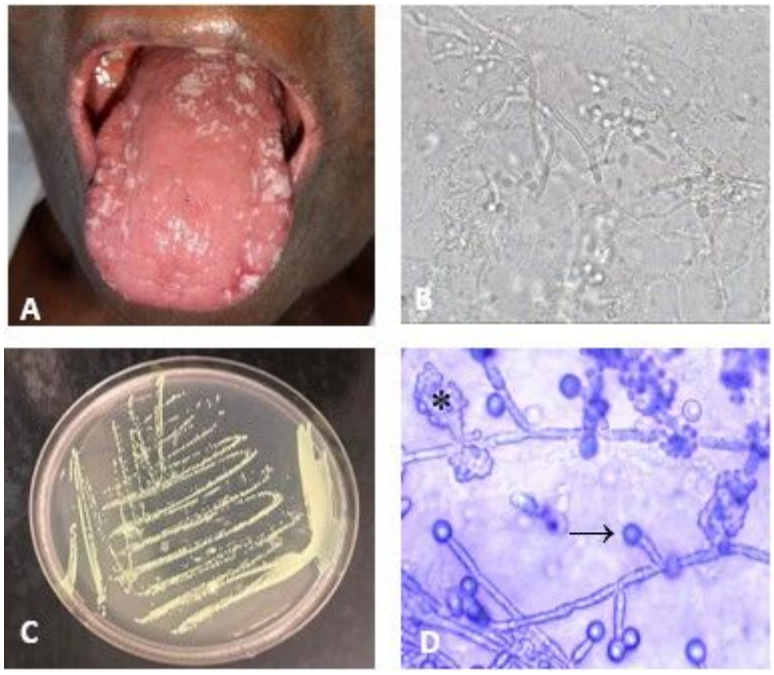


Figure 2

Clinical features and microbiological findings of fungal characterisation methods. A: Clinical presentation of pseudomembranous candidosis, showing widespread non-adherent white plaques on dorsal tongue. B: Direct examination revealing yeast hyphae. C: identification of fungal species utilising CHROMagar, here exhibiting Candida albicans in green. D: Microculture showed presence of clamidoconids (arrow) and blastoconids (asterisk) within fungal clusters.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.pdf
- Table1.pdf