

Molecular detection of *Candida aaseri* in oral cavity of Immunocompromised

Patients

Abstract

The impaired human immune system, resulting from diabetes mellitus, can lead to the transition of *Candida* from a commensal to a pathogenic status, causing oral fungal infections. *Candida aaseri* is a dimorphic yeast with lipolytic activity which has not been previously reported in clinical infections. This study reports an extremely rare oral fungal infection associated with *C. aaseri* in a patient with type II diabetes mellitus. The patient, a 42-year-old female with diabetes mellitus type II from Basrah, Iraq, was identified with a fungal oral infection. Swab samples were collected from the patient's oral cavity for microbial investigation and cultured on Sabouraud Dextrose Agar (SDA) at 37°C for 48 hours. The results revealed pure yeast growth. Blue colonies were observed on CHROMagar Candida. The genomic DNA of the isolated yeast was extracted for molecular-level diagnosis, utilizing the mRNA gene's internal transcribed spacer (ITS) region. *C. aaseri* was the sole yeast species identified in this clinical case. Anti-fungal sensitivity tests indicated that *C. aaseri* was sensitive to Nystatin, Posaconazole, and Fluconazole while exhibiting significant resistance to Colistin Sulfate. This study represents the first global report of isolating *C. aaseri* from an oral infection in an immunocompromised patient.

Keyword: Antifungal, Candida, Fungi, Oral infection, immunocompromised, ITS, Diabetes, Basrah.

Introduction

The human oral cavity is a unique site that becomes colonized with many microbes, such as fungi, bacteria, and viruses. Among these microbes, *Candida* species are the most frequent colonizers in the human oral cavity that adapted to exist harmlessly within mucus membranes. Overgrowth of *Candida* in the oral cavity can result in various clinical symptoms such as discomfort, pain, changes in taste perception, and difficulty in swallowing (dysphagia) (Lu 2021). *C. albicans* is considered the most predominant opportunistic yeast for causing candidiasis compared with other species such as *C. tropicalis*, *C. glabrata*, *C. pseudotropicalis*, *C. guillierimondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. stellatoidea* due to its ability to biofilm and hyphae form and produce hydrolytic enzymes and candidalysin (Patel 2022). *Candida* species members have the ability to cause infection due to possess a wide range of virulence factors, such as the expression of adhesions and invasions on the cell surface, the morphological transition between yeast and hyphal forms, thigmotropism, biofilms formation, and the secretion of hydrolytic enzymes (Silva et al. 2019).

The commensalism relation between the host and *Candida* species bases immune status for both innate and adaptive immune systems (Mei-Sheng 2021). The status of the immune system is key to developing fungal infections. Among immunocompromised individuals, the infection can spread through the bloodstream or upper digestive system, causing a severe infection with higher risks of illness that is associated with an increase in the mortality rate (Jerônimo et al. 2022). Diabetic patients are more susceptible to the development of various oral infections, including bacterial and fungal infections, due to the decreasing salivary flow rate and the absence of antimicrobial effects. In addition, poor metabolic control, an impaired defence mechanism, and a defect in the immune system may play an important role in developing infection (Rohani 2019). For this reason, the rate of *Candida* colonization increases with immunocompromised individuals who suffer from diabetes mellitus and become a serious pathogenic fungus, leading to disseminated candidiasis (Contaldo et al. 2023). Therefore, early diagnosis of pathogenic fungal infections results in prevention and management treatment that is key to removing or alleviating the predisposing conditions (Al-Rifaie and Al-Maqtoufi 2018).

The oral mucosa is composed of both oral epithelial cells, keratinocytes and immune cells, including cells such as macrophages, dendritic cells (DCs), natural killer (NK) cells and polymorphonuclear neutrophils, which contribute to the plethora of immunomodulatory cytokines found in the oral cavity and the production of biological mediators such as antimicrobial peptides (Casaroto et al. 2019). There are four classes of antifungals that differ in their mechanisms of action: polyene amphotericin B which has a broad spectrum and a potent fungicidal effect (Carolus et al. 2020). Fluorocytosine (flucytosine) inhibits fungal protein synthesis and is also a potent inhibitor of fungal DNA synthesis through the inhibition of thymidylate synthetase (Barantsevich and Barantsevich 2022). Systemic azoles and currently widely used triazoles have several antifungal formulations, including fluconazole, itraconazole, voriconazole, posaconazole, ravuconazole, and isavuconazole. Azoles inhibit lanosterol 14 α demethylase, a key enzyme of ergosterol biosynthesis and has a fungistatic activity against *Candida* spp. (Hassanmoghadam et al. 2019). Echinocandins (micafungin, anidulafungin, caspofungin) present the newest class of antifungals and feature a fungicidal effect in *Candida* species (Ham et al. 2021). Some studies focus on using natural products for treating *Candida* infections using honey (Albosolemy et al. 2022) or chemically synthesized antimicrobials (Yossra et al. 2019) with no side effects. *C. aaseri* is commonly known as a saprophytic yeast on many plants, such as oil palms (Lee et al. 2017). The only report of isolation of *C. aaseri* and closely related species *C. pseudoaaseri* from the sputum of Norwegian patients (Pfüller et al. 2011). There is currently no global research addressing the identification of dimorphic *C. aaseri* as a potential etiological agent of oral infections in an immunocompromised patient with diabetes mellitus type 2 in Basrah, Iraq.

Methods

Samples Collection

Swab samples from patients with clinically confirmed cases of fungal oral cavity infections were collected from a 42-year-old woman with a five-year history of Diabetes mellitus II presented at A-Sadr Teaching Hospital in Basrah, Iraq, in January 2021. After obtaining ethical approval and

reviewing the patient's medical history, it was noted that she was taking Glucophage 500 mg twice daily for her Diabetes. She had not taken any antibacterial or antifungal medications for more than a week, and she did not have any immunosuppressive conditions other than Diabetes. Furthermore, she did not have any heart, liver, or renal diseases, and she did not consume alcohol or smoke. Upon examination, the patient's tongue appeared white with red spots at the edges. A random blood sugar test (RBS) revealed a 300 mg/dL blood sugar level. Oral swab samples were collected and cultured on Sabouraud Dextrose Agar (SDA) at 37°C for 48 hours to investigate the microbial composition.

Molecular Identification of Isolated Endophytic Fungi

Recovered yeast on Cornmeal tween 80 agar for one week at 25°C appeared as colonies with the ability to produce pseudo-hyphae, but no chlamydo spores were formed. No germ tube was formed when grown in human serum for three hrs. at 37 °C. Molecular identification using the ITS regions of the rRNA-encoding region showed that *C. aaseri* was the only microbial colony recovered from this patient using the primers ITS5 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3'. PCR amplification condition of the ITS region was conducted according to procedures described by Al-Maqtoofi and Thornton (2016). DNA sequence data were aligned for comparison with those stored in the National Center for Biotechnology Information (NCBI) databases using NCBI BLAST® (<https://blast.ncbi.nlm.nih.gov>). Phylogenetic analyses were conducted in MEGA 11 (Kumar et al. 2018).

Results

A yeast colony was isolated and cultured on CHROM agar™ Candida at 37°C for 48 hours. This resulted in the recovery of distinctive blue colonies, and the letter C3 was used to indicate this isolate (Figure.1). After genomic analysis, the data sequence of isolate (C3) was deposited to GenBank for Accession Number verification(OR398895).

A combination of bioinformatics tools was employed for initial editing and analysis to construct the phylogenetic tree. The analysis result was compatible with *C. aaseri* with per cent identity (100 – 99.43%) (Figure 2). Antifungal sensitivity test showed that *C. aaseri* was significantly sensitive to fluconazole and posaconazole followed by nystatin (43, 41, 23 mm, inhibition zone) respectively, while exhibiting a notable resistance to Colistin sulphate (Figure 3).

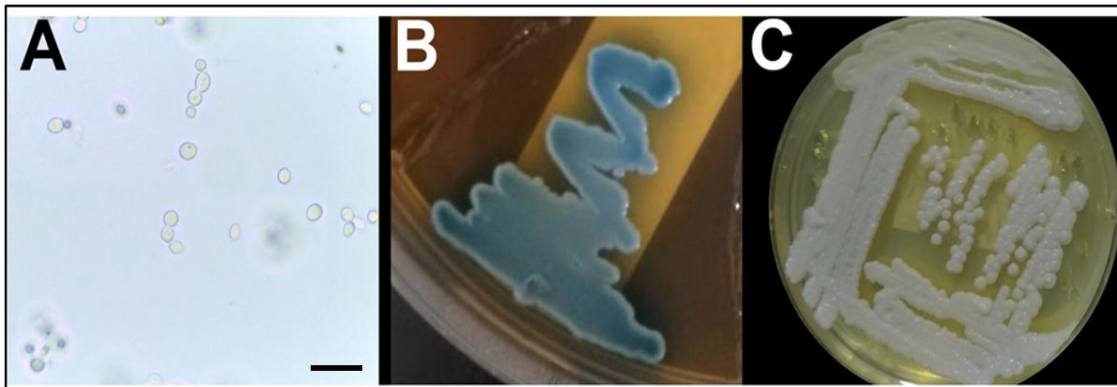


Figure 1. *C. aaseri* under light microscope. **A:** *C. aaseri* (100x). **B:** *C. aaseri* on CHROMagar™ Candida; **C:** *C. aaseri* on Sabouraud Dextrose Agar. Scale bar= 10 µm.

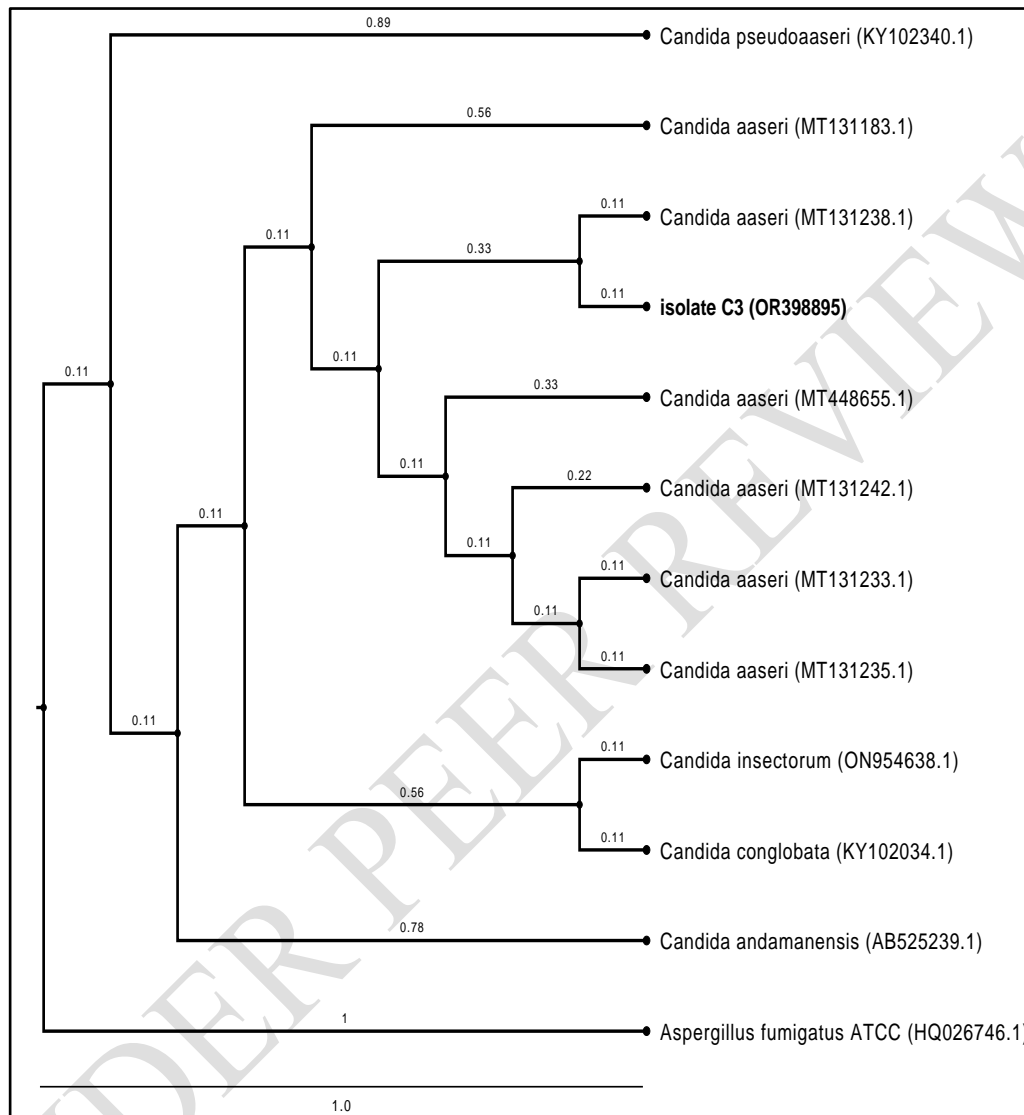


Figure 2. Phylogenetic tree drawn from neighbour-joining analysis of ITS domain sequences depicting the relationships of *Candida aaseri* (C3) (OR398895) with closely related *Candida* species. Bootstrap percentages over 50% from 1000 bootstrap replicates are shown. Reference sequences were retrieved from GenBank with the accession numbers indicated. The phylogenetic tree was rooted with *Aspergillus fumigatus* as an outgroup.

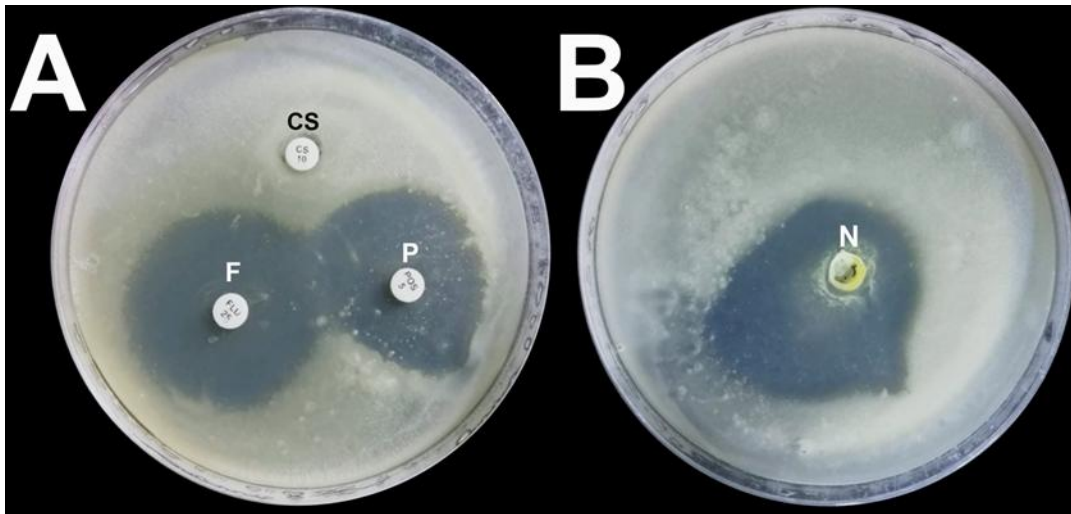


Figure 3. Antifungal susceptibility test on Mueller-Henton agar for *C. aaseri* where A: Antifungal susceptibility test Fluconazole (F) 25 µg, Posaconazole (P) 5µg and Colistin Sulphate (CS) 10µg; and B: Antifungal susceptibility test of Nystatin (N) 0.1 mg.

Discussion

Candidiasis infections are a significant concern among immunocompromised individuals who have an impaired immune system, making them more susceptible to opportunistic infections, including those caused by *Candida* species leading to a high morbidity and mortality rate exceeding 70% (Chavda et al. 2023). The incidence of disseminated candidiasis has an increasing trend along with the ever-expanding populations of immunocompromised such as those with diabetic patients (Abdoli et al. 2022). Among the pathogenic species, *C. albicans* is the prevailing and frequently encountered *Candida* species causing candidiasis in human immunodeficiency individuals followed by *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* (Miruka et al. 2023). In Basrah, a study reported a very rare eye infection by yeast, *Hanseniaspora uvarum* (Israa et al. 2022). In a unique clinical case, an extremely rare *Candida* infection was reported in the oral cavity of an immunocompromised patient with diabetes mellitus in Basrah, Iraq. This particular infection is associated with *C. aaseri*. This species of *Candida* has not been reported as a human fungal pathogen. To our knowledge, this study is the only one that showed isolation, identification and successful in-vitro treatment of *C. aaseri* from oral infection of immunocompromised patients with diabetes mellitus in the world.

Conclusions

Candida aaseri has not been reported as a human fungal pathogen. To our knowledge, this study is the only study that showed isolation, identification and successful in-vitro treatment of *C. aaseri* from oral infection of immunocompromised patient with diabetes mellitus in the world.

Abbreviations

SDA: Sabouraud Dextrose Agar
 ITS: Internal Transcribed Spacer
 DCs: Dendritic Cells
 NK: Natural Killer

RBS: Random Blood Sugar
NCBI: National Center for Biotechnology Information
CS: Colistin Sulphat

Consent

Written informed consent was obtained prior to the interview.

Ethics approval

The study was approved by the University of Basrah, College of Science, Department of Biology Ethic Committee

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