

Case Report

The first case of isolation of *Magnusiomyces capitatus* from the oral cavity of an addicted patient

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Abstract

Magnusiomyces capitatus (*M. capitatus*) is an emerging opportunistic yeast, rarely found as a causal agent of invasive fungal infection. In this study, we report a 31-year-old man infected with *M. capitatus* in the oral cavity, with a history of heroin and amphetamine abuse. *M. capitatus* was isolated through culture and microscopic analysis and identified by PCR amplification of the ITS DNA region. Based on the *in vitro* antifungal susceptibility test, the lowest MICs for *M. capitatus* were recorded for nystatin, itraconazole, and amphotericin, while higher MICs were observed for caspofungin and fluconazole. Treatment with nystatin successfully eliminated *M. capitatus* and relieved the clinical symptoms. This study presents the first case of *M. capitatus* in a patient with substance use disorder, manifesting as a plaque-like ulcer in the oral cavity.

Key words: *Geotrichum*; *Magnusiomyces capitatus*; diagnosis; addiction; nystatin.

J Infect Dev Ctries 2024; 18(2):309-314. doi:10.3855/jidc.18099

(Received 18 February 2023 – Accepted 14 May 2023)

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Introduction

In recent years, the incidence of oral fungal infections has increased due to changing patterns in risk factors, such as alterations in epidemiology, an increased number of organ transplantations, and the more widespread use of immunosuppressive therapy [1]. It is now well established that most opportunistic oral mucosal fungal infections are due to *Candida albicans* and *Aspergillus fumigatus* species [2]. *Magnusiomyces capitatus* (previously known as *Blastoschizomyces capitatus*, *Dipodascus capitatus*, *Geotrichum capitatum*, *Saprochaete capitata*, *Trichosporon captiatum*) is an arthroconidial yeast-like fungus commonly isolated from soil, beach sand, wood, and poultry feces [3]. It is rarely found as a causal agent of invasive fungal infections [4]. *Magnusiomyces capitatus* (*M. capitatus*) is an emerging opportunistic fungal pathogen in immunocompromised patients,

particularly those with hematological malignancies. Surgery, neutropenia, catheters, corticosteroids or broad-spectrum antibiotic therapy are indeed predisposing factors for this infection [5]. Due to its close relation to other fungi such as *Saprochaete clavata*, conventional methods for diagnosing *M. capitatus*, such as microscopic observation and biochemical tests, have not consistently provided accurate results. To address this issue, molecular methods have been employed for the consistent, timely, and accurate identification of *M. capitatus* [6]. *M. capitatus* has not previously been reported as a causative agent of oral infection in drug-abusing patients worldwide. Here, we describe the first case of oral fungal infection caused by *M. capitatus*, in an addicted patient in Iran. This emphasizes the importance of close monitoring for the timely and accurate diagnosis of various uncommon fungal

infections to determine appropriate treatment with antifungals in patients with substance abuse.

Case Report

A 31-year-old man with substance use disorder presented at the addiction treatment camp in Ahvaz, southwest Iran, with a history of several weeks of plaque-like oral ulcers. Physical evaluation revealed a white to gray discolored plaque-like ulcer in the anterior supragingival area with poor dentition (Figure 1A). Table 1 presents a few of the white lesions that require additional consideration in terms of their definition and terminology. Specifically, it is recommended to differentiate between a provisional clinical diagnosis and a definitive diagnosis of oral leukoplakia [7]. Making this distinction is important to ensure accuracy in diagnosis and appropriate treatment for patients.

Notably, the patient had not used any medication to treat the ulcer before the infection was diagnosed. He had no history of underlying disease or prior hospitalizations. According to the patient's medical records, there was no evidence of HIV infection. He had a ten-year history of daily heroin and amphetamine use. He denied alcohol dependence and started smoking at the age of 18 and currently smokes 5 to 10 cigarettes per day. His social profile indicates unemployment, primary education, and married status. He was not taking any routine medications. Hematological and biochemical tests revealed normal results, except for a

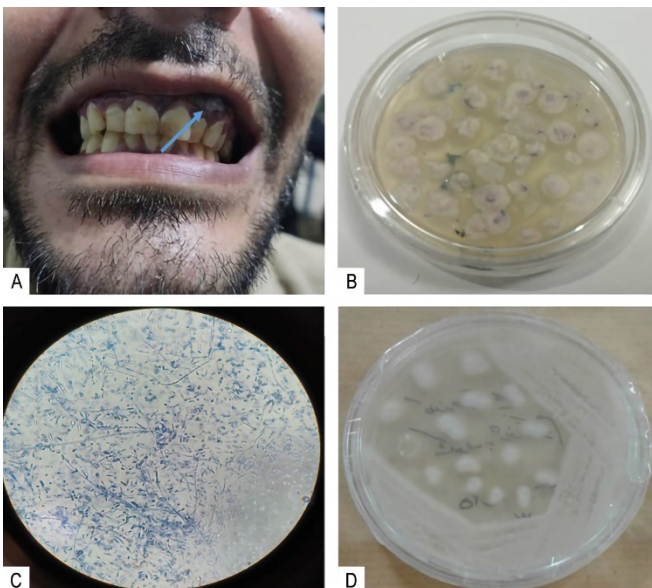
slight elevation of leucocytes to $14.8 \times 10^3/\mu\text{L}$ (reference value: $4.0 - 11 \times 10^3/\mu\text{L}$) with 64% lymphocytes, creatinine of 1.9 mg/dL (reference value: 0.7 - 1.4 mg/dL), and γ -glutamyl transferase (γ -GT) of 66 IU/L (reference value: 10 - 49 IU/L). The patient's neutrophil count was within normal limits.

Samples from the oral cavity of the mentioned case were collected using a sterile cotton swab, which was then seeded onto CHROMagar™ *Candida* plates (CHROMagar™, Pioneer, Paris, France) and incubated aerobically at 35 °C. Sample characterization was performed by examining the microscopic properties of pure cultures on CHROMagar™ medium after four days of incubation (Figure 1B). The isolate produced white/pink colonies with remarkable mycelial growth and arthroconidia (Figure 1C). The next step involved the molecular characterization of the sample; a single colony was subcultured on Saburo dextrose agar medium (SDA) (Lifoilchem, Roseto Degli Abruzzi, Italy) at 35 °C for 3 - 5 days (Figure 1D). Based on the patient's medical history, clinical examination, and microbiological analysis, it is probable that the infection is caused by *M. capitatus*. We employed molecular and sequencing methods to achieve an accurate diagnosis.

Genomic DNA was extracted using the phenol-chloroform/isoamyl alcohol method from the purified colony as previously described [8]. The identification of the isolate as *M. capitatus* was verified through PCR amplification, followed by DNA sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA. These gene regions were amplified with ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-2 (5'-GCATCGATGAAGAACGC AGC-3') primers [9]. The amplification of the DNA template was conducted under the following conditions: initial denaturation at 94 °C for 3 minutes, 30 cycles of 94 °C for 1 minute, 60 °C for 1 minute, and 72 °C for 1 minute, followed by a final extension at 72 °C for 3 minutes. The ITS fragments were partially sequenced via Sanger sequencing (Bioneer®, Seoul, Korea). The species were identified using the BLAST sequence analysis tool (<http://www.ncbi.nlm.nih.gov/BLAST/>). The isolate showed 100% identity with the sequence from reference *M. capitatus* strain sequences in GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>). The sequence has been deposited in the GenBank database with the accession number OQ184727.

The antifungal susceptibility of the *M. capitatus* isolate was assessed *in vitro* against nystatin, itraconazole, fluconazole, amphotericin B, and caspofungin using the microtiter broth dilution method,

Figure 1. A: white to gray discoloration and plaque-like ulcer on the front part of the supragingival; **B:** white and pink colonies of *M. capitatus* on CHROMagar™ *Candida* medium after 4 days of incubation; **C:** Microscopy of *M. capitatus* (hyphae and arthroconidia); **D:** white colonies of *M. capitatus* from a subculture in SC medium after 3 days of incubation.



in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (Approved Standard M27-A3) [10]. Table 2 summarizes the minimum inhibitory concentrations (MICs) of various antifungal agents. MIC values for nystatin, itraconazole, and amphotericin B against *M. capitatus* were lower than those of fluconazole and caspofungin (0.25 µg/mL, 0.032 µg/mL, and 0.125 µg/mL, 4 µg/mL and 2 µg/mL, respectively).

The patient responded to empiric nystatin therapy, and the ulcer healed after two weeks. Based on the *in vitro* susceptibility results and clinical response, administering nystatin 100,000 units/ml oral suspension four times a day was determined to be the most effective treatment.

Discussion

M. capitatus is one of the opportunistic fungal infections (OFIs), mainly affecting immunocompromised patients with malignancies [11].

Table 2. *In vitro* susceptibility of *M. capitatus* to five antifungal drugs according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Approved Standard M27-A3).

Antifungal agent	Minimum Inhibitory Concentration
Nystatin	0.25 µg/mL
Itraconazole	0.03 µg/mL
Amphotericin B	0.12 µg/mL
Fluconazole	4 µg/mL
Caspofungin	2 µg/mL

Infections caused by *M. capitatus* have been well documented in Mediterranean regions such as Spain, France, and Italy [12]. However, recent reports have documented cases of *M. capitatus* infection in countries where it is not typically found, including Slovakia, the Czech Republic, Switzerland, Kuwait, India, Nepal, China, and the United States [13]. Some reported predisposing factors for OFIs include diabetes, immunodeficiency syndrome, soft tissue injury, loss of natural defensive barriers, dryness and lack of saliva, artificial teeth, smoking cigarettes, and addiction. Addiction can make a patient vulnerable to invasion by

Table 1. Most common well-defined white diseases and disorders that may have a leukoplakic appearance and their main diagnostic criteria.

Differential Diagnosis	Evaluation Strategy
Candidiasis, pseudomembranous	Clinical aspect (pseudomembranous, often symmetrical pattern), microbiological analysis, histopathology, PCR
Alveolar ridge keratosis	Primarily a clinical diagnosis of a flat, white aspect of the mucosa of an edentulous part of the alveolar ridge; may overlap frictional keratosis
Frictional lesion	Presence of mechanical irritation (e.g., habit of vigorous toothbrushing)
Hairy Leukoplakia ("Greenspan lesion")	Clinical aspect (bilateral localization on the tongue), culture, histopathology, PCR
Oral Lichen Planus	Clinical aspect (often symmetrical pattern), histopathology
Oral Submucous Fibrosis	Clinical aspect, histopathology
Squamous Cell Carcinoma	Clinical aspect, histopathology
Verrucous Carcinoma	Clinical aspect, primarily histopathological entities
Papilloma and allied lesions	Clinical aspect, medical history, histopathology, HPV typing
Morsicatio	History of habitual chewing or biting, clinical aspect
Leukoedema	Clinical diagnosis (incl. symmetrical pattern) of a veil-like aspect of the buccal mucosa
Nicotine or Tobacco-Associated Lesions	Disappearance of the lesion within a period of 4-8 weeks after cessation of the tobacco habits (retrospective diagnosis only), clinical aspect, history of smoking
White Sponge Nevus	Family history, clinical aspect (often symmetrical pattern)
Lesion caused by a dental restoration (often amalgam)	Disappearance of the anatomically closely related (amalgam) restoration; clinical aspect (relation to dental restoration)
Cutaneous Lupus Erythematosus	History of skin lesion, clinical appearance (incl. bilateral pattern), histopathology
Skin graft, e.g., after a vestibuloplasty	History of a previous skin graft, clinical aspect
Syphilis, secondary ('mucous patches')	Clinical aspect, presence of <i>T. pallidum</i> , serology
Linea alba	Clinical aspect (located on the line of occlusion in the cheek mucosa; almost always bilateral)
Aspirin burn	History of prolonged local application of aspirin tablets (paracetamol may cause similar changes)
Glassblowers lesion	Occurs only in glassblowers (disappears within a few weeks after cessation of glassblowing)
Contact Stomatitis	Clinical aspect, patch testing
Chemical or Thermal Injury	Clinical aspect, history of exposure
Idiopathic Leukoplakia	Clinical aspect, histopathology
Snuff dippers' lesion	Clinical aspect, site where snuff is placed

opportunistic fungi [14,15]. Addictive drugs suppress the immune system, the body's innate defense against infections [16]. Immunosuppressed patients appear to be less resistant to *M. capitatus*.

Opiates are a class of drugs, including heroin, fentanyl, morphine, and many others derived from *Papaver somniferum* [17]. The mechanisms of immunomodulation induced by these drugs are mainly receptor-mediated, primarily via interaction with specific receptors on both humoral and cellular immune cells, or secondary, by reactions with similar receptors on nervous system cells [18]. Numerous *in vivo* and *in vitro* studies have confirmed the significant role of opiates in receptor-mediated suppression of phagocytosis, macrophages, cytokines, chemotaxis, and chemokines [18-20]. Prolonged neutropenia is one of the most common risk factors for *M. capitatus* infection [11]. The favorable outcome for eliminating *M. capitatus* depended on the host's immune system status and degree of neutropenia. According to the study conducted by Vagi *et al.* (2013), exposure to levamisole in patients who use illicit drugs, such as heroin and cocaine, can lead to unexplained neutropenia [21]. These effects modulate host resistance to bacterial, viral, fungal, and protozoan infections in addicted patients [20-22]. In our study, the patient's neutrophil count was within the normal range, in contrast to others. It should be noted that the use of heroin products has been linked to the development of neutropenia, although not all individuals who use heroin will necessarily experience this condition. Factors such as the severity and duration of heroin use, or following a healthy diet and lifestyle, can affect the immune system's response [23].

As mentioned above, the patient had a long history of amphetamine use. Substantial evidence suggests that amphetamine use can lead to vasoconstriction of blood vessels in the teeth and contribute to the development of oral health issues. A previous study has indicated that chronic vasoconstriction of the arteries supplying blood to the upper front teeth, caused by frequent snorting of methamphetamine (the N-methyl derivative of amphetamine), can reduce arterial blood supply to this area. In addition, the alpha-adrenergic receptors in the vasculature of salivary glands can undergo vasoconstriction, leading to reduced saliva secretion. A decrease in oral saliva can create an environment conducive to the growth of pathogenic microorganisms, including bacteria and fungi. Clinicians may potentially identify drug abuse by recognizing the symptoms of "meth mouth," characterized by severe tooth decay often associated with methamphetamine use [24,25].

Mortality from emerging OFIs in immunocompromised patients remains alarmingly high due to improper identification of the causative agent or delayed diagnosis. Conventional methods for identifying *M. capitatus* in clinical samples have not consistently produced accurate results. For example, *Saprochaete clavata* is often mistaken with *M. capitatus* due to their close relation and the difficulty in distinguishing them based on macroscopic and microscopic features [26]. Consequently, molecular methods are employed to identify rare fungal infections. Examining specimens through DNA sequencing of the internal transcribed spacer region of the rRNA gene can help accurately differentiate *M. capitatus* from other strains of OFIs.

Recently, certain antifungal-resistant fungal species, such as *M. capitatus*, have emerged as causative agents of OFIs [26]. Inadequate treatment of OFIs can lead to bloodstream infections, disseminated multi-organ involvement, and invasive fungal infections. Consequently, precise and prompt diagnosis, as well as treatment, emerge as pivotal challenges [27]. As susceptibility breakpoints have not yet been established for *M. capitatus* [28], we employed breakpoints recommended by the CLSI M27-A3 document for other related yeast species to overcome this limitation. Based on the *in vitro* susceptibility results, the highest MICs were observed for fluconazole and caspofungin. These findings align with earlier research by Kaplan *et al.* (2017), which reported an *M. capitatus* isolate with high MICs for fluconazole and micafungin [29]. Furthermore, our study suggests that *M. capitatus* had the lowest MICs with nystatin, itraconazole, and amphotericin B, indicating the potential efficacy of these antifungal agents against this fungal species.

Sancak *et al.* (2009) demonstrated the effects of itraconazole and voriconazole on *M. capitatus* isolated from clinical specimens, although the *in vitro* activity of fluconazole was found to be limited [30]. To date, there are no therapeutic guidelines available for the treatment of *M. capitatus* infections. According to the *in vitro* susceptibility results, we administered nystatin 100,000 units/mL oral suspension to manage the infection, and recovery was achieved after two weeks.

One limitation of our study is the lack of a histopathology test to confirm the diagnosis of oral *Magnusiomyces* infection. Nevertheless, we employed a combination of other diagnostic methods, such as medical history review, physical examination, microbiological analysis, molecular methods, and clinical response to antifungal therapy, to reach an

accurate diagnosis. Future studies should endeavor to incorporate histopathology testing to confirm the diagnosis of oral fungal infections and enhance diagnostic accuracy.

In conclusion, this study represents the first reported case in Iran of OFIs caused by *M. capitatus* in an addicted patient. The findings of this study highlight the potential role of risk factors such as addiction in the emergence of *M. capitatus* infection, which should serve as a warning for physicians and infectious disease specialists. Furthermore, this report emphasizes the impact of ITS region-based molecular techniques on clinical specimens for accurately diagnosing *M. capitatus*. The patient exhibited a favorable response to nystatin, which supports its use as a treatment recommendation for *M. capitatus*. It is important to acknowledge that untreated mucosal candidiasis can lead to systemic infection. Given that the clinical manifestations of systemic candidiasis are non-specific, early diagnosis of infection during the initial stages of mucosal colonization will effectively prevent infection.

Acknowledgements

We would like to thank the Cellular and Molecular Research Center, Medical Basic Sciences Research Institute, Ahvaz, Jundishapur University of Medical Sciences for the support. (Ethical code: IR. AJUMS.MEDICINE.REC.1400.047).

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Conflict of interests: No conflict of interests is declared.