First Isolation of *Candida nivariensis*, an Emerging Fungal Pathogen, in Kuwait

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Highlights of the Study

- *Candida nivariensis* is an emerging fungal pathogen which is often misidentified by conventional phenotypic methods.
- Molecular testing or MALDI-TOF MS with updated software is required to accurately detect *C. nivariensis*.
- The first clinical *C. nivariensis* isolate from Kuwait exhibited high MIC against fluconazole, a commonly used antifungal drug.
- Accurate identification and antifungal susceptibility testing are essential for the treatment of infections caused by rare yeast pathogens.

Keywords

Candida nivariensis · Rare yeasts · Matrix-assisted laser desorption ionization-time of flight mass spectrometry · Antifungal resistance · Kuwait

Abstract

Objective: *C. nivariensis* is a rare *Candida* species which is phenotypically closely related to *Candida glabrata* and *Candida bracarenensis*. The 3 species form the *C. glabrata* sensu lato complex. Here, we describe the first isolation and characterization of a *C. nivariensis* isolate cultured from the tracheal aspirate obtained from a young man in Kuwait. **Materials and Methods:** The yeast isolate was initially tested by VITEK 2 followed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and multiplex PCR. The identification was confirmed by sequencing of internal transcribed spacer (ITS) region of rDNA. Antifungal susceptibility testing was performed by Etest, and phylo-

genetic comparison with other international strains was carried out by using MEGA version 7 software. **Results:** The *C. nivariensis* isolate was misidentified by VITEK 2, but correctly identified by MALDI-TOF MS with updated software and multiplex PCR. The identity was confirmed by sequence comparisons of ITS region of rDNA. Antifungal susceptibility testing revealed high minimum inhibitory concentration (MIC) against fluconazole, but low MICs against amphotericin B and echinocandins. Phylogenetically, our isolate was closely related to Indian isolates. **Conclusions:** This report extends the geographic distribution of *C. nivariensis* to the Arabian Peninsula. MALDI-TOF MS with updated software and molecular tests are needed to correctly identify *C. nivariensis*. Since *C. nivariensis* may exhibit reduced susceptibility to antifungal agents, accurate identification and antifungal susceptibility testing are essential, particularly for isolates from sterile sites, for optimal patient management.

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Introduction

Candida nivariensis is a rare *Candida* species which belongs to the *Nakaseomyces* clade. Phylogenetically, it is closely related to *Candida glabrata*, *Candida bracarensis*, and nonpathogenic *Nakaseomyces delphensis* [1]. *C. nivariensis* was first described in Spain in 2005. It was isolated from 3 different clinical samples during the period 1999–2002 [2]. Subsequently, this species was identified among clinical isolates of *C. glabrata* sensu lato, which includes *C. glabrata* sensu stricto, *C. nivariensis*, and *C. bracarensis*, mostly by the application of molecular methods in various countries across the world including Japan, UK, USA, Indonesia, Malaysia, India, China, Australia, Italy, Poland, Iran, Egypt, Brazil, and Argentina [3–15]. *C. nivariensis* has been implicated in different types of infections including invasive infections such as candidemia, peritonitis, and pelvic abscess [4, 8, 12]. It has also been implicated in mucocutaneous infections such as vulvovaginal candidiasis and oropharyngeal candidiasis [5, 16]. Accurate identification of *C. nivariensis* by commonly used phenotypic methods in routine clinical microbiology laboratories is difficult as it is usually misidentified [2]. *C. nivariensis* strains isolated from clinical specimens often exhibit reduced susceptibility to commonly used antifungal drugs [2–4]. Here, we describe the first isolation and characterization of *C. nivariensis* from a clinical specimen indicating the emergence of this species in Kuwait. We also briefly discuss the laboratory diagnostic methods for accurate species-specific identification and compare antifungal drug susceptibility patterns among clinical *C. nivariensis* isolates from other geographical locations within the Middle East and India.

Materials and Methods

Case Description

A 35-year-old Kuwaiti male was brought to the Medical Casualty Department of Mubarak Al-Kabir Hospital because of generalized tonic-clonic convulsions, vomiting, and aspiration. He was previously diagnosed with epilepsy but was noncompliant with his medication. On arrival, the patient received diazepam, was intubated, and shifted to intensive care unit (ICU). During his ICU stay, blood, tracheal aspirate, and urine samples were collected for culture. The patient was treated with ceftriaxone, which was later switched to meropenem and vancomycin. The tracheal aspirate culture grew a yeast, while other cultures were negative. Five days later, the patient became afebrile, was extubated, and his antibiotic therapy was discontinued. He did not receive any antifungal therapy.

Mycological Workup and Antifungal Susceptibility Testing

The yeast isolate was subcultured on Sabouraud dextrose agar and CHROMagar chromogenic media (CHROMagar, Paris, France) according to the manufacturer's instructions and as described previously [17]. VITEK 2 and VITEK MS (bioMérieux) were used for species identification, and antifungal susceptibility testing was performed by Etest (bioMérieux) according to the manufacturer's instructions and as described previously [18, 19].

PCR and DNA Sequencing

To confirm the species identification, molecular testing was performed. Genomic DNA was extracted from the isolate by the rapid boiling method using Chelex 100, as described previously [20]. Subsequently, multiplex PCR (mPCR) was performed using mCGLF, mCNIF, mCBRF, and mCGCR primers which yields amplicons of ~360 bp, ~288 bp, and ~299 bp from *C. glabrata* sensu stricto, *C. nivariensis*, and *C. bracarensis*, respectively [21]. PCR amplification results were confirmed by DNA sequencing of the internal transcribed spacer (ITS) region of rDNA using panfungal primers, as described previously [22].

Phylogenetic Comparisons

The genotypic relationship of our isolate with some other previously described strains was analyzed. The ITS region sequence was compared with the corresponding sequences from 13 *C. nivariensis* isolates retrieved from GenBank. Pairwise comparisons and multiple sequence alignments were performed with MUSCLE (<https://www.ebi.ac.uk/Tools/msa/muscle/>) [23]. Phylogenetic tree was constructed by employing the MEGA (<https://www.megasoftware.net/>) software version 7 [24] using the neighbor-joining method, and the robustness of tree branches was assessed by bootstrap analysis with 1,000 replicates, as described previously [20].

Results

The yeast isolate (Kw261-1/20) produced creamy colored colonies on CHROMagar medium. VITEK 2 and VITEK MS with updated database identified the isolate as *Candida magnoliae* with a score of 97% and *C. nivariensis* with a score of 99.9%, respectively. The mPCR amplification yielded an amplicon of ~288 bp which is a characteristic of *C. nivariensis*. Furthermore, basic local alignment search tool (BLAST) searches (<http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi>) [25] of DNA sequence data for the ITS region of our isolate showed >99% identity with reference strains of *C. nivariensis* (CBS 9983 and CBS 10161 corresponding to GenBank Accession No. MH545923 and GU199441, respectively). Based on the previous observations that fungal strains belonging to the same species exhibit >99% nucleotide identity in the ITS region of rDNA [26], the molecular identity of our isolate (Kw261-1/20) was established as *C. nivariensis*. Multiple sequence alignment was carried out to compare the genotypic rela-

Fig. 1. Neighbor-joining phylogenetic tree based on sequence data for the ITS region of rDNA for Kw261-1/20 (GenBank Accession No. LR792666) together with 13 other *C. nivariensis* isolates. The GenBank Accession No./Isolate No. and the country of isolation/Strain No. for reference strains are shown. The countries are abbreviated as AR, Argentina; CN, China; IN, India; IR, Iran; MY, Malaysia; SP, Spain; and USA, United States of America. The bootstrap frequencies (>50%) on the node branches are also shown. *CBS9983, Iran; CBS9985, Iran; CBS10161, Indonesia. ITS, internal transcribed spacer.

tionship of our isolate with 13 other *C. nivariensis* strains isolated at diverse geographical locations. Our isolate was genetically more closely related to the isolates from India but was different from those from Iran, Spain, USA, Argentina, Malaysia, and China (Fig. 1). The DNA sequencing data for the isolate (Kw261-1/20) have been submitted to GenBank/EMBL/DDJB under Accession No. LR792666.

Discussion

The incidence of opportunistic infections in humans by rare yeasts has increased in recent years [4, 9, 17, 19, 20]. These infections are difficult to diagnose and treat due to the large number and broad diversity of yeast species and resistance to one or several antifungal drugs [9, 17, 27]. Rapid and accurate detection of the infecting agent and its drug susceptibility profile are thus crucial for proper patient management [27]. *C. nivariensis* is a rare yeast which has a global distribution [21]. It has been implicated in both invasive infections such as candidemia, peritonitis, and pelvic abscess as well as mucocutaneous infections such as vulvovaginal candidiasis and oropharyngeal candidiasis [4, 5, 8, 12, 16, 28]. Although a large collection of phenotypically identified *C. glabrata* sensu lato isolates were previously screened by molecular methods in a recent study from Kuwait, *C. nivariensis* was not detected [21]. This study now describes the first isola-

tion of *C. nivariensis* from a clinical specimen in Kuwait. The isolate was recovered from a nonsterile site, and the patient's condition improved without any antifungal treatment suggesting mere colonization of the patient with *C. nivariensis*. The origin of *C. nivariensis* in Kuwait remained uncertain as the travel history of the patient was not available. The present report extends the geographic distribution of this emerging fungal pathogen to the Arabian Peninsula as no similar cases have previously been reported from other countries in this region.

Although *C. nivariensis* was discovered nearly 15 years ago [2], the number of total cases is still relatively small. The true prevalence, however, might be underestimated because the biochemical testing methods currently in use in most clinical laboratories fail to identify rare yeast species, including *C. nivariensis*, accurately [27]. *C. nivariensis* shares many phenotypic characteristics with *C. glabrata* including the inability to form germ tube, pseudohyphae, chlamydospore, or ascospores. However, in chromogenic CHROMagar *Candida*, the colonies of *C. glabrata* are purple, while *C. nivariensis* and *C. bracarenensis* form white/cream-colored colonies [2]. Furthermore, in commercial tests based on biochemical utilization of different compounds such as AuxaColor2 and API 20C systems, *C. nivariensis* assimilates only glucose among carbohydrates in contrast to *C. glabrata* which can assimilate glucose and trehalose [4].

Currently, the method of choice for detecting *C. nivariensis* among yeast isolates is application of PCR-based

Table 1. Comparison of antifungal susceptibility patterns of *C. nivariensis* isolates from Kuwait, other Middle Eastern countries, and India*

Country	Isolates, n	Amphotericin B	Fluconazole	Itraconazole	Voriconazole	Caspofungin	Anidulafungin	Micafungin	References
India	2	0.5	1–2	0.25	0.03	–	–	–	[7]
India	4	0.03–0.125	0.5–16	0.03–0.5	0.03–0.5	0.25–0.5	0.06–0.125	0.015	[16]
Iran	4	0.5–1	0.06–1	≤0.016–0.06	≤0.016–0.03	–	≤0.016	≤0.016	[12]
Egypt	3	0.25–1	16	0.5	–	–	–	–	[13]
Kuwait	1	0.25	6	1	0.125	0.19	0.032	0.006	Present study

* The isolate from Kuwait was tested by Etest, while all other isolates were tested by the broth microdilution method.

methods [21, 29–31] or PCR sequencing of the ITS and/or D1/D2 domains of rDNA [21, 26]. Besides PCR-based methods, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) with an updated database has also been shown to accurately detect *C. nivariensis* [12, 32]. In this study, *C. nivariensis* was accurately detected by VITEK MS since the database was updated recently.

C. nivariensis isolates exhibit variable susceptibility to azole antifungal agents. In the case report from Japan, the isolate was resistant to fluconazole and voriconazole with minimum inhibitory concentrations (MICs) of ≥128 and 4 µg/mL, respectively [3]. Borman et al. [4] also reported similar findings from UK with MIC₉₀ of >64 and 4 µg/mL for fluconazole and voriconazole, respectively. However, a study from China reported lower MICs against fluconazole (MIC₉₀: 2 µg/mL) and voriconazole (MIC₉₀: 0.06 µg/mL) but elevated amphotericin B MIC₉₀ of 2 µg/mL among 12 *C. nivariensis* isolates [8].

A recent study from UK used the CLSI method and showed that resistance rates against fluconazole, itraconazole, voriconazole, and posaconazole were detected in 30, 14.8, 51.4, and 7.7% of isolates, respectively. However, no resistance was detected against amphotericin B or anidulafungin [33]. Our isolate also exhibited high MIC against fluconazole (MIC of 6 µg/mL). The antifungal susceptibility profile of our isolate was compared with *C. nivariensis* isolates from other Middle Eastern countries and India, and the data are presented in Table 1. While the 3 isolates tested from Egypt were resistant to fluconazole with an MIC of 16 µg/mL [13], the Iranian isolates were susceptible to fluconazole (MIC of 0.0625–1 µg/mL) and voriconazole (MIC of ≤0.016–0.03 µg/mL) [12]. The isolates from India [7, 16] exhibited variable patterns (MIC of 0.5–16 µg/mL) with some isolate exhibiting susceptibility while others showing resistance to fluconazole (Table 1).

Conclusion

C. nivariensis is an emerging opportunistic pathogen that can cause invasive infections and can be resistant to azoles. The true prevalence is still unclear, but seems to be underestimated. The inability of phenotypic methods to accurately identify such potential yeast pathogens clearly indicates the importance of molecular testing including MALDI-TOF MS in the diagnosis of this emerging species. Antifungal susceptibility testing of rare yeast species is also essential for proper patient management and epidemiological surveillance.

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Statement of Ethics

Specific ethical approval for this study was not required as the isolate was sent to the Mycology Laboratory and tested as a routine laboratory service. The data are reported on a deidentified sample, the privacy of the patient was respected, and confidentiality was maintained.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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