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ORIGINAL RESEARCH

First Report of *Cystobasidium slooffiae* in Human Wounds from China: Molecular Identification and Clinical Insights

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Background: *Cystobasidium* spp. are rare yeasts recently recognized as emerging human pathogens. This study presents the first report from China of *Cystobasidium slooffiae* isolated from human wound infections, and characterizes its microbiological profile, phylogenetic identity, and antifungal susceptibility.

Methods: Two strains were isolated from skin wounds of immunocompromised patients. They were characterized based on colony morphology on Sabouraud dextrose agar, Gram staining, MALDI-TOF MS analysis, Erg11 amino acid sequences analysis, and phylogenetic analysis using combined sequences of 18S rDNA, D1/D2 domains, and ITS regions. Antifungal susceptibility testing was performed according to CLSI guidelines (M27-A3/M60).

Results: The colonies transitioned from light yellow to orange within 48–96 h with Gram-positive budding cells. MALDI-TOF MS failed to identify accurately any of these strains. However, phylogenetic analysis of ITS confirmed that both strains were *C. slooffiae*. Both strains exhibited high minimum inhibitory concentrations (MICs) for all three echinocandins (>8 μg/mL) and fluconazole (32–64 μg/mL), whereas the MICs for isavuconazole were in the range 0.75–1 μg/mL. Erg11 sequence analysis revealed they formed a distinct clade that was genetically distant from Rhodotorulaceae.

Conclusion: Our findings showed that *C. slooffiae* could be an important emerging, opportunistic human invasive fungal pathogen because of its reduced susceptibility to echinocandins and fluconazole.

Keywords: *Cystobasidium slooffiae*, skin wounds, superficial infections, *Rhodotorula*

Introduction

The global prevalence of superficial fungal infections (SFIs) ranges from 20 to 25%.[1,2] Superficial fungal infections are defined as infections in which a pathogen is restricted to the stratum corneum with little or no tissue reaction.[3] The Basidiomycota phylum is predominant in the skin system, and *Cryptococcus* spp. have been commonly reported.[4] However, the current view is that SFI encompasses mucosal infections caused by fungi that can lead to chronic recurrent infections and the emergence of new drug resistance.[1]

Cystobasidium slooffiae, previously classified under the genus *Rhodotorula* due to its ability to synthesize and accumulate carotenoid pigments within its cells, endows the yeast colonies with colors ranging from coral to orange, pink, or red.[5] These pigments provide certain advantages to cells, such as resistance to various environmental stresses (including ultraviolet radiation).[5] Several clinically relevant red-pigmented *Rhodotorula* spp. can cause opportunistic infections such as meningitis, endocarditis, fungemia, central venous catheter infections, keratitis,[6] and non-healing oral

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ulcers.[7] Since 2011, this has been reclassified and transferred to the class Cystobasidiomycetes within the order Cystobasidiales, family Cystobasidiaceae, and genus *Cystobasidium*.[3] To date, 22 species of *Cystobasidium* have been identified and described, including *C. fimetarium, C. minutum, C. slooffiae, C. calyptogenae, C. pinicola, C. laryngis, C. benthicum, C. pallidum, C. lysinophilum, C. portillonense, C. oligophagum, C. alpinum, C. psychroaquaticum, C. ritchiei, C. tubakii, C. ongulense, C. keelungense*,[3,8–11] *C. halotolerans*,[12] *C. iriomotense, C. onofrii*,[13] *C. raffinophilum*, and *C. terricola*.[14] *Cystobasidium* yeasts commonly occur in natural environments and are associated with various habitats and ecosystems such as ice sediments,[9] aquatic environments,[8] soil,[10] and leaf surfaces,[3] indicating their strong adaptability to diverse environments.

Owing to the increasing number of patients with weakened immune systems after transplantations, surgeries, or severe conditions in the last decade, there is an increase in infections by opportunistic or rare yeast species.[15] These pathogenic rare yeasts encompass genera from both Basidiomycota (*Saprochaete, Magnusiomyces, Trichosporon, Malassezia, Rhodotorula, Sporobolomyces*) and Ascomycota (*Kodamaea, Saccharomyces, Moesziomyces*).[16] However, reports of human infections caused by *Cystobasidium* are rare, and its clinical characteristics and antifungal susceptibility profile are poorly understood. Therefore, this study aimed to present the first documented cases of *C. slooffiae* isolated from human wound infections in China. We characterized these clinical isolates through conventional microbiological methods, molecular phylogenetic analysis (based on 18S rDNA, ITS, and D1/D2 regions), and comprehensive antifungal susceptibility testing. Our findings aim to improve the understanding of this emerging pathogen's microbiological profile and to provide antifungal susceptibility data to guide effective therapeutic strategies.

Materials and Methods

Cultivation and Microscopic Examination of Fungi

Two clinical *Cystobasidium* isolates (Z30790 and Z30215) were isolated from wound secretions of the patients. Clinical isolates were inoculated onto Sabouraud dextrose agar (SDA) medium and incubated at 35 °C for approximately 96 h. Changes in the morphology, texture, and color of the colony surface were observed daily. A single colony was selected for smearing, and its morphological characteristics were observed under a microscope with oil immersion after Gram staining. The cultures were stored at –80 °C. This study was approved by the Human Research Ethics Committee of Peking Union Medical College Hospital (no. S-263).

Identification by MALDI-TOF MS and Molecular Methods

Two matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI TOF MS) systems were utilized: the MALDI Biotyper (Bruker) and VITEK MS IVD (bioMérieux), following the manufacturers' instructions. Spectra analysis was performed using the MALDI Biotyper database library V.3.3.1.2 and the VITEK® MS IVD v2.0 database, respectively.

Genomic DNA of *C. slooffiae* was extracted using the Fungi Genomic DNA Extraction Kit (Solarbio Science & Technology, Beijing, China) according to the manufacturer's recommended protocols.[17] For molecular identification, three rDNA region sequences were analyzed: (i) 18S rDNA, (ii) D1/D2 domain of 26S rDNA, and (iii) rDNA internal transcribed spacer (ITS) regions. Primer sets and PCR conditions were used as previously described,[18–20] and DNA sequencing was performed on the ABI 3730xl DNA Analyzer platform (Applied Biosystems, USA). The sequences were searched against the NCBI database using the Basic Local Alignment Search Tool for Nucleotides (BLASTn, http://blast.ncbi.nlm.nih.gov) for species identification.[21]

Molecular evolutionary genetic analysis software (MEGA, version 11.0)[22] was used for alignment and to remove gaps from the dataset using the neighbor-joining (NJ) method. The statistical support of the cluster nodes was estimated using 1000 randomized bootstrappings. Strains of *C. slooffiae* and related strains from the genus *Cystobasidium* were selected from those publicly available before December 13, 2024 (n = 20) in GenBank databases (https://www.ncbi.nlm.nih.gov/genbank). The ITS sequences of the type species of *Rhodotorula mucilaginosa* (GenBank accession number NR_073296) was used as the out-group.[23]

Antimicrobial Susceptibility Testing

The susceptibility to nine antifungal agents was assessed using the broth microdilution method with Sensititre YeastOne™ YO10 panels (Thermo Scientific, Cleveland, OH, USA). Colonies were suspended in sterile saline, and the turbidity was adjusted to a 0.5 McFarland standard. This suspension was then further diluted according to the manufacturer's instructions to achieve a final inoculum concentration of approximately 1.5×10^3 to 8×10^3 CFU/mL in the test wells. The nine agents included in the panel were three echinocandins (caspofungin, micafungin, and anidulafungin), four azoles (fluconazole, voriconazole, itraconazole, and posaconazole), amphotericin B, and 5-fluorocytosine. Additionally, susceptibility to isavuconazole was determined independently using E-test strips (Liofilchem, Roseto Degli Abruzzi, Italy) following the manufacturer's instructions. *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used for quality control, and their MIC results were within the published reference ranges. Due to the slow growth of *C. slooffiae*, all antifungal susceptibility tests were recorded after 72 h incubation to obtain accurate results.[24] As clinical breakpoints and epidemiological cutoff values have not yet been established for *C. slooffiae*, the interpretation criteria for *Candida* spp. outlined in the CLSI M27[25] guidelines were applied in this study. Specifically, an MIC value of ≥32 μg/mL was interpreted as resistance to both fluconazole and 5-flucytosine. Additionally, an MIC ≥ 2μg/mL was used to define resistance to amphotericin B.[26]

Sequence Analysis of Erg11

To study the sequence variability of Erg11, which is the drug target of azoles, Erg11 amino acid sequences were extracted from the gene prediction files of in-house sequenced *Rhodosporidiobolus* genome.[17] Additional Erg11 amino sequences from different Basidiomycetous yeast species were retrieved from the NCBI and FungiDB databases < https://fungidb.org/fungidb/app>. The Erg11 sequence of *Cryptococcus neoformans* (accession number: WLK77786.1) was included as an outgroup. The optimal amino acid substitution model was determined using IQtree v2.1.2[27] and then employed to carry out a maximum-likelihood phylogenetic analysis on MEGA 11 with 1000 bootstrap replicates.

Nucleotide Sequence Accession Numbers

The 18S rDNA, D1/D2 domain, and ITS region sequences of strains Z30790 (from patient 1) and Z30215 (from patient 2) were deposited in GenBank. The sequence accession numbers are PQ805459, PQ805460, and PQ805461for strain Z30790; and PQ805456, PQ805457, and PQ805458 for strain Z30215.

Results

Case Presentation

Patient 1 (Z30790), a 15-year-old male patient, was injured in a car accident in May 2021 and underwent symphysis pubis separation. On November 18, 2021, the patient was admitted to the Intensive Care Unit of Peking Union Medical College Hospital because of wound infection, which led to a sinus tract and aggravated bleeding. CT tomography revealed rupture of the right iliac artery pseudoaneurysm. After admission, the patient underwent surgery for a vascular occlusion. On November 30, 2021, a fungal culture of sinus tract secretions was conducted, which revealed pink colonies on chromogenic medium. Despite the unsuccessful matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) test, the organism was confirmed to be *C. slooffiae* by rDNA sequencing. The erythrocyte sedimentation rate (ESR) was 27 mm/h and the high-sensitivity C-reactive protein (hsCRP) concentration was 63.27 mg/L. Treatment included wound debridement, negative-pressure suction, and antibiotics such as tigecycline, meropenem, and amikacin sulfate. The wound healed on January 28, 2022 (Table 1).

Patient 2 (Z30215), a 67-year-old male patient, was diagnosed with connective tissue disease and diabetes at Peking Union Medical College Hospital on October 29, 2019. After receiving methylprednisolone pulse therapy and cyclophosphamide treatment, the patient's symptoms were significantly relieved, but he did not continue the medication regularly. The patient was re-admitted to the hospital on November 15, 2021. The patient had been experiencing intermittent joint pain for six months, and multiple painful red nodules and non-healing ulcers had newly emerged on his calves and feet two weeks previously. The nodules were punctured independently and treated with high-dose glucocorticoids and

naproxen. His symptoms slightly improved; however, his feet remained red, swollen, hot, and painful. On November 17, 2021, the fungal culture of wound secretions was morphologically identified as *Rhodotorula*. However, MALDI-TOF MS analysis reported "no identification". The 1,3-β-D-glucan test results were negative. Subsequently, it was identified as *C. slooffiae* using rDNA sequencing. After discharge from the hospital, treatment with glucocorticoids, ciprofloxacin, and sulfamethoxazole tablets was continued. By January 20, 2022, the ulcers had still not healed but the symptoms had improved (Table 1).

Colony Morphology and Microscopic Characteristics

The colony morphologies of Z30215 and Z30790 in SDA medium were largely identical. Within 48 h, light-yellow colonies that were visible to the naked eye were formed, and after 96 h distinct orange colonies were formed. The colonies exhibited a spherical shape characterized by a central elevation, neatly trimmed edges, moist and non-transparent consistency, and a lustrous surface (Figure 1a–d). Upon Gram staining, the organisms displayed a positive reaction, with morphologies ranging from spherical to oval. They can exist either singly, in pairs, or arranged in chains, demonstrating budding reproduction (Figure 1e and f).

Identification Results of Fungal 18S rDNA, D1/D2 and ITS Region Sequences

The 18S rDNA, D1/D2, and ITS regions were amplified from Z30215 and Z30790, respectively. The amplicon sizes were approximately 880 bp, 600 bp, and 560 bp, respectively. Consensus sequences were subjected to BLAST comparison in the GenBank database, and a phylogenetic tree was constructed to determine the genetic relationship between strains Z30215 and Z30790 and other known fungi. The two clinical strains had identical 18S rDNA, D1/D2, and internal transcribed spacer (ITS) sequences. The phylogenetic tree showed that strains Z30790 and Z30215 clustered with other *C. slooffiae* strains from Taiwan, China, Japan, and Argentina (Figure 2). The tree also revealed that these two *C. slooffiae* isolates differed from other species within *Cystobasidium*. Despite small sample size and the lack of polymorphism in ITS region, *C. slooffiae* strains may not be differentiated from their geographic origin or source of isolation (Figure 2).

Table 1 Clinical Features of Two Patients Infected with *C. slooffiae*

Clinical Feature	Patient 1	Patient 2
Isolate ID number	Z30790	Z30215
Age	15	67
Gender	Male	Male
Reason for hospital admission	Rupture and bleeding of the right iliac artery pseudoaneurysm	Thromboangiitis obliterans
Underlying disease	Multiple traumas after a car accident; Wound infection	Diabetes; Wound infection
First isolation of *C. slooffiae* (date[a])	6/12/2021 (19)	17/11/2021 (3)
Clinical status at time of positive culture		
Immunosuppressive state	Yes	Yes
Neutropenia ($<10^9$/L)	No	No
Broad-spectrum antibiotics	No	Yes
Glucocorticoid	No	Yes
Parenteral nutrition	No	No
Surgery within 30 days	Yes	No
Intensive care	Yes	No
Previous antifungal agents within 30 days	No	No
Therapy		
Antifungal therapy after culture	Yes	Yes
Outcome of patient	Cured	Get better

Note: [a]Numbers in parentheses indicate the days from the beginning of hospitalization.

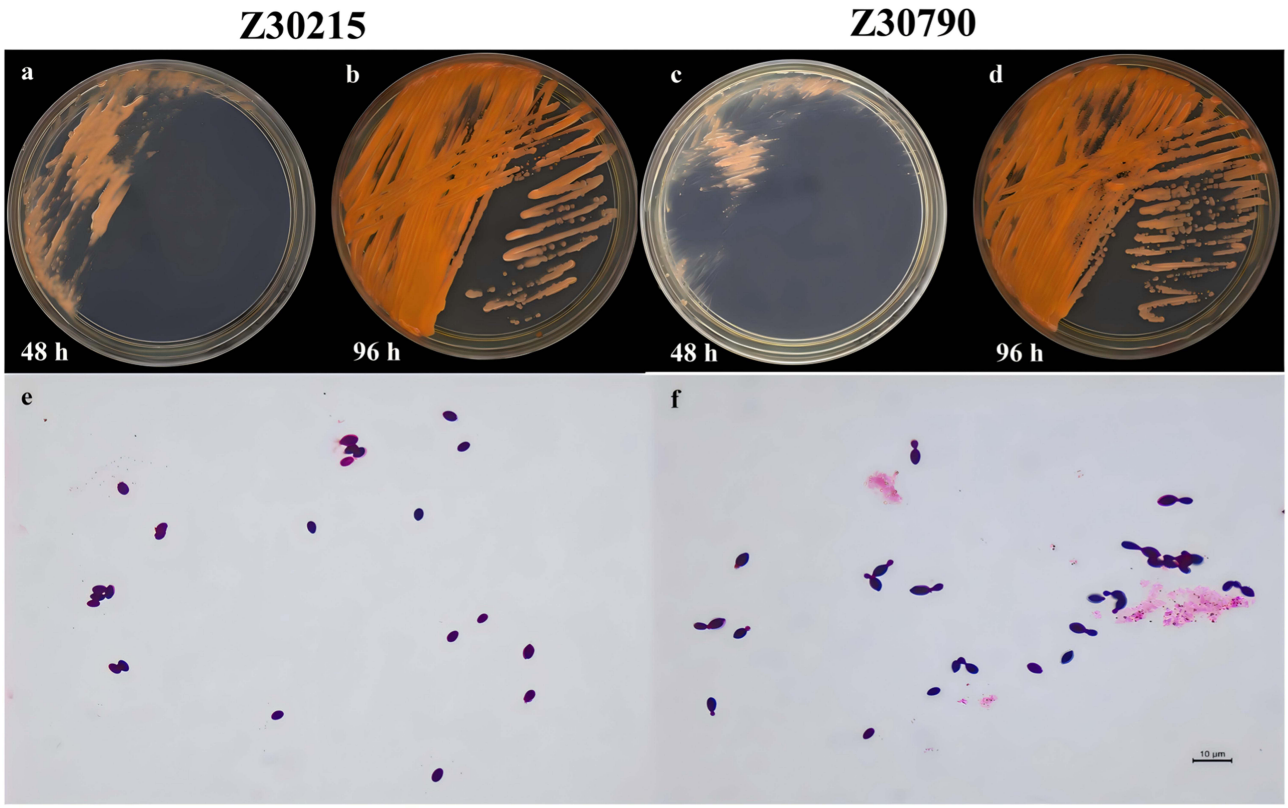

Figure 1 Colonial and microscopic morphology of *C. slooffiae* isolates. (**a-d**) Colonial morphology on SDA after 96 hours of incubation at 35 °C, showing Orange, spherical colonies with a raised center, entire edge, and a glossy surface. (**e** and **f**) Gram staining of the isolates reveals Gram-positive, oval to spherical yeast cells, which occur singly, in pairs, or in short chains. Budding reproduction is evident (black arrow in panel f). Scale bar = 10 μm (**f**).

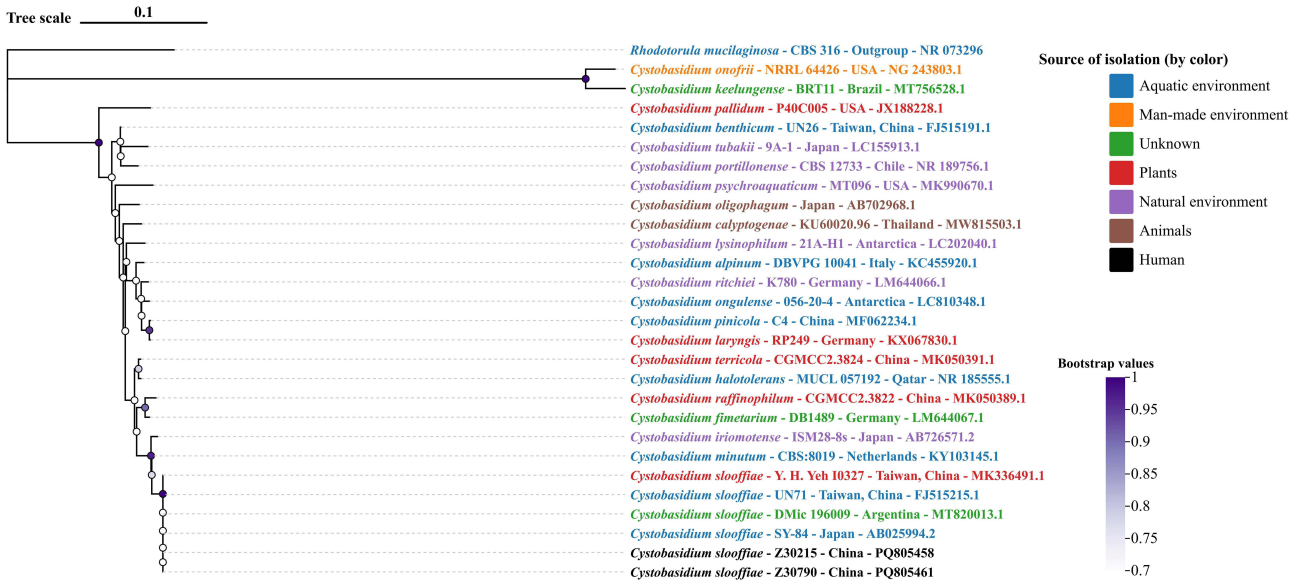

Figure 2 Phylogenetic analysis of Z30215 and Z30790 with related *Cystobasidium* species based on sequences of the ITS region. The taxa were colored according to the source of isolation.

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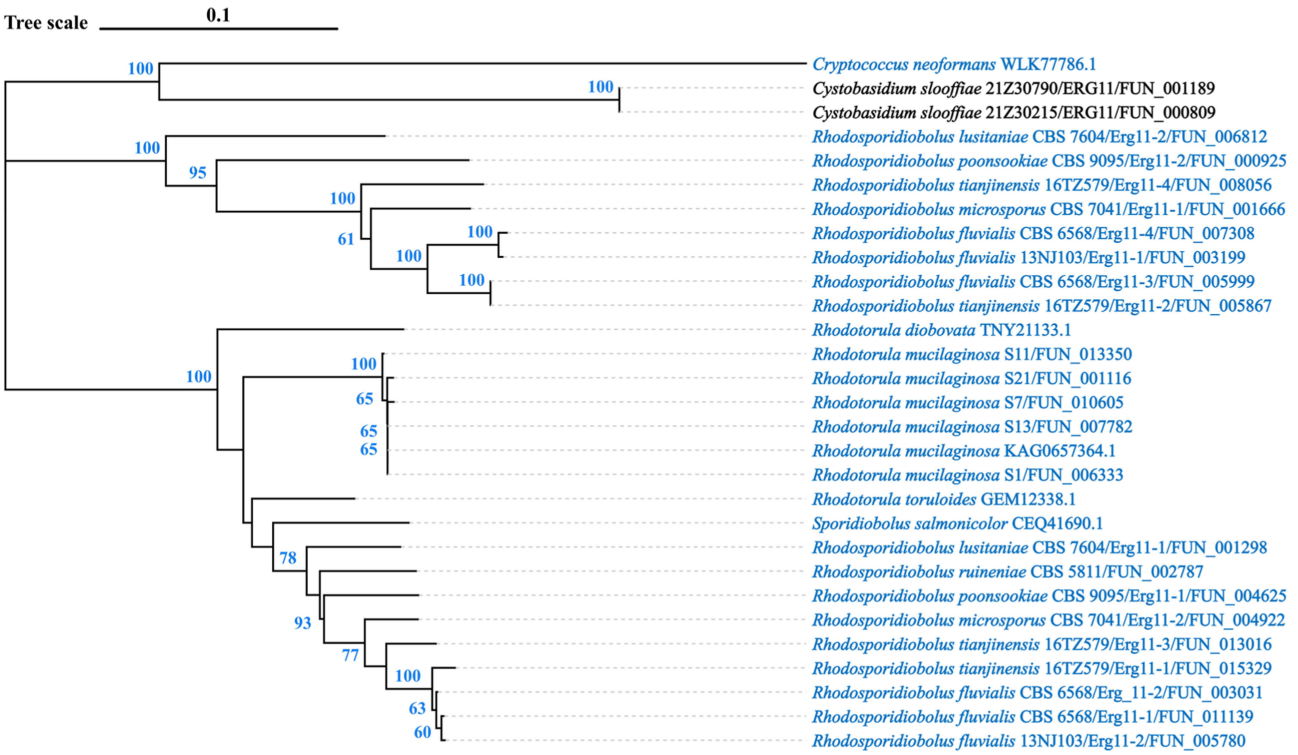

Figure 3 Phylogenetic relationship of selected basidiomycetous yeasts using Erg11 amino acid sequences.

Phylogenetic Analysis Based on the Amino Acid Sequences of Erg11

During sequence analysis of Erg11 (Figure 3), *C. slooffiae* formed a highly supported independent clade (bootstrap value ≥90%) that did not cluster with the major branches of *Rhodosporidiobolus* or *Rhodotorula* (the representative genera of Sporidiobolaceae).

Antimicrobial Susceptibility Testing

Table 2 shows that the strains Z30215 and Z30790 in this study exhibited high MICs for echinocandins (MIC > 8 μg/mL) and fluconazole (MICs of 128 and 64 μg/mL, respectively). In contrast, these strains demonstrated low MICs for itraconazole (MIC 0.12 μg/mL), voriconazole (MIC 0.5 μg/mL), posaconazole (MIC 0.25 μg/mL), and isavuconazole (MICs of 1 and 0.75 μg/mL, respectively). The MICs for 5-flucytosine and amphotericin B were <0.06 μg/mL and 0.5 μg/mL, respectively.

Table 2 The Susceptibility of the Z30215 and Z30790 Strains to Antifungal Agents

Antifungal Susceptibility (μg/mL)	Z30215	Z30790
Azoles		
FLC	128	64
VOR	0.5	0.5
ITCZ	0.12	0.12
POS	0.25	0.25
ISA	1	0.75
Flucytosine		
5-FC	< 0.06	< 0.06

(*Continued*)

Table 2 (Continued).

Antifungal Susceptibility (μg/mL)	Z30215	Z30790
Echinocandins		
AND	> 8	> 8
MCF	> 8	> 8
CAS	> 8	> 8
Polyenes		
AMB	0.5	0.5

Abbreviations: FLC, fluconazole; VOR, voriconazole; ITCZ, itraconazole; POS, posaconazole; ISA, isavuconazole; 5-FC, 5-flucytosine; AND, anidulafungin; MCF, micafungin; CAS, caspofungin; AMB, amphotericin B; MIC, minimum inhibitory concentration.

Discussion

Case 1 (Z30790) involved a patient who experienced multiple fractures following a car accident. The symptoms were remarkably similar to those of bacterial infections, with sinus tract formation and bleeding. The test results indicated an erythrocyte sedimentation rate (ESR) of 27 mm/h and high-sensitivity C-reactive protein (hsCRP) concentration of 63.27 mg/L. Despite multiple debridement and negative pressure wound therapy surgeries, the wound infection persisted, suggesting the presence of a difficult-to-control infectious agent fungus. Case 2 (Z30215) involved extensive glucocorticoid use, diabetic foot disease, and connective tissue disease. Clinical manifestations include redness, swelling, heat, and pain in both lower limbs, along with multiple ulcers resistant to healing, thus serving as diagnostic evidence for yeast infection. Sequencing of the 18S rDNA, D1/D2, and ITS regions of strains Z30215 and Z30790 confirmed that both strains were *C. slooffiae*.

The clinical *C. slooffiae* strains isolated in this study were misidentified as *Rhodotorula* species by the commercially available biochemical method, and species identification could not be achieved using MALDI-TOF MS. Although MALDI-TOF MS is a powerful tool for yeast identification, its performance mostly relies on the protein fingerprint database, and the profiles of *C. slooffiae* are not included in the databases used in mainstream commercial products, such as Vitek MS or Bruker Biotyper. This is likely why the identification failed; infections due to *Cystobasidium* may be underestimated. Therefore, rDNA sequence analysis remains the most reliable method to correctly identify *C. slooffiae* strains and other emerging pathogens in clinical laboratories.

In 2019, *C. slooffiae* was isolated from clinical samples collected from various locations in the United Kingdom.[28] The high MIC values observed during fluconazole testing (MIC ≥ 64 μg/mL) were consistent with the fluconazole MICs of the Z30215 and Z30790 isolates in our study (MICs of 128 and 64 μg/mL, respectively). The resistance profile of our isolates suggests that commonly employed first-line agents (echinocandins and fluconazole) may have limited efficacy against *C. slooffiae* infections. In contrast, the potent in vitro activity of isavuconazole and amphotericin B positions them as superior therapeutic alternatives. This susceptibility profile is critically important clinically, providing essential guidance for both empiric and targeted therapy, particularly in cases of breakthrough or persistent infection following standard treatment. Consequently, clinicians should consider alternative agents such as isavuconazole or amphotericin B when *C. slooffiae* is identified or suspected.

Given the observed resistance to fluconazole and susceptibility to isavuconazole, we further reviewed the properties of the latter drug. Isavuconazole (ISA), a triazole antifungal, inhibits cytochrome P450-dependent 14α-demethylase activity which is essential for ergosterol synthesis. This disruption leads to alterations in fungal membrane structure and function, resulting in fungal cell death.[29] ISA has demonstrated in vitro activity against various yeasts, molds, and dimorphic fungi.[30] Compared to commonly used triazoles, ISA exhibits favorable tolerability and linear pharmacokinetics.[31] Michael A. Pfaller assessed the susceptibility to ISA of 4856 clinical fungal isolates collected globally from 2015 to 2016 and found that ISA had low minimum inhibitory concentrations (MICs) against *Aspergillus* spp. (model MIC of 0.5 μg/mL), Mucorales (model MIC of 1 μg/mL), *Candida* and other yeasts (MIC_{50} ≤ 0.5 μg/mL), and *Cryptococcus* (MICs ≤ 0.5 μg/mL).[30] From January 2015 to October 2017, M. Desnos-Ollivier et al determined the

MICs of ISA against 1457 clinical yeast isolates collected from French hospitals, showing that the MICs for *Candida* spp. (MIC_{90} < 0.5 μg/mL) was relatively low.[29] In contrast, the strains Z30215 and Z30790 in this study exhibited higher MICs for ISA (MICs of 1 μg/mL and 0.75 μg/mL, respectively). However, as neither patient received antifungal treatment after the culture results were reported, the current clinical data are insufficient to predict the correlation between in vitro susceptibility and in vivo therapeutic response.

Azole resistance mechanisms in *Candida* spp. primarily are often associated with *ERG11* mutations that impair drug binding to lanosterol 14α-demethylase and copy number amplification/transcriptional upregulation that increases *ERG11* production.[32,33] In contrast, *C. slooffiae* occupies a phylogenetically distinct Erg11 lineage (Figure 3; p-distance > 0.3 vs *Rhodosporidiobolus* clinical cluster). Its sequence divergence from the homologues in *Rhodosporidiobolus* and *Rhodotorula* may alter sterol uptake and metabolism, necessitating further studies on target binding affinities and drug efficacy.

Compared with other *Candida* and *Cryptococcus* species, the yeast *Cystobasidium* genus has rarely been reported worldwide. In 2020, the first case of fungemia caused by *C. minutum* was reported in Brazil.[34] In 2022, researchers reported for the first time that an atypical yeast, *C. calyptogenae*, was isolated from the oral samples of patients with angular cheilitis.[35] *C. slooffiae*, a well-known producer of carotenoids and known as "red yeast", has recently been extensively studied for its synthesis of carotenoid mixtures from low-cost carbon sources.[36] The *C. slooffiae* isolates promote plant growth and seed germination.[37] *C. slooffiae* is prevalent in other natural environments and can be found in washing machines.[38] This feature is believed to enhance *C. slooffiae* to different ecological niches and hosts as well as its geographic spread and potential to cause human infections. This ability to thrive in various habitats could underpin its potential to persist in the wound environment. In 2012, Kim Hyung Joo reported that individuals with oily skin were susceptible to *C. slooffiae* infection.[39] Once the body touches the source of infection due to skin trauma, older individuals with underlying conditions and low immunity are susceptible to infection.

Several limitations of this study should be acknowledged. First, the conclusions are drawn from only two clinical cases, which underscores the rarity of this pathogen. More importantly, neither patient received targeted antifungal therapy after correct identification and susceptibility data. Therefore, we could not correlate the in vitro susceptibility data with clinical treatment outcomes. Future studies that include a larger number of cases and detailed documentation of antifungal treatment and patient response are essential to validate these in vitro findings and to establish clinical breakpoints for this rare yeast.

Conclusion

This report presents the first isolation of *C. slooffiae* from skin wound infections in Chinese patients. The high MIC values for echinocandins and fluconazole underscore the critical need for precise identification to guide effective treatment protocols, especially in cases where fluconazole is commonly used as first-line therapies. Beyond traditional laboratory diagnostics, the application of molecular techniques is essential for the accurate identification and comprehensive characterization of this fungal species. Therefore, clinical laboratories should consider sequencing-based identification when unconventional or poorly identified yeasts are encountered, especially in cases of persistent or refractory infections.

Abbreviations

MIC, minimum inhibitory concentration; SFI, superficial fungal infections; SDA, Sabouraud dextrose agar; PCR, Polymerase chain reaction; BLAST, Basic Local Alignment Search Tool; MEGA, Molecular evolutionary genetic analysis; CT, Computed Tomography; ESR, erythrocyte sedimentation rate; hsCRP, high-sensitivity C-reactive protein; 5-FC, 5-fluorocytosine.

Data Sharing Statement

The rDNA region sequences of *C. slooffiae* strains Z30790 (from patient 1) and Z30215 (from patient 2) obtained in this study were deposited in the NCBI GenBank database under accession numbers PQ805459 and PQ805456 (for 18S rDNA), PQ805460 and PQ805457 (for the D1/D2 domain), and PQ805461 and PQ805458 (for the ITS region), respectively.

Ethical Approval and Informed Consent

The study protocol was approved by the Institutional Review Board of Peking Union Medical College Hospital (no. S-263), and followed the Declaration of Helsinki Ethical Principles for Medical Research involving Human Subjects. Written informed consent was obtained from both patients for the publication of their case details.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of work. The specific contributions are as follows: JH, LG, and C.K.M.T were involved in study design, data interpretation, and the writing and critical revision of the manuscript. GZ was primarily responsible for conducting experiments and patient sample collection. WH, KL, XJ, YL, XC, and CT assisted in data acquisition, analysis, and experimental procedures. YX and NL supervised the project, secured funding, and provided critical feedback on the manuscript.

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Disclosure

The authors report no conflicts of interest concerning the materials or methods used in this study, or the findings specified in this paper.

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