Clinical characteristics of the first cases of invasive candidiasis in China due to pan-echinocandin-resistant *Candida tropicalis* and *Candida glabrata* isolates with delineation of their resistance mechanisms

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Abstract: Echinocandin antifungal agents have become the first-line therapy for invasive candidiasis (IC) in many countries. Despite their increasing use, resistance to this class of drug is, overall, still uncommon. Here, we report two patients from the People’s Republic of China with IC, one with infection caused by pan-echinocandin-resistant *Candida tropicalis* and the other by pan-echinocandin-resistant *Candida glabrata*. We also describe the mechanisms of drug resistance of these isolates. The echinocandin-resistant *C. glabrata* isolate was cultured from ascitic fluid of a 46-year-old male patient with intra-abdominal IC developing after surgery in 2012. This patient had had no prior antifungal exposure. The echinocandin-resistant *C. tropicalis* isolate was cultured from chest drainage fluid of a 60-year-old female patient with severe coronary disease and lung infection. Prior to culture and identification of the isolate, the patient had received micafungin treatment for 19 days. Both isolates were cross-resistant to micafungin, anidulafungin, and caspofungin, with minimum inhibitory concentration values of ≥2 µg/mL. The amino acid substitution E655K was found adjacent to the FKS1 HS1 region of the *C. glabrata* isolate, while the substitution S80P were found in the FKS1 HS1 region of the *C. tropicalis* isolate. This report highlights the emergence of echinocandin resistance in two important non-albicans *Candida* species. Although the overall prevalence of echinocandin resistance is low in the People’s Republic of China, monitoring of antifungal susceptibility trends in all *Candida* species is warranted.

Keywords: *Candida tropicalis*, *Candida glabrata*, echinocandins, antifungal resistance, People’s Republic of China

Introduction

Invasive candidiasis (IC) is increasing in prevalence, especially among immunocompromised patients and those with serious underlying disease. In addition, mortality from IC remains high.¹ *Candida tropicalis* and *Candida glabrata* are two major pathogenic non-albicans *Candida* species. *C. glabrata* is the second most common cause of candidemia in Europe and America, while *C. tropicalis* has become the first and second leading cause of IC in India and the People’s Republic of China, respectively.¹⁻³ These species are notable for their resistance or reduced susceptibility to azole antifungal agents.⁴⁻⁵

As such, the echinocandins are increasingly used as first-line therapy for IC, because of their good efficacy and safety profiles.⁴⁻⁶ However, reduced susceptibility
and resistance among Candida species to these agents has also been noted, linked to mutations within specific hotspot (HS) regions of the Candida FKS genes. The emergence of echinocandin resistance has been most concerning for C. glabrata, especially in North America, while echinocandin resistance in other Candida species and in other geographical regions remains more uncommon.

In the China Hospital Invasive Fungal Surveillance Net (CHIF-NET) 2013 program, one C. tropicalis and C. glabrata isolate each was found to be resistant to all three licensed echinocandin agents, being the first pan-echinocandin-resistant isolates identified in the country. Here, we report the clinical features of the patients affected by these pan-echinocandin-resistant strains. We also determined the mechanism of resistance in the isolates.

### Case presentation

#### Case 1: Infection with echinocandin-resistant C. glabrata

On September 27, 2012, a 46-year-old male was admitted to the Department of Pancreatic Surgery in a hospital in the Northeast region of the People's Republic of China with fever and an abdominal wound infection (Table 1). He had experienced a right hepatectomy, subtotal gastrectomy, transverse colon loop ostomy, and right thoracic cavity drainage and closure 38 days prior in another hospital owning to severe trauma. He was immunocompetent. Before admission, the patient had received imipenem therapy for 10 days because of Escherichia coli bacteremia, but there was no history of exposure to antifungal agents.

### Table 1 Clinical features of two patients with echinocandin-resistant candidiasis and in vitro susceptibilities of two isolates

| Characteristics                          | Candida glabrata patient | Candida tropicalis patient |
|------------------------------------------|--------------------------|---------------------------|
| Age (years)                              | 46                       | 69                        |
| Gender                                   | Male                     | Female                    |
| Date of admission                        | September 27, 2012       | May 10, 2013              |
| Department of admission                  | Department of Pancreatic Surgery | ICU                     |
| Reason for hospital admission            | Fever and abdominal incision infection | Asthma, pulmonary infection |
| Underlying disease                       | Right hepatectomy, subtotal gastrectomy, transverse colon loop ostomy, right closed chest drainage | Coronary heart disease, cardiac valve replacement, multiple organ dysfunction syndrome |
| CHIF-NET strain no.                      | 12Z1132                  | 13TJ350                   |
| Site of isolation                        | Ascitic fluid            | Left chest drainage       |
| Date of isolation                        | October 1, 2012          | July 18, 2013             |

**Clinical status at time of positive culture**

| Immunosuppressive state                | No                        | No                        |
| Neutropenia (<10^9/L)                 | No                        | No                        |
| Presence of CVC                       | No                        | No                        |
| Broad-spectrum antibiotics             | Yes                       | Yes                       |
| Total parenteral nutrition             | No                        | Yes                       |
| Mechanical ventilation                | No                        | Yes                       |
| Surgery within 30 days                | Yes                       | Yes                       |
| Previous antifungal agents within 30 days | No                     | Micafungin, 18 days      |
| Indwelling urinary catheter            | No                        | Yes                       |

**Therapy**

- Antifungal CVC removal: Not applicable
- Antifungal after culture: Fluconazole, 200 mg/d
- Outcome: Recovered

**Antifungal susceptibilities (MIC [mg/L]/category)**

| Antifungal                  | MIC [mg/L]/category |
|-----------------------------|---------------------|
| Micafungin                  | ≥ 8/R               |
| Anidulafungin               | ≥ 8/R               |
| Caspofungin                 | ≥ 8/R               |
| Fluconazole                 | 32/SDD              |
| Voriconazole                | 1/WT                |
| Itraconazole                | 1/WT                |
| Posaconazole                | 2/WT                |
| Amphotericin B              | 0.5/WT              |
| 5-Flucytosine               | ≤ 0.06/WT           |

**Abbreviations:** ICU, intensive care unit; CHIF-NET, China Hospital Invasive Fungal Surveillance Net; CVC, central venous catheter; MIC, minimum inhibitory concentration; S, susceptible; SDD, susceptible dose-dependent; R, resistant; WT, wild-type.
On day 4 of admission, the local laboratory reported growth of *C. glabrata* in the ascitic fluid of the patient collected on day 2; however, antifungal susceptibility testing was not performed. Fluconazole therapy 200 mg/d was initiated immediately on day 4 for 23 days duration. No other cultures were positive for fungi. The patient made good clinical recovery and was discharged on October 24 (day 27).

**Case 2: Echinocandin-resistant *C. tropicalis***

On May 10, 2013, a 69-year-old female was admitted to the intensive care unit of a hospital in the Middle region of the People’s Republic of China with severe asthma and pulmonary infection (Table 1). She suffered from severe coronary disease, underwent aortic valve replacement 1 month prior in another hospital, and had since developed multiple organ dysfunction. A previous sputum culture within the last 30 days was positive for *Acinetobacter baumannii* and *Candida albicans*. From day 1 of admission, she received meropenem 3 g/d for 22 days and micafungin 100 mg/d for 18 days.

On day 8 of admission, a chest tube was placed in the left chest wall for drainage of a pleural effusion from unresolved pneumonia. On day 33, *C. tropicalis* was cultured from the pleural drainage fluid. The local laboratory performed antifungal susceptibility testing of the isolate by the disk diffusion method, but only for fluconazole, and reported a “susceptible” result. Voriconazole therapy (200 mg/d) was initiated and continued till day 99 of admission when the patient passed away from heart failure.

**Materials and methods**

**Ethics statement**

Written informed consent to publish their case details was obtained from the patient, or next of kin where the patient was unable to consent. The Human Research Ethics Committee of Peking Union Medical College Hospital has provided permission to publish this report (S-628).

**Detection of the pan-echinocandin-resistant *Candida* isolates from CHIF-NET national surveillance**

CHIF-NET was established as a nationwide surveillance program in the People’s Republic of China to monitor the trends in the epidemiology of invasive yeast infections and to provide up-to-date susceptibility data on antifungal agents. CHIF-NET 2013 comprised the fourth consecutive surveillance year of the program, held from August 1, 2012 to July 31, 2013. Generally, all yeast isolates collected in CHIF-NET 2013 were forwarded to the central laboratory, the Department of Clinical Laboratory, Peking Union Medical College Hospital. Confirmation of species identification was carried out by an algorithm of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Vitek MS; bioMérieux, Marcy l’Etoile, France) supplemented with rDNA internal transcribed spacer (ITS) sequencing. The in vitro susceptibility to nine antifungal drugs, including three echinocandins (caspofungin, micafungin, and anidulafungin), amphotericin B, and 5-flucytosine, was determined using Sensititre Yeast-One™ YO10 methodology (Sensititre; Thermo Scientific, Cleveland, OH, USA) following the manufacturer’s instructions. Current available species-specific clinical breakpoint or epidemiological cut-off values were used for interpretation of results (Table S1). Only one *C. glabrata* (CHIF-NET study no. 12Z1132) and one *C. tropicalis* (study no. 13TJ350) isolate, which were isolated from the patients described above, were resistant to all the echinocandins (Table 1).

**Analysis of the FKS gene**

For *C. glabrata* strain 12Z1132, sequencing of FKS1 and FKS2 genes was carried out as described by Zimbeck et al; sequences of *C. glabrata* strain CBS 138 (GenBank accession numbers XM_446406 and XM_448401 for FKS1 and FKS2, respectively) were the wild-type (WT) reference sequences. For *C. tropicalis* strain 13TJ350, sequencing of FKS1 gene was carried out as described by Jensen et al, with the sequence of *C. tropicalis* ATCC 750 (GenBank accession number EU676168) representing the WT reference sequence.

**Nucleotide sequences**

The partial FKS2 gene sequence of *C. glabrata* 12Z1132 and partial FKS1 gene sequence of *C. tropicalis* strain 13TJ350 have been deposited in GenBank database, with GenBank accession numbers of MF667536 and MF667537, respectively.

**Results**

**Echinocandin-resistant isolates**

In CHIF-NET 2013, 2,687 yeast isolates were collected from 48 hospitals in the People’s Republic of China where *C. tropicalis* (413 isolates, 15.4%) and *C. glabrata* sensu stricto (254 isolates, 9.5%) were the third and fourth most common species. However, only *C. tropicalis* isolate 13TJ350 and *C. glabrata* isolate 12Z1132 were echinocandin-resistant. These were the first pan-echinocandin-resistant *C. tropicalis* and *C. glabrata* isolates identified in the People’s Republic of China.
Antifungal susceptibilities

C. glabrata strain 1Z1132 was resistant to all three echinocandin agents, with minimum inhibitory concentrations of ≥8 µg/mL (Table 1). C. tropicalis strain 13TJ350 was also pan-echinocandin resistant, with minimum inhibitory concentrations of 4 µg/mL, 2 µg/mL, and 2 µg/mL to caspofungin, micafungin, and anidulafungin, respectively (Table 1). Both strains were susceptible or of WT phenotype to 5-flucytosine, amphotericin B, and four azoles tested, except for C. glabrata strain 1Z1132 which was susceptible in a dose-dependent manner to fluconazole (Table 1).

Analysis of FKS genes

A mutation leading to the amino acid substitution S80P was found in HS1 region of the FKS1 gene of C. tropicalis strain 13TJ350. However, no amino acid substitution was detected in HS regions of FKS1 and FKS2 genes in C. glabrata strain 12Z1132. Instead, there was a mutation leading to the amino acid substitution E655K adjacent to HS1 region of the FKS2 gene.

Discussion

Because there are only a few classes of antifungal agents, for example, the azoles, echinocandins and polyenes, in clinical use, options for antifungal therapy are relatively limited. Facing the challenges posed byazole resistance, echinocandins have become the preferred therapy for IC. Overall, resistance to the echinocandins appears to be uncommon. From a recent global surveillance report, echinocandin susceptibility rates were over 95% for C. albicans as well as for the more commonly encountered non-albicans Candida species. However, since the first report of reduced susceptibility to caspofungin in C. albicans in 2004, echinocandin resistance among Candida isolates has been increasingly identified among different Candida species, raising global concern. Of note, most echinocandin resistance in Candida species worldwide have been documented in C. glabrata. In addition, the prevalence of echinocandin resistance has varied with geographic region: echinocandin-resistant C. glabrata is more common in North America (7%–10%) than in other continents (0%–2%). Echinocandin resistance is typically associated with prior antifungal drug exposure. However, in our patient with infection due to an echinocandin-resistant C. glabrata, no previous antifungal use was evident. The infection was thought to be acquired after the patient’s abdominal operation, supporting the notion that, even without typical risk factors, patients may become infected by echinocandin-resistant strains.

Echinocandin resistance in C. tropicalis appears to be uncommon (<1%). Reports of infections caused by such strains have included three cases of breakthrough candidemia in allogeneic stem cell recipients, and a case of candidal esophagitis in a patient with acute myelogenous leukemia. Compared with published reports, our patient (patient 2) had exposure to at least one echinocandin, but the patient was not immunosuppressed.

It is well established that mutations in the FKS gene account for echinocandin resistance and that their presence is linked with failure of echinocandin therapy. Although other resistant mechanisms, for example, alterations in the mismatch repair gene msh2, have also been reported, their role in conferring resistance is less certain. In C. albicans and C. tropicalis, amino acid substitutions associated with resistance have mainly been documented in two HS regions (HS1 and HS2) of the FKS1 gene, while in C. glabrata, these mutations are in homologous regions of FKS1 and FKS2 genes. In the present study, the S80P substitution was detected, while no strain was found to be resistant to caspofungin or micafungin. Since then, themsg2 role in conferring resistance is less certain. In C. albicans and C. tropicalis, amino acid substitutions associated with resistance have mainly been documented in two HS regions (HS1 and HS2) of the FKS1 gene, while in C. glabrata, these mutations are in homologous regions of FKS1 and FKS2 genes. In the present study, the S80P substitution was detected, while no strain was found to be resistant to caspofungin or micafungin.
in C. glabrata strain 12Z1132, the E655K substitution was identified upstream to FKS2 gene HS1 region compared with the WT strain. However, as a limitation of the study, the correlation between the novel E655K substitution identified in the C. glabrata isolate and the strain’s resistance phenotype remains to be confirmed.

The emergence of multidrug resistant Candida isolates, for example, isolates resistant to both azoles and echinocandins that occurred in C. glabrata, has also been a concern.4,5 Fortunately, both our pan-echinocandin-resistant Candida isolates were not resistant to any of the azoles tested. Although the C. tropicalis infected patient finally passed away because of poor health, the C. glabrata infected patient exhibited good clinical response to fluconazole treatment. Importantly, since the writing of this report, an additional isolate of C. glabrata resistant to all azoles and all echinocandins has been identified.10 This emergence of multi class drug resistance signals the need for increased vigilance by clinicians and scientists alike.

Conclusion
In conclusion, we describe the clinical presentation of the first patients with IC caused by pan-echinocandin-resistant C. tropicalis and C. glabrata isolates. Taking the rapid increase of azole resistance in these two species into account, the emergence of echinocandin resistance will pose challenges for management of patients with IC in the People’s Republic of China.

Acknowledgments
This work was supported by a CAMS Innovation Fund for Medical Sciences (2016-I2M-1-014), a Beijing Innovation Base Cultivation and Development Special Fund (Z171100002217068), a PUMCH Out-standing Young Talents Program (JQ201703), and a National Key Research and Development Program of China (No. 2016YFC0901500). The funders had no role in study design, data collection, and interpretation, or the decision to submit the work for publication.

Author contributions
All authors contributed toward data analysis, drafting, and critically revising the paper, and agree to be accountable for all aspects of the work.

Disclosure
The authors report no conflicts of interest in this work.

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## Supplementary material

Table S1 CBPs and ECVs used in the present study

| Antifungal agents | Candida tropicalis | Candida glabrata |
|-------------------|--------------------|------------------|
|                   | CBPs (mg/L) | ECVs (mg/L) | CBPs (mg/L) | ECVs (mg/L) |
|                   | S | SDD | I | R | S | SDD | I | R |
| Fluconazole       | ≤2 | 4 | – | ≥8 | – | – | ≤32 | – | ≥64 | – |
| Voriconazole      | 0.125 | 0.125–0.5 | – | ≥1 | – | – | – | – | – | 2 |
| Itraconazole      | – | – | – | – | 1 | – | – | – | – | 4 |
| Posaconazole      | – | – | – | – | 2 | – | – | – | – | 4 |
| Caspofungin       | ≤0.25 | – | 0.5 | ≥1 | – | ≤0.12 | – | 0.25 | ≥0.5 | – |
| Micafungin        | ≤0.25 | – | 0.5 | ≥1 | – | ≤0.06 | – | 0.12 | ≥0.25 | – |
| Anidulafungin     | ≤0.25 | – | 0.5 | ≥1 | – | ≤0.12 | – | 0.25 | ≥0.5 | – |
| 5-Flucytosine     | – | – | – | – | 0.5 | – | – | – | – | 0.25 |
| Amphotericin B    | – | – | – | – | 2 | – | – | – | – | 2 |

**Abbreviations:** CBP, clinical breakpoint; ECV, epidemiological cut-off value; S, susceptible; SDD, susceptible dose-dependent; I, intermediate; R, resistant.