Identification of the rare yeast *Cutaneotrichosporon (Trichosporon) debeurmannianum* from diabetic foot infection

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Abstract

Background: Diabetic foot infection is the most common complications of diabetes mellitus. Although most of the diabetic foot infections has been known to be caused by aerobic and anaerobic bacteria, mycotic diabetic foot infection caused by *Candida* species has also been reported recently. Here, we present the first case of diabetic foot infection caused by *Cutaneotrichosporon debeurmannianum* (previously known as *Trichosporon debeurmannianum*).

Methods: A 68-year-old diabetic male patient was admitted for management of the necrosis of the big toe. Wound swab culture was performed three times, and each time after 5 days of incubation, beige-colored, wrinkled, and rough colonies were observed on chocolate agar plate.

Results: The isolate was identified as *C. debeurmannianum* with the matrix-assisted laser desorption ionization-time of flight mass spectrometry system (MicroIDSys, ASTA corp.). For confirmation, the sequencing for ITS1/ITS2 and D1/D2 ribosomal DNA was also performed, and the isolate was confirmed as *C. debeurmannianum* with 100% identity. The isolate exhibited low minimum inhibitory concentrations (MICs) for azoles and high MICs for all echinocandins.

Conclusion: Considering that usual incubation time for bacterial culture of open wound specimens is only 48 h, it is important to include the request for fungus culture to detect pathogen in diabetic foot lesion.

**KEYWORDS**

*Cutaneotrichosporon debeurmannianum*, diabetic foot infection, MIC, *Trichosporon debeurmannianum*

1 | BACKGROUND

Diabetic foot infection is the most common complications of diabetes mellitus with a lifetime incidence of 25% and high mortality and morbidity.1 Most of the diabetic foot infections are known to be caused by aerobic and anaerobic bacteria and in most cases polymicrobial.2 Recently, a few studies have reported mycotic infections of the diabetic foot, mainly *Candida* species.3–5

*Trichosporon debeurmannianum* was first isolated from bronchial aspirate, but it had not been determined whether it was pathogenic or not.6 Recently, Liu et al.7 reclassified *Trichosporon* into three genera: *Apiotrichum*, *Cutaneotrichosporon*, and *Trichosporon*.
T. debeurmannianum was renamed to Cutaneotrichosporon debeurmannianum based on the phylogenetic analysis. The genus Cutaneotrichosporon contains 15 species,\(^7\) of which C. cutaneum, C. dermatis, and C. mucoides are currently regarded as potentially pathogenic for humans.\(^8\)\(^–\)\(^12\) The accurate identification and discrimination of these species by conventional method (e.g., morphological and biochemical method) is difficult, leading to inconclusive identification especially for infrequent species.\(^13\) Recently, the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) system is considered a reliable method for rapid identification of fungal species and used as an alternative to molecular method.

Here, we present the first case of diabetic foot infection caused by C. debeurmannianum. This case report was approved by the Institutional Review Board of Seoul St. Mary’s Hospital, Korea (IRB No. KC22ZASI0555), and informed consent was waived.

## CASE PRESENTATION

A 68-year-old man was transferred from another hospital for wound management of big toe gangrene. The patient presented with diabetes mellitus and end-stage renal disease undergoing hemodialysis. A wound swab culture was performed on the necrotic part of the big toe showing swelling, redness, and purulent discharge. Gram stain of the swab specimen showed many white blood cells and some yeasts. The swab specimen was inoculated onto blood, McConkey, and chocolate agar plates. Also, smooth with mucoid texture colonies grown on CHROMagar Candida® medium were mucoid and brown-colored.

The yeast was identified as C. debeurmannianum by the MicroIDSys MALDI-TOF MS system (ASTA corp., Suwon, Korea) using in-tube ethanol/formic acid protein extraction method (in-tube E/FA method). For the in-tube E/FA method, a single colony was mixed with 300 µl of distilled water and 900 µl of 100% ethanol. The sample was centrifuged (15,000 g for 2 min), and supernatant was removed. The pellet was air-dried at room temperature for 10 min, and resuspended in 20 µl of 70% formic acid and incubated for 5 min. The sample volume of 100% acetonitrile was then added to the sample and incubated for 5 min. After centrifugation at 15,000 g for 5 min, 1.0 µl of the supernatant was spotted in duplicate onto the target plate and overlaid with 1.0 µl of a CHCA matrix. The target plate was placed in the MicroIDSys Elite system (ASTA corp.) and was analyzed. They were identified as C. debeurmannianum with 156/150 scores (over the cutoff value of 140).

This isolate was not identified by Vitek 2 YST system (bioMérieux, Marcy l’Etoile, France) and VITEK MS MALDI-TOF system (bioMérieux), given that the C. debeurmannianum was not in the database. The culture of the wound swab was repeated on two other days, and C. debeurmannianum grew each time. To confirm the identification, we performed sequencing of the internal transcribed spacer (ITS1/ITS2) regions and D1/D2 region of the 28S ribosomal DNA. Analysis of the ITS1/ITS2 and D1/D2 ribosomal DNA sequences showed 100% identity with C. debeurmannianum (GenBank accession no. KY103002.1 and NR_154752.1 for ITS1/ITS2, GenBank accession no. NG_058998.1, AY143554.1, and AB044568.1 for D1/D2). We performed antifungal susceptibility testing with Sensititre YeastOne plate (Thermo Scientific, Cleveland, OH, U) according to the manufacturer’s instructions. The minimum inhibitory concentration of each antifungal agent was measured after 72 h of incubation at 35 °C (when growth control appeared as positive after 72 h of incubation). The minimum inhibitory concentrations (MIC) were 1.0 µg/ml of amphotericin B, 1.0 µg/ml of fluconazole, 0.03 µg/ml of posaconazole, 0.06 µg/ml of itraconazole, 0.015 µg/ml of voriconazole, and >8.0 µg/ml of all echinocandins (Table 1). The follow-up cultures were serially performed on hospital Days 2 and 4, then the same isolate was also identified. However, as the patient was transferred to another hospital for amputation, we could not follow up the clinical course.

## DISCUSSION AND CONCLUSIONS

Since Sugita et al.\(^6\) reported two cases of T. debeurmannianum (a previous name of C. debeurmannianum), it has been rarely found in clinical samples. Nath et al.\(^14\) reported that nine isolates from three blood and six urine samples in patients who were diagnosed as septicemia and urinary tract infection over a 3-year period. In addition, Noy et al.\(^15\) reported the case of a subcutaneous mycotic infection caused by C. debeurmannianum in an immunocompetent man. In our study, the isolated C. debeurmannianum showed high echinocandins MICs. Azoles had good in vitro activity against this isolate, especially voriconazole (MIC = 0.015 µg/ml). Previous study on antifungal susceptibility testing for C. debeurmannianum reported that these isolates were susceptible to fluconazole and voriconazole performed by disc diffusion method.\(^14\) Despite the lack of defined clinical breakpoints for Cutaneotrichosporon spp. and Trichosporon spp., previous studies reported that all Trichosporon spp. exhibited high MICs for all echinocandins.\(^5\)\(^,\)\(^16\) We summarized the results of the antifungal susceptibility testing for the Cutaneotrichosporon spp. isolated from clinical specimens (Table 1). Except only two isolates isolated from patients treated with antifungal agents, most Cutaneotrichosporon spp. show relatively low MICs for azoles.

The main limitation of this study is that we could not determine the clinical response to conventional antifungal agents (especially azoles) for this isolate. Nevertheless, it is meaningful in that we tried to identify the C. debeurmannianum isolated from diabetic foot by culturing more than 2 days for wound culture. For clinicians, it is important to suspect fungal infections in diabetic foot lesion, especially in patients unresponsive to antibacterial treatment with
| Species         | Case no. (reference) | Origin        | MIC method                  | MIC result (μg/ml) | Previous antifungal treatment |
|-----------------|----------------------|---------------|-----------------------------|--------------------|-------------------------------|
|                 |                      |               | AMB | 5-FC | FLZ  | ITZ  | POS | VOR | CAS | MIF   |
| *C. debeurmannianum* | Case 1<sup>6</sup>  | Bronchial secretion | Eiken kit   | 0.125 | >64  | 2   | 0.06 | -  | -   | -    | -    | None             |
|                 | Case 2<sup>6</sup>  | Bronchial secretion | Eiken kit   | 0.25  | >64  | 4   | 0.125 | -  | -   | -    | -    | None             |
|                 | This case            | Wound         | Sensititre YeastOne | 1     | 8    | 1   | 0.06  | 0.03 | 0.015 | >8  | >8   | None             |
|                 | Case 3<sup>12</sup> | Nail          | BMD (CLSI)<sup>a</sup> | 4     | -    | 1   | 0.25  | 0.25 | 0.25  | -   | -    | Unknown          |
| *C. dermatis*   | Case 4<sup>17</sup> | Pericardial fluid | BMD (EUCAST)<sup>b</sup> | 2     | -    | 0.25 | 0.25  | 0.5  | 0.032 | -   | -    | Unknown          |
|                 | Case 5<sup>10</sup> | Abdominal fluid | Sensititre YeastOne | 0.25  | >64  | >256 | >16   | >8   | >16  | >16  | -   | Yes (Azoles) |
|                 | Case 6<sup>12</sup> | Wound         | BMD (CLSI)<sup>a</sup> | 4     | -    | 1   | 0.25  | 0.25 | 0.12  | -   | -    | Unknown          |
| *C. mucoides*   | Case 7<sup>18</sup> | Nail          | E-test | 0.32  | -    | 256 | 32    | -   | -    | 32   | -    | -    | Yes (Azoles) |
|                 | Case 8<sup>19</sup> | Blood Heart Skin | BMD (CLSI)<sup>a</sup> | 1     | -    | 16  | -     | -   | 0.25  | >8  | >8   | None             |
|                 | Case 9<sup>12</sup> | Blood         | BMD (CLSI)<sup>a</sup> | 2     | -    | 1   | 0.12  | 0.25 | 0.25  | -   | -    | Unknown          |

Abbreviations: 5-FC, 5-flucytosine; AMB, amphotericin B; CAS, caspofungin; FLZ, fluconazole; ITZ, itraconazole; MIC, minimum inhibitory concentration; MIF, micafungin; POS, posaconazole; VOR, voriconazole.

<sup>a</sup>As per the CLSI guidelines. 20

<sup>b</sup>As per the EUCAST guidelines. 21
longstanding ulcers. Early detection and appropriate fungal treatment are critical for the prevention of severe consequences such as amputation.

**AUTHOR CONTRIBUTIONS**
IYY wrote the first draft of the article; IYY assisted in the acquisition of data; WH, MRL, and JAK performed the laboratory analyses; YJP reviewed and improved the case report. All authors read and approved the final article.

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**CONFLICT OF INTEREST**
The authors have no relevant financial or non-financial interests to disclose.

**DATA AVAILABILITY STATEMENT**
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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