Candida dubliniensis Isolated from the Sputum of a Patient with End-stage Liver Cirrhosis

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Abstract

Candida dubliniensis is rare and very similar to *C. albicans*. To date, detailed clinical reports on *C. dubliniensis* recovered from an immunocompromised patient have not been described in Japan. A 71-year-old man with end-stage liver cirrhosis had been treated for suppurative omarthritis due to methicillin-resistant *Staphylococcus aureus* (MRSA). Anti-MRSA agents and broad-spectrum antimicrobials but no antifungal agents had been administered. *C. dubliniensis*, isolated from the sputum, was eliminated by selective digestive decontamination and supportive therapy. This case emphasizes the need to recognize this emerging *Candida* sp., *C. dubliniensis* in cases of opportunistic infection.

Key words: susceptibility, micafungin, methicillin-resistant *Staphylococcus aureus*, selective digestive decontamination

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Introduction

Candida albicans has been the predominant etiologic agent of candidiasis. The incidence of candidiasis caused by other species of *Candida*, including *C. glabrata*, *C. krusei* and *C. tropicalis*, has increased (1). However, *C. dubliniensis* has not been known in Japan because of the difficulty in discriminating it from *C. albicans*. Although it is said that *C. dubliniensis* colonies appear dark green on the commercially available chromogenic agar medium (CHROMagar™, Paris, France) in contrast to light blue-green colonies of *C. albicans*, it is not easily distinguishable in practice. Therefore, *C. dubliniensis* has possibly been misidentified as *C. albicans* (2, 3). If so, it is suspected that some *C. albicans* isolates resistant to fluconazole (FLCZ) may involve *C. dubliniensis* isolates because of their ability to rapidly develop FLCZ resistance in vitro (4). Furthermore, there is a controversy of which antifungal agents are necessary for treatment of candidiasis due to *C. dubliniensis* because the patients have multiple underlying conditions such as human immunodeficiency virus (HIV)-infected and with end-stage liver disease. As far as we know, this is the first Japanese case report demonstrating the process to yield the identification as

C. dubliniensis from the clinical specimen.

Case Report

A 71-year-old man was transferred to our ICU because of respiratory failure. He was diagnosed as having alcoholic liver injury and left suppurative omarthritis, which developed 5 days after artificial head replacement for humeral comminuted fracture. The curettage and drainage were performed for suppurative omarthritis at the previous hospital. Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* had been isolated from the pus on the wound and 200 mg per day of arbekacin (ABK) and 600 mg per day of ciprofloxacin (CPFX) had been administered.

On admission, he appeared restless. His heart rate was 80 beats/min, respiratory rate was 24 breaths/min, blood pressure was 175/90 mmHg, and body temperature was 37.6°C. His breath sounds were weak bilaterally. There were no abnormal findings on his heart, abdomen, or nervous system but epidermolysis and swelling on his left shoulder were seen. His laboratory data on admission are shown in Table 1. These data showed hypoalbuminemia, liver dysfunction and impaired oxygenation. White-cell count, C-reactive

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Table 1. Laboratory Data on Admission

[Peripheral blood]		[Biochemistry]		[Coagulation]	
White-cell count	13,740 / μ l	Urea nitrogen	9 mg/dl	Prothrombin time	15.4 secs
Stab neutrophils	6 %	Creatinine	0.6 mg/dl	Control	12.1 secs
Segmented neutrophils	84.5 %	C-reactive protein	7.63 mg/dl	Partial thromboplastin time	41.2 secs
Eosinophils	0.5 %	Glucose	146 mg/dl	Control	29.3 secs
Monocytes	4 %	Total protein	6.2 g/dl	D-dimer	57.7 μ g/ml
Lymphocytes	5 %	Albumin	2.3 g/dl	Antithrombin III	44.1 %
Red-cell count	271×10^4 / μ l	Total bilirubin	2.4 mg/dl	Fibrinogen	300 mg/dl
Hemoglobin	9.1 g/dl	Conjugated bilirubin	1.1 mg/dl	[Blood gas analysis (under 10 L/min O ₂)]	
Hematocrit	27.4 %	Aspartate aminotransferase	75 IU/L		
Platelet count	15.3×10^4 / μ l	Alanine aminotransferase	45 IU/L	pH	7.48
[Infection]		γ -glutamyl transpeptidase	98 IU/L	PaO ₂	137 mmHg
1, 3- β -D-glucan	4.9 pg/ml	Lactate dehydrogenase	350 IU/L	PaCO ₂	31 mmHg
HBs antigen	negative	Alkaline phosphatase	559 IU/L	HCO ₃ ⁻	25 mmol/L
HCV antibody	negative	Total cholesterol	71 mg/dl	Base excess	-0.5 mmol/L
HIV antibody	negative	Cholinesterase	37 IU/L		

HCV, hepatitis C virus; HIV, human immunodeficiency virus

protein and D-dimer levels were high. His chest radiograph showed a large cardio-thoracic ratio. The chest and abdominal CT scans revealed the findings of cardiogenic pulmonary edema and liver cirrhosis with ascites. The cultures of the pus on the left shoulder and the sputum but not of the urine and blood showed the growth of MRSA. The electrocardiogram showed no abnormal findings but the echocardiography showed 40% of ejection fraction and normal wall motion.

He was treated with 7 cmH₂O of continuous positive airway pressure (CPAP) by the noninvasive positive pressure ventilator (BiPAP Vision™, Respironics, Murrysville, PA, U.S.A.). The fraction of inhaled oxygen (FIO₂) was 0.4. He was diagnosed as having the following: 1) sepsis due to suppurative omarthritis, 2) cardiogenic pulmonary edema, 3) end-stage liver cirrhosis, 4) disseminated intravascular coagulation (DIC). An antibiotic regimen of intravenous vancomycin (VCM) and meropenem (MEPM) was started at 0.5 g twice per day (Fig. 1). On Day 4, division of laboratory reported that *Candida* sp., similar to *Candida albicans*, was detected in the sputum culture from Day 2. An antifungal agent was not administered because of negative serum β -D-glucan test and a single positive site of *Candida* spp. Furthermore, MRSA was considered pathogenic. Prosthesis evulsion operation was performed and he was returned to the ICU on the invasive ventilator on Day 4. The ventilator setting was volume controlled, FIO₂ 1.0, and positive endexpiratory pressure (PEEP) 7 cmH₂O. The ABG analysis revealed; PaO₂ 135 mmHg, PaCO₂ 43 mmHg, pH 7.38. He developed acute respiratory distress syndrome (ARDS) and was treated with steroid pulse therapy (methylprednisolone 1 g daily for 3 days). *C. dubliniensis* was isolated from the aspirated sputum of Day 2 and Day 7. Selective digestive decontamination (SDD) had been performed using 0.5 g of VCM, 100 mg of amphotericin B (AMPH-B) and 1000,000 units of polymyxin B twice per day since Day 9. Although the trough and peak concentrations of VCM were 14.9 and 25.6 μ g/ml, respectively, the sputum cultures still remained

positive for MRSA but not for *C. dubliniensis*. β -D-glucan tests were all negative on Day 4 and Day 18. He was extubated on Day 10 and discharged to the general ward on Day 17.

Microbiology Results

Broth microdilution minimal inhibitory concentrations (MICs) were determined according to Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) document M27-A guidelines (ASTY™, Kyokuto, Tokyo, Japan). MICs of AMPH-B, flucytosine (5-FC), FLCZ, itraconazole (ITCZ), miconazole (MCZ) and micafungin (MCFG) were 0.25, $\leq$ 0.125, 0.25, 0.25, 0.125 and 2 μ g/ml, respectively.

The automated method for the identification of yeasts (API ID 32C™, bioMérieux, Lyon, France) showed 99.9% identification for *C. dubliniensis* after 48 hours incubation. The identification was confirmed by the nested polymerase chain reaction (PCR), using primer mixes of *Candida* DNA topoisomerase II genes (5). Furthermore, it was reconfirmed to be germ tube positive and to produce abundant chlamydospores on cornmeal agars (Fig. 2).

Discussion

This is the first report describing *C. dubliniensis* isolated from the sputum of a HIV-negative patient in Japan as far as we know. The incidence of infection or colonization due to *C. dubliniensis* is not known in Japan, largely because of the difficulty in distinguishing this species from the morphologically similar *C. albicans*. First, this *Candida* sp. was also misidentified as *C. albicans* by green colony color on CHROMagar™ *Candida* medium at our division of laboratory. However, the susceptibility of the *Candida* sp. against MCFG was different from that of *C. albicans* and they performed a further examination of a sugar assimilation test with the API ID 32C™, which showed 99.9% identification

Figure 1. Clinical course of the patient. VCM, vancomycin; MEPM, meropenem; mPSL, methylprednisolone; SDD, selective digestive decontamination; CPAP, continuous positive airway pressure; SIMV, synchronized intermittent mandatory ventilation.

Figure 2. Chlamydospore productions of *Candida dubliniensis* and *Candida albicans* on cornmeal agar plates incubated for 4 days. The production of abundant chlamydospores by *C. dubliniensis* was found (x400).

for *C. dubliniensis*. Therefore, it resulted in the isolation of *C. dubliniensis*.

C. dubliniensis, first isolated from oropharyngeal lesions of AIDS patients in Dublin, Ireland in 1995 (6), and has a worldwide distribution (7). *C. dubliniensis* has been recovered from not only HIV-infected individuals with/without oral candidiasis but also HIV-negative healthy individuals without oral candidiasis (8). *C. dubliniensis* isolates were recovered from the blood, urine, sputum, tracheal aspirates, high vaginal swabs and so on in HIV-negative patients (2).

The two patients, in whom *C. dubliniensis* was recovered from the blood cultures, had end-stage liver disease (9). They speculated that the gastrointestinal (GI) tract was the source of the *C. dubliniensis* in these patients. The present patient had not been treated with antifungal agents and had end-stage alcoholic liver cirrhosis. *C. albicans* and other less pathogenic species such as *C. glabrata*, *C. krusei* and *C. tropicalis* were not identified but MRSA was isolated. The relationship between *C. dubliniensis* and MRSA, and interaction between anti-MRSA agents and environmental conditions for *C. dubliniensis* were not known. However, we suggested that the source of *C. dubliniensis* was the GI tract or oral cavity. The reason for positive *C. dubliniensis* from the sputum was considered to be that it had been aspirated from the GI tract or oral cavity.

Suppurative omphalitis due to MRSA and colonization of MRSA and *C. dubliniensis* in the respiratory tract were finally diagnosed for the infection in this patient. Accordingly, he was treated using SDD for *C. dubliniensis* colonization according to Japanese “guidelines for the diagnosis and treatment of deep-seated mycosis” (10). There were several risk factors for invasive candidiasis, including elderly (≥ 70), prolonged use of broad spectrum antimicrobials (more than 2 classes, ≥ 7 days), total parenteral nutrition, severely malnourished, use of H2-blockers, prolonged mechanical ventilation (≥ 48 hrs) and intravascular catheterization, urinary catheterization (≥ 1 week). Although mortality benefit of SDD for the patients undergoing liver transplantation has not been shown, it has been shown to decrease the number of infections (11). Actually, *C. dubliniensis* was not recovered 7 days after the initiation of SDD and it was not identified from the other sites. Not only SDD but also supportive care such as nutrition might be effective for its elimination.

The clue to identify *C. dubliniensis* was the incomprehen-

sible MIC of MCFG (2 µg/ml) if it had been *C. albicans*. Pfaller et al reported the validation of in vitro methods for determining the susceptibilities of *Candida* spp. to caspofungin (CSP), one of the echinocandin-class antifungal agents (12). They showed the usefulness of RPMI medium, MIC endpoint criterion of partial inhibition and incubation for 24 hours. Consequently, they revealed 0.25 µg/ml of MIC at which 90% of *C. dubliniensis* isolates are inhibited

(MIC₉₀) against CSP (13). When we reevaluated the MIC of MCFG by their methods, the MIC was decreased to 0.016 µg/ml. Taken together, encountering the puzzling susceptibility of *C. albicans* against MCFG, it may be necessary to discriminate it from *C. dubliniensis* by use of API ID 32C™. The present case emphasizes the need to recognize the new *Candida* sp., *C. dubliniensis* when encountering immunocompromised patients.

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