Frequency of clinically isolated strains of oral Candida species at Kagoshima University Hospital, Japan, and their susceptibility to antifungal drugs in 2006–2007 and 2012–2013

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Abstract

Background: The isolation frequency and susceptibility to antifungal agents of oral Candida isolates from patients with oral candidiasis (OC) were compared between studies conducted in 2006–2007 and 2012–2013.

Methods: A total of 158 strains was isolated from 112 patients who visited Kagoshima University Hospital for the treatment of OC during the 14-month period from February 2012 and March 2013, and evaluated on the isolation frequency of each Candida strain and the susceptibility against antifungal drugs as compared to those evaluated in 2006–2007.

Results: There was a higher frequency of xerostomia as a chief complaint and of autoimmune disease in the 2012–2013 study than in the 2006–2007 study. More than 95% of Candida isolates were C. albicans and C. glabrata. In addition, the proportion of the latter increased from 12.3% in the 2006–2007 study to 23.4% in the 2012–2013 study, while the proportion of the former decreased from 86.2% to 72.8%, respectively. C. albicans was isolated in almost all patients, while C. glabrata was only isolated concomitantly with C. albicans. Minimal inhibitory concentrations (MICs) were not significantly different between groups with a few exceptions. Candida isolates, of which MICs surpassed break points, apparently increased for miconazole and itraconazole against C. glabrata in the 2012–2013 study, but this was not statistically significant. As a result, more cases of autoimmune disease, a greater number of C. glabrata isolates, and higher resistance to azoles were seen in the 2012–2013 study than in the 2006–2007 study.

Conclusion: These data indicate that with recent increases in C. glabrata infection, a causative fungus of OC, and in C. glabrata resistance to azoles, caution is needed in the selection of antifungal drugs for the treatment of OC.

Keywords: Candida glabrata, Candida albicans, Clinically isolated strains, Minimal inhibitory concentration, Resistant strains

Background

In the early 1990s, Candida albicans resistant to fluconazole was reported to be frequently isolated from the oral cavity of acquired immunodeficiency syndrome patients. An epidemiological study was conducted on the susceptibility to antifungal drugs of Candida strains isolated from patients with invasive candidiasis [1]. Global surveillance programs have been implemented for dealing with candidal resistance to antifungal drugs, such as the SENTRY program on fungi resistant to amphotericin B in 2006–2007 [2] and reports on candidal susceptibility to candin-type antifungals [3–6]. There are 2 reports on domestic surveillance in Japan: Surveillance 99 by the Japan Invasive Mycosis Surveillance Study Group (JMS) [7] and Report by Japan Antifungal Surveillance Program (JASP) [8–10]. However, these surveillance programs focused on strains isolated from clinical cases of deep-seated mycosis, and only 1 report, our previous study
performed in 2006–2007, was conducted on oral candidiasis [11].

The isolation frequency of Candida strains is likely to change over time, as shown by the comparisons of previous studies conducted in different time periods. For example, C. glabrata was rarely isolated from AIDS patients with oral candidiasis (OC) or esophageal candidiasis in Sudo’s study published in 1997 [12]; in contrast, C. glabrata was detected in 12.3% of patients with OC in our study, in which itraconazole (ITCZ) was effective despite the existence of resistant C. glabrata against ITCZ [11]. In addition, while resistant C. glabrata against ITCZ occurred with high frequencies of 50% and 72.7% in OC and non-OC patients of this study, respectively, [11], a previous Japanese surveillance study demonstrated resistant C. glabrata in only 1.0–9.3% of patients with mycosis, mainly deep-seated mycosis [7-10]. Thus, the isolation frequency of Candida species and their resistant strains varies over time, but no study has reported variations in frequency of OC over time in the same health facility.

The present study was undertaken to evaluate antifungal drugs against Candida strains isolated from the oral cavity of OC patients who visited Kagoshima University Hospital in 2012–2013 (14 months) and to assess the isolation frequency of Candida species and their resistant strains in comparison with our previous study conducted in 2006–2007 (24 months) in order investigate the use antifungal drugs in the treatment of OC.

**Methods**

**Subjects**

This study analyzed 158 strains isolated from 112 patients who visited the Department of Oral Surgery, Kagoshima University Hospital, for the treatment of OC during the 14-month period from February 2012 and March 2013. Strains collected from patients having received antifungal drug therapy within 3 months from collection were excluded from this study.

**Analysis of background variables of Candida-isolated patients**

An analysis was conducted for age, gender, underlying systemic disease, steroid hormone use, denture use, chief complaints, and oral disease in the 112 patients from whom Candida was isolated.

**Collection of Candida from the oral cavity and bacterial culture**

Prior to the diagnosis of OC, the affected area of the oral cavity was wiped with a sterile cotton swab pre-immersed in sterile physiological saline. Fluid sample collected on the swab was smeared onto a CHROMagar Candida Culture Medium (Nippon Becton and Dickinson Company, Ltd.) and incubated for 48 hours at 36°C.

**Identification of Candida species**

Positive culture of Candida species was determined by colorimetric observation of colonies in the CHROMagar Candida culture medium containing synthetic colorimetric substrates which are changed to be chromogenic by enzymes contained in Candida species. Confirmation of the determination of Candida species was then done by PCR based on the method reported from Kanbe et al., briefly fungal cells grown in the CHROMagar Candida culture medium were harvested by centrifugation, washed three times with distilled water, and the DNAs were extracted and purified according to the protocol of a DNA purification kit, Fast DNA Kit (BP Biomedical, U.S.). Then, the DNA samples were amplified by PCR and the PCR products were analyzed by agarose gel electrophoresis to determine the kind of Candida species [13].

**Diagnosis of OC**

Fluid collected on the swab from the affected area was transferred onto a glass slide for Gram staining by a routine method. A diagnosis of OC was made when colony formation was noted during incubation and the presence of pseudo mycelium was shown by Gram staining [14].

**Evaluation of susceptibility to antifungal drugs**

Candida strains detected by the above-mentioned methods were re-seeded onto Sabouraud agar medium (Nippon Becton and Dickinson Company, Ltd.), followed by 48-hour incubation at 36°C. Then the culture was diluted and adjusted to a McFarland factor of 0.5 by measuring the optical density at 530 nm using a spectrophotometer ViSpec II (Kyokuto, Japan), and applied for the assay to determine the susceptibility. A minimum inhibitory concentration (MIC) of each of AMPH-B, 5-FC, FLCZ, MCZ, ITCZ, and MCFG against these Candida strains were determined with a Yeast-like Fungal Sensitivity Kit ASTY (Kyokuto Pharmaceutical) compatible with the National Committee for Clinical Laboratory Standards (NCCLS) M-27A method [15]. The kit includes 11 concentrations of each antifungal drug in a 96-well microplate, i.e. final concentrations 0, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 μg/mL for amphotericin B, miconazole, and micafungin, 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 μg/mL for fluconazole and 5-FC, and 0, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, and 8 μg/mL for itraconazole and micafungin. The concentration “0 μg/mL” is regarded as a control. Strains with high MIC were subjected to re-measurement of MIC with a Yeast-like Fungal DP Eiken (Eiken Chemical) compatible with the Clinical and Laboratory Standards Institute (CLSI) M27-A3 method [10,16], MIC50 and MIC90, MICs required to inhibit the growth of respective 50% and 90% of Candida species, were calculated. Resistant strains were determined by the following MIC criteria: AMPH-B,
FLCZ, ≥64 μg/mL; 5-FC, ≥32 μg/mL; MCZ, ≥4 μg/mL; MCFG, >2 μg/mL; and IT CZ, ≥1 μg/mL.

**Informed consent and ethical guideline**

All enrolled patients provided written informed consent upon directly providing verbal and written detailed explanation of this investigation. This study was approved by the Human Ethics Committee of the Kagoshima University Medical and Dental Hospital (No.23-18 and 23–27).

**Statistical analysis**

Fisher’s exact probability test was employed to determine differences in gender (2 × 2), chief complaints (2 × 8), systemic disease (yes or no; 2 × 2), steroid use (2 × 2), presence of dentures (2 × 2), oral candidiasis (2 × 4), composition of isolated *Candida* species (2 × 7), and the incidence of resistant strains between the 2006–2007 study and the 2012–2013 study. The difference in age between the 2 studies was examined by Student’s *t*-test. *P* value less than 0.05 was regarded as statistically significant.

**Results**

**Background variables**

As shown in Table 1, there were no statistical differences in background variables in terms of gender, age, use of steroid hormone treatment, presence and the kind of systemic diseases and presence of dentures between patients in the 2006–2007 study and those of the 2012–2013 study. Among patients with 1 chief complaint, xerostomia was higher in the 2012–2013 study (23.2%) than in the 2006–2007 study (8.8%), while tylosis linguae was lower at 7.0% and 0%, respectively. Collectively, the composition of chief complaints was significantly different between the 2006–2007 patients and 2012–2013 patients as analyzed by Fisher’s exact probability test (*p* < 0.05). Despite no significant difference in the occurrence of systemic disease between the 2 studies (77.2% vs. 86.6%), the presence of autoimmune diseases was higher in the 2012–2013 study than in the 2006–2007 study (27.6% vs. 6.7%, respectively).

**Classification of oral candidiasis (OC)**

Classification of diagnosed OC cases is also shown in Table 1. Among the 57 patients in the 2006–2007 study, OC was most frequently classified as pseudomembranous OC (73.7%), followed by erythematous OC (19.3%). In the 2012–2013 study, however, erythematous OC accounted for half (46.4%) of OC cases, followed by pseudomembranous OC (37.5%) and mixed OC (erythematous and pseudomembranous, 12.5%), but no statistical difference was noted.

**Isolated *Candida* species**

In total, 158 strains of *Candida* were isolated in patients in the 2012–2013 study. The most frequently isolated *Candida* species were *Candida albicans* (C. albicans, 116 strains, 73.4%), followed by *C. glabrata* (36 strains, 22.8%), *C. tropicalis* (3 strains, 1.9%), *C. parapsilosis* (2 strains, 1.3%), and *C. krusei* (1 strain, 0.6%) (Table 2). When analyzed on a patient basis, *C. albicans* was isolated in all patients (*n* = 112), while *C. glabrata* was never found on its own, only concomitantly with *C. albicans* in 36 patients. More than one kind of *Candida* species was isolated in 41 patients, with concomitant *C. albicans* and *C. glabrata* being the most common combination in approximately 90% of these patients. In contrast, among 65 strains of *Candida* isolated in patients in the 2006–2007 study, 56 strains of *C. albicans* (86.2%), 8 strains of *C. glabrata* (12.3%), and 1 strain of *C. tropicalis* (1.5%) were identified, and no strains of *C. parapsilosis* and *C. krusei* were detected. Thus, in the 2012–2013 study, the number of isolated strains of *C. glabrata* was almost double that of the 2006–2007 study, and the ratio of *C. albicans* to all *Candida* strains was therefore significantly lower in the 2012–2013 study when analyzed on a strain-basis or on a patient-basis. Thus, in both patient-basis and strain-basis analyses, a statistical difference was observed between the 2006–2007 study and the 2012–2013 study (*p* < 0.01).

**Susceptibility to antifungal drugs**

Since few (1 to 3 strains) of the *Candida* species other than *C. albicans* and *C. glabrata* were isolated in either study, MIC values of antifungal drugs against *C. tropicalis*, *C. parapsilosis*, and *C. krusei*, which were never isolated alone but only in combination with *C. albicans* and *C. glabrata*, were not reliable. Therefore, the results of susceptibility testing mainly refer to the MICs of *C. albicans* and *C. glabrata* (Table 3).

**Amphotericin B (AMPH-B)**

The MIC was found to be low (≤2 μg/mL) in 63 (96.9%) of the 65 strains in the 2006–2007 study and in 153 (99.4%) of the 154 strains in the 2012–2013 study. Two strains of *C. albicans* in the 2006–2007 study and 1 strain of *C. glabrata* in the 2012–2013 study demonstrated high MIC of over 16 μg/mL. However, the presence of resistant strains could not be detected in the concentration range used in this analysis. There was no obvious difference in the susceptibility of the 2 main *Candida* species, *C. albicans* and *C. glabrata*, between the 2 studies, with MIC90 values of 0.5 μg/mL and 2.0 μg/mL, respectively, in the 2006–2007 study and of 1.0 μg/mL and 1.0 μg/mL, respectively, in the 2012–2013 study.
Flucytosine (5-FC)

Two strains of *C. albicans* in the 2006–2007 study and 2 strains of *C. albicans* and 1 strain of *C. glabrata* in the 2012–2013 study demonstrated high MIC of more than 32 μg/mL. There was no obvious difference in the susceptibility of *C. albicans* and *C. glabrata* between studies, with the 2006–2007 study demonstrating MIC90 of 0.5 μg/mL and 16 μg/mL, respectively, and the 2012–2013 study, of 1.0 μg/mL and 8.0 μg/mL, respectively.

Fluconazole (FLCZ)

MIC values were found below 64 μg/mL in 60 (92.3%) of the 65 strains in the 2006–2007 study and in 146 (92.4%) of the 158 strains in the 2012–2013 study. High MIC ≥64 μg/mL was recorded in 5 strains (9.5%) in the 2006–2007 study, of which 3 strains were *C. albicans*, 1 strain was *C. glabrata*, and 1 strain was *C. tropicalis*, and in 12 strains (7.6%) in the 2012–2013 study, of which 3 strains were *C. albicans* and 9 strains were

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Table 1 Background variables of patients with oral candidiasis enrolled in the 2006–2007 and 2012–2013 studies

| Subjects number | Total  | 2006-2007 | 2012-2013 | Test |
|-----------------|--------|-----------|-----------|------|
| Gender          |        |           |           |      |
| Male            | 19 (33.3) | 30 (26.8) |           | Fisher’s exact probability test |
| Female          | 38 (66.7) | 82 (73.2) |           | [2 × 2] N.S. |
| Age             |        |           |           |      |
| Average [max-min] | 67.9 [26–90] | 69.6 [21–97] | Student’s t-test | N.S. |
| Chief Complaints|        |           |           |      |
| Heat sensation(HS) | 23 (40.4) | 46 (41.1) |           | Fisher’s exact probability test |
| Taste abnormality (TA) | 2 (3.5) | 0 (0) |           | |
| Xerostomia (X) | 5 (8.8) | 26 (23.2) |           | |
| Tylosis linguae (TL) | 4 (7.0) | 0 (0) | Fisher’s exact probability test |
| Rubor/Erosion (R/E) | 4 (7.0) | 8 (7.1) | [2 × 8] | |
| HS + TA + X | 9 (15.8) | 16 (14.3) | p < 0.05 | |
| HS + TA + R/E | 5 (8.8) | 8 (7.1) |           | |
| HS + TL | 5 (8.8) | 8 (7.1) |           | |
| Total          | 57 (100) | 112 (100) |           | |
| Systemic disease |        |           |           |      |
| Yes            | 44 (77.2) | 97 (86.6) | Fisher’s exact probability test |
| None           | 13 (28.8) | 15 (13.4) | [2 × 2] | N.S. |
| Total          | 57 (100) | 112 (100) |           | |
| Endocrine      | 22 (29.3) | 22 (17.3) |           | |
| Cardiovascular | 18 (24.0) | 28 (22.0) |           | |
| Gastrointestinal | 123 (16.0) | 12 (9.4) | Fisher’s exact probability test |
| Malignant tumor | 8 (10.7) | 12 (9.4) | [2 × 7] | |
| CNS            | 4 (5.3) | 10 (7.9) | N.S. | |
| Viral          | 6 (8.0) | 8 (6.3) |           | |
| Autoimmune     | 5 (6.7) | 35 (27.6) |           | |
| Total          | 75 (100) | 127 (100) |           | |
| Steroid        |        |           |           |      |
| Dose           | 12 (21.1) | 28 (25.0) | Fisher’s exact probability test |
| Non-dosed      | 45 (78.9) | 84 (75.0) | [2 × 2] | N.S. |
| Total          | 57 (100) | 112 (100) |           | |
| Denture        |        |           |           |      |
| Wearer         | 37 (64.9) | 57 (50.9) | Fisher’s exact probability test |
| Non-wearer     | 20 (35.1) | 55 (49.1) | [2 × 2] | N.S. |
| Total          | 75 (100) | 112 (100) |           | |
| Oral candidiasis |      |           |           |      |
| Pseudomembranous | 42 (73.7) | 42 (37.5) | Fisher’s exact probability test |
| Erythematous   | 11 (19.3) | 52 (46.4) | [2 × 2] | N.S. |
| Hypertrophic   | 2 (3.5) | 4 (3.6) | [2 × 2] | |
| Mixed          | 2 (3.5) | 14 (12.5) | N.S. | |
| Total          | 55 (100) | 112 (100) |           | |

Figure in parentheses represents the percentage except the age.
C. glabrata, and which were all found to be FLCZ-resistant strains. A higher proportion of resistant C. glabrata strains were found in the 2012–2013 study (9/36, 25.0%) than in the 2006–2007 study (1/8, 12.5%). An increase in MIC 50 of C. glabrata from 2 μg/mL in 2006–2007 to 16 μg/mL in 2012–2013 was also observed.

Miconazole (MCZ)
The MIC of 60 (92.3%) of the 65 strains in the 2006–2007 study and in 139 (88.0%) of the 158 strains in the 2012–2013 study were below 4 μg/mL. High MIC (≥4 μg/mL) was recorded for 3 strains of C. albicans (4.6%) and 2 strains of C. glabrata (25.0%) in 2006–2007, and for 5 strains (3.2%) in 2012–2013, of which 3 strains were C. albicans (10.7%), 1 strain was C. glabrata (33.3%), and 1 strain was C. tropicalis (33.3%). Although MIC 90 against C. glabrata in the 2012–2013 study (16 μg/mL) was higher than that in the 2006–2007 study (4.0 μg/mL), no other significant differences were seen between studies. The incidence of resistant C. glabrata was similar between the 2 studies at 25.0% (2/8 strains) and 30.6% (11/36 strains), respectively.

Itraconazole (ITCZ)
The MIC of 54 (83.1%) of the 65 strains in the 2006–2007 study and of 127 (80.4%) of the 158 strains in the 2012–2013 study were less than 1 μg/mL. High MIC (≥1 μg/mL) was recorded in 11 strains (16.9%) in 2006–2007, of which 6 strains were C. albicans (10.7%), 4 strains were C. glabrata (50.0%), and 1 strain was C. tropicalis (100%), and in 31 strains (19.6%) in 2012–2013, of which 5 strains were C. albicans (4.5%), 24 strains were C. glabrata (66.7%), and 2 strains were C. tropicalis (66.7%). There was a higher proportion of resistant strains of C. glabrata against ITCZ in the 2012–2013 study (24/36 strains, 66.7%) than in the 2006–2007 study (4/8 strains, 50.0%), but the difference was not statistically different. The MIC 90 against C. glabrata was similar in both studies at >8.0 μg/mL, but that against C. albicans in the 2012–2013 study (0.25 μg/mL) was lower than that in the 2006–2007 study (1.0 μg/mL).

Micafungin (MCFG)
The MIC of 63 (96.9%) of the 65 strains in the 2006–2007 study and of 157 (99.4%) of the 158 strains in the 2012–2013 study were >4 μg/mL. High MIC (≥4 μg/mL) was recorded in only 2 strains (4.6%) in the 2006–2007 study and 31 strains (19.6%) in the 2012–2013 study, of which 5 strains were C. albicans (4.5%), 24 strains were C. glabrata (66.7%), and 2 strains were C. tropicalis (66.7%).
Table 3 Antifungal susceptibility profile, against amphotericin B, flucytosine, fluconazole, miconazole, itraconazole, and micafungin, of Candida species isolated from patients with oral candidiasis in the 2006–2007 and 2012–2013 studies

### a. amphotericin B (AMPH-B)

| Year       | Strain   | No. of strains | MIC (µg/mL) |    |     |     |     |     |     |     |     | MIC50 | MIC90 |
|------------|----------|----------------|-------------|----|-----|-----|-----|-----|-----|-----|-----|-------|-------|
|            |          |                | <0.03       | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1   | 2   | 4   | 8    | 16   | >16  |
| 2006-2007  | C. albicans | 56             | 8            | 43  | 3   | 2    | 0.5 | 0.5 |     |     |     |     |     |     |
|            | C. glabrata  | 8              | 1            | 2   | 2   | 2    | 1   |     |     |     |     |     |     |     |
|            | C. tropicalis | 1             | 1            |     |     |     |     |     |     |     |     |     |     |     |
| 2012-2013  | C. albicans | 112            | 4            | 12  | 86  | 9    | 1   |     |     |     |     |     |     |     |
|            | C. glabrata  | 36             | 1            | 2   | 16  | 14   | 2   |     |     |     |     |     |     |     |
|            | C. tropicalis | 3             | 1            | 2   |     |     |     |     |     |     |     |     |     |     |
|            | C. parapsilosis | 2          | 2            |     |     |     |     |     |     |     |     |     |     |     |
|            | C. krusei  | 1             | 1            |     |     |     |     |     |     |     |     |     | 0.5  | 0.5  |

### b. flucytosine (5-FC)

| Year       | Strain   | No. of strains | MIC (µg/mL) |    |     |     |     |     |     |     |     |     | MIC50 | MIC90 |
|------------|----------|----------------|-------------|----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-------|
|            |          |                | <0.125      | 0.125 | 0.25 | 0.5 | 1   | 2   | 4   | 8   | 16  | 32   | 64   | >64  |
| 2006-2007  | C. albicans | 56             | 18           | 8   | 24  | 3   | 1   | 2   | 0.25 | 0.5 |     |     |     |     |     |
|            | C. glabrata  | 8              | 4            | 1   | 1   |     | 2   |     | 0.125 | 16 |     |     |     |     |     |
|            | C. tropicalis | 1             | 1            |     |     |     |     |     |     | 0.125 | 0.125 |     |     |     |     |
| 2012-2013  | C. albicans | 112            | 56           | 1   | 43  | 3   | 3   | 4   | 1   | 1   |     | 0.25 | 1   |     |     |
|            | C. glabrata  | 36             | 20           | 6   | 6   | 2   | 1   | 1   |     |     |     | 0.125 | 8  |     |     |
|            | C. tropicalis | 3             | 3            |     |     |     |     |     |     |     |     | <0.125 | <0.125 |     |     |
|            | C. parapsilosis | 2          | 2            |     |     |     |     |     |     |     |     |     | 2   |     |     |
|            | C. krusei  | 1             | 1            |     |     |     |     |     |     |     |     |     | 0.25 | 0.25 |     |

### c. fluconazole (FLCZ)

| Year       | Strain   | No. of strains | MIC (µg/mL) |    |     |     |     |     |     |     |     | MIC50 | MIC90 |
|------------|----------|----------------|-------------|----|-----|-----|-----|-----|-----|-----|-----|-------|-------|
|            |          |                | <0.125      | 0.125 | 0.25 | 0.5 | 1   | 2   | 4   | 8   | 16  | 32   | 64   | >64  |
| 2006-2007  | C. albicans | 56             | 22           | 22  | 5   | 1   | 1   | 1   | 1   | 3   | 0.5 |     | 4    |     |     |
|            | C. glabrata  | 8              | 1            | 2   | 2   | 1   | 1   | 1   | 1   |     | 2   |     | >64  |     |     |
|            | C. tropicalis | 1             | 1            |     |     |     |     |     |     |     |     | 1   | >64  | >64  |     |
| 2012-2013  | C. albicans | 112            | 4            | 33  | 51  | 2   | 1   | 3   | 3   | 0.5 | 2    |     |     |     |     |
|            | C. glabrata  | 36             | 4            | 1   | 5   | 6   | 7   | 2   |     | 16  | 64  |     |     |     |     |
|            | C. tropicalis | 3             | 1            | 2   |     |     |     |     |     |     | 32  | 32  |     |     |     |
|            | C. parapsilosis | 2          | 2            |     |     |     |     |     |     |     |     |     | 2   |     |     |
|            | C. krusei  | 1             | 1            |     |     |     |     |     |     |     |     |     | 2    |     |     |

### d. miconazole (MCZ)

| Year       | Strain   | No. of strains | MIC (µg/mL) |    |     |     |     |     |     |     |     | MIC50 | MIC90 |
|------------|----------|----------------|-------------|----|-----|-----|-----|-----|-----|-----|-----|-------|-------|
|            |          |                | <0.03       | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1   | 2   | 4   | 8    | 16   | >16  |
| 2006-2007  | C. albicans | 56             | 2            | 22  | 20  | 8    | 1   |     |     |     |     |     |     |     |
|            | C. glabrata  | 8              | 1            | 2   | 2   | 1   | 1   |     |     |     |     | 1   |     |     |
|            | C. tropicalis | 1             | 1            |     |     |     |     |     |     |     |     | 0.125 | 0.125 |     |     |
| 2012-2013  | C. albicans | 112            | 2            | 20  | 64  | 18  | 1   | 1   | 1   | 2   | 3   |     | 0.25 | 0.5  |
|            | C. glabrata  | 36             | 1            | 5   | 12  | 7   | 3   | 3   | 2   | 3   | 2   | 16   |     |     |
|            | C. tropicalis | 3             | 1            | 1   |     |     |     |     |     |     |     | 1    |     |     |
|            | C. parapsilosis | 2          | 1            | 1   |     |     |     |     |     |     | 1-2 | 2    |     |     |
|            | C. krusei  | 1             | 1            |     |     |     |     |     |     |     |     |     | 4    |     |     |
study, of which 1 strain each was *C. albicans* and *C. glabrata*, and 1 strain of *C. glabrata* in the 2012–2013 study. Although the MIC90 against *C. glabrata* in 2012–2013 (1.0 μg/mL) was lower than in 2006–2007 (>16 μg/mL), there was no other obvious differences in the susceptibility of *C. albicans* and *C. glabrata* between studies.

**Discussion**

Non-*C. albicans* Candida (NCAC) strains have been isolated in increasing numbers in medically compromised patients including those with OC and denture stomatitis. *C. albicans* remains the most common fungus associated with infection, followed by *C. glabrata*. In clinically isolated samples from the blood, urine, and tracheal secretion of candidemia patients, the isolation frequency of *C. glabrata* has been found to be 16–26%, which is the highest among NCAC strains [17-20]. In the Prospective Antifungal Therapy Alliance (PATH Alliance*), in which 3,648 patients with candidemia were enrolled from 2004 to 2008 in the US, the most common *Candida* species was *C. albicans* (42.1%), followed by *C. glabrata* (26.7%), and the proportion of candidemia caused by NCAC strains was higher than that caused by *C. albicans* [21]. Such a high emergence of NCAC strains, especially of *C. glabrata*, in candidemia has recently been reported, with a higher incidence of *C. glabrata* found from 2006 to 2010 (range: 4.8–23.5%, *P* = 0.024) in Brazil [22]. In addition, the prevalence of infection due to a specific *Candida* species may vary by geographic region [23]. However, few reports exist on differences in variation of isolation frequency and its relation to candidemia in oral candidiasis. In contrast, there are several reports on oropharyngeal *Candida* infections due to *C. glabrata* [24,25]. In the present study, the isolation frequency of *C. glabrata* increased from 14.1% in 2006–2007 to 32.1% in 2012–2013 when assessed by the number of patients, indicating a similar tendency observed in Japanese OC patients. The tendency of a shift of NCAC species would also be attributable to frequent and wide use of azole antifungal drugs, such as fluconazole, in the treatment of mycosis [26]. Moreover, the isolation frequency of NCAC strains, especially *C. glabrata*, was reported to increase [26-28]. In Japan, as a result of a wide use of azole antifungal drugs as the standard remedy for deep-seated mycosis, it is supposed that the isolation frequency of *C. glabrata* became high.

By comparing background data of OC patients in the 2012–2013 study and in the 2006–2007 study, the
following differences were observed: (1) the frequency of xerostomia as a chief complaint increased from 24.6% to 37.5% of patients, (2) autoimmune disorders as a systemic disease increased from 6.7% to 27.6%, and (3) erythematous OC as a type of OC increased from 19.3% to 46.4%. Differences in the frequency of xerostomia and autoimmune disorders were associated with an increase in patients with Sjögren’s syndrome, a chronic and progressive autoimmune disorder mainly characterized by xerostomia. Differences in erythematous OC may be partly attributed to an increase in simultaneous identification of C. albicans and C. glabrata as observed in 32.1% of patients in the 2012–2013 study. In fact, cases of mixed infection suggest that C. albicans enhances the invasiveness of C. glabrata, and leads to increased lactate dehydrogenase (LDH) release from a reconstituted human oral epithelium (RHOE) in vitro [29]. Furthermore, LDH release paralleled the observed histological damages in RHOE, which may be associated to the increase in erythematous OC.

By comparing the susceptibilities of patients in the 2012–2013 study with that of the 2006–2007 study, azole antifungal drugs (FLCZ, MCZ, and ITCZ) demonstrated high MIC$_{90}$ values against C. glabrata in both studies. In particular, the MIC$_{90}$ value of MCZ against C. glabrata increased from 4 μg/mL (2006–2007) to 16 μg/mL (2012–2013). Such high MIC values of azole antifungal drugs, which suggest a decrease in the susceptibility of C. glabrata to these drugs, have been recognized in several studies [17–19,30]. Decreased susceptibility to FLCZ was mainly seen against C. glabrata isolated from patients with blood stream infections, with a dose-dependent susceptibility rate of 76.5%, while the susceptibility rates of FLCZ were 100% for C. albicans and C. parapsilosis [19]. Similar results were also found for systemic candidiasis [18,20,23]. Decreases in the susceptibility of NCAC, especially of C. glabrata, resulted from resistance to FLCZ, leading to oropharyngeal Candida infections, which are more severe and more difficult to treat in HIV-infected and cancer patients [30]. The development of resistance in C. glabrata was observed against other azoles, such as ITCZ and ketoconazole [20,31]. In addition, in the present study, although statistical significance was not obtained, resistant strains of C. glabrata increased from 50.0% (4/8) in the 2006–2007 study to 66.7% (24/36) in the 2012–2013 study. In cases of other azole antifungal drugs, FLCZ and MCZ, resistant strains of C. glabrata increased slightly from 12.5% to 25.0% and from 25.0% to 30.6%, respectively. Thus, a tendency for increased isolation frequencies of resistant C. glabrata against azole antifungal drugs, especially ITCZ have been observed. Frequent exposure of antifungal drugs, especially azoles, is known to lead to an increase in resistant Candida strains, which has been reported since fluconazole was clinically available in 1989. After that new azole antifungal drugs, such as itraconazole and voliconazole, have been widely used for deep-seated mycosis. Thus, increases in clinically available antifungalazole drugs and in their clinical usages for the treatment of deep-seated mycosis could lead to an increment of resistant Candida strains. This tendency would be still continuing during the period of 2006–2013.

Conclusions
In oral Candida species isolated from OC patients, the ratio of C. glabrata to C. albicans increased in the 2012–2013 study as compared to the 2006–2007 study, accompanying the tendency of increases in tolerant strains of C. glabrata against azole antifungal drugs and in cases of autoimmune disease. The higher isolation frequency of C. glabrata, especially of co-isolation frequency with C. albicans, may be associated with the increase in erythematous OC. Considering the increase in C. glabrata resistant strains against azoles, selection of appropriate antifungal drugs and dosing regimens against resistant C. glabrata, as well as against C. albicans is important.

Competing interests
The authors declare that they have no competing interests.

Author’s contribution
KY and MY participated in the design of the study, conducted the study, and conducted the data collection and analyses, and drafted the manuscript. NT, FJ, HD, SR, HT, and SK, participated in the design of the study and carried out the data collection. All authors contributed to the writing of the manuscript and critically reviewed the final version. All authors read and approved the final manuscript.

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