Original Article

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Investigation of Clonal Relationship Between *Candida Parapsilosis* and *Candida Glabrata* Strains Isolated from Blood Culture by Pulse Field Gel Electrophoresis

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Abstract

Aim: Candidemia may occur when endogenous *Candida* species in the intestinal microbiota enter the bloodstream in patients with risk factors. Apart from endogenous transmission, patients can also transmit it exogenously. In the current study, we aimed to investigate the fluconazole susceptibility and genotypes of *Candida parapsilosis* (*C.parapsilosis*) and *Candida glabrata* (*C. glabrata*) strains that cause candidemia.

Methods: Twenty-six *C. parapsilosis* and sixteen *C. glabrata* strains were included in the study. Fluconazole sensitivity was determined by the broth microdilution method. Genotyping of strains was done by pulsed-field gel electrophoresis.

Results: Nine (34%) of *C. parapsilosis* strains and 3 (19%) of *C. glabrata* strains were found to be resistant to fluconazole. Among 26 *C. parapsilosis* isolates, 17 different genotypes were detected and clustered isolates were collected in five clusters. Fourteen out of the 26 *C. parapsilosis* isolates are placed in one of the clusters, with a clustering rate of 53.8%. Among 16 *C. glabrata* isolates, 11 different genotypes were detected and divided into four clusters. Nine out of the 16 *C. glabrata* isolates are placed in one of the clusters, with a clustering rate of 56.2%.

Conclusion: Our data indicated the possibility of nosocomial transmission of *C. parapsilosis* and *C. glabrata* among intensive care unit patients in our hospital. Infection control policies should be strictly applied in our hospital to prevent cross-transmission.

Keywords: *Candida parapsilosis*, *Candida glabrata*, fluconazole susceptibility, genotype

Introduction

Bloodstream infections caused by *Candida* are less common than those caused by other pathogens. They are seen especially in patients who have undergone surgery, those in intensive care for long periods, those who are cancer patients, and immunocompromised patients who have undergone allogeneic stem cell transplantation. *Candida* species are isolated from 4.5% of hospital-acquired bloodstream infections (1). Among *Candida* spp., *Candida albicans* (*C. albicans*) is the most common species worldwide, although recently non-albicans *Candida* species have been reported to be more common (2). Additionally, the incidence of non-albicans *Candida* species varies from region to region. While *Candida parapsilosis (C. parapsilosis)* is more common in Asia (including Japan and China), Latin America (including Brazil), and Southern Europe, *C. glabrata* is more common in the United States, Australia, and Northern Europe (3).

Candida parapsilosis strains can spread more easily in hospitals than other *Candida* species because they can stick to surfaces, attach to the hands of healthcare workers and medical devices, and last on surfaces for up to 28 days.

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Some studies have shown that *C. parapsilosis* strains have high minimal inhibitory concentrations for echinocandin drugs due to naturally occurring FKS1 polymorphism. Additionally, very high azole resistance rates have been reported in some regions. This makes it difficult to choose empiric antifungal drug treatment in *C. parapsilosis* infections (4-7). Recently, the rate of isolation of *Candida glabrata* (*C. glabrata*) from bloodstream infections has been increasing. *Candida glabrata* is isolated from 18-25% of candidemia cases in North America. It is the second most common *Candida* species causing candidemia. In addition, *C. glabrata* is the main bloodstream fungal pathogen in patients with hematological malignancies. Azole group antifungals are frequently used for prophylaxis and treatment of candidemia. As a result, azole resistance is gradually increasing among *C. glabrata* isolates. While the azole resistance rate of *C. glabrata* strains worldwide is around 8%, in some centers, this rate exceeds 20% (8,9).

Candidemia can happen when *Candida* species that normally live in the intestines get into the bloodstream of patients who have certain risk factors like cancer, long stays in intensive care, or long-term use of antibiotics. In addition to endogenous transmission, it can also be transmitted exogenously from patient to patient. Genotyping can help clarify potential *Candida* outbreaks in hospitalized patients and whether exogenous transmission is occurring (10).

We hypothesized that there is a link between fluconazole resistance and genotypes in *C. parapsilosis* and *C. glabrata* strains. We aimed to investigate the fluconazole susceptibility and genotypes of *C. parapsilosis* and *C. glabrata* strains that cause candidemia. Additionally, it was investigated whether there was clustering among genotypes. This will contribute to the strict implementation of infection control policies such as hand washing, using protective equipment, and staff training to prevent cross-contamination in hospitals and to antimicrobial resistance surveillance to prevent the spread of resistant isolates.

Materials and Methods

Compliance with Ethical Standards

For the study, ethics committee approval was obtained from the Tokat Gaziosmanpasa University Faculty of Medicine Clinical Research Ethics Committee (approval no.: 23-KAEK-027, date: 16.02.2023). Written consent was obtained from all participants, and the study complied with the Declaration of Helsinki.

Study Design

It is a cross-sectional study that included *C. parapsilosis* and *C. glabrata* strains causing candidemia between November 2019 and September 2021. Firstly, identification of strains isolated from blood culture was performed, and antifungal susceptibility tests were performed. Identification of *Candida* strains was performed with the YST8 diagnostic panel (Diagnostics, Slovenia), using their characteristic appearance in cornmeal Tween-80 (Himedia, India) medium and the Vitek II automatic device (bioMérieux, France). Fluconazole sensitivity was determined by the broth microdilution method (Sigma Aldrich, St. Louis, MO, USA) and interpreted using Clinical and Laboratory Standards Institute M27-A Supplement 4 clinical breakpoints. *Candida* krusei American Type Culture Collection (ATCC) 6258 and *C. parapsilosis* (ATCC 22019) were used as quality control strains.

Then, the genotypes of the strains were determined by pulse-field gel electrophoresis. Electrophoretic karyotyping for discrimination of isolates was performed as previously described in the literature with minor modifications (11,12). Electrophoresis was performed in 0.5×TBE buffer using the contour-clamped homogeneous electric field method with a Contour-Clamped Homogeneous Electric Field DRII system (Bio-Rad, Richmond, USA). The electrophoresis conditions were 12°C at 6 V/cm² for 48 hours. The initial and final switch times were 90 seconds and 360 seconds, respectively. For all methods, each gel was stained with ethidium bromide (5 mg/mL) and destained in tap water and then viewed and photographed under ultraviolet light.

Analysis of arbitrarily primed polymerase chain reaction band profiles was performed with the GelCompar software package (version 6.5; Applied Maths, Sint-Martens-Latem, Belgium). The Dice correlation coefficient was used for similarity calculations for band analysis, and the unweighted pair group method with arithmetic mean was used for clustering analysis. If the Dice similarity coefficient value was below 95%, isolates were identified as different genotypes.

Statistical Analysis

Statistical analysis of the data was performed with IBM SPSS Statistics version 20 (IBM, USA). Whether the numerical data were normally distributed was evaluated with the Shapiro-Wilk test. The expression of normally distributed data is "mean ± standard deviation", and the expression of non-normally distributed data is "median (25th percentile-75th percentile)." Student's t-test was used to compare numerical data that followed a normal distribution, and the Mann-Whitney U test was used to compare numerical data that did not follow a normal distribution. To compare qualitative data, the chi-square test was used. When more than 20% of cells had expected frequencies <5, Fisher's exact test was used.

Results

While the number of *C. parapsilosis* that caused candidemia between November 2019 and September 2021 was 26, the number of *C. glabrata* was 16. The median age of the patients with *C. parapsilosis* was found to be 74 years (65-83). The median age of the patients with

C. glabrata was 65 years (56-74). The median age of the patients with *C. parapsilosis* was significantly higher than that of patients with *C. glabrata* (p=0.036). Twenty-five (96%) of the patients with *C. parapsilosis* were receiving treatment in intensive care, and 1 (4%) in the oncology service. 14 (88%) of the patients with *C. glabrata* were receiving treatment in the intensive care unit (ICU) and 2 (12%) in the oncology service (Table 1).

Of the *C. parapsilosis* strains, 13 (50%) were susceptible to fluconazole, 4 (15%) were dose-dependent susceptible, and 9 (35%) were resistant. Of the *C. glabrata* strains, 13 (81%) were susceptible in a dose-dependent manner to fluconazole, and 3 (19%) were resistant. The minimum inhibitory concentration (MIC) range, MIC_{50}, MIC_{90}, and geometric mean values of the strains are given in Table 2.

Among 26 *C. parapsilosis* isolates, 17 different genotypes were detected, which were collected into five clusters (tolerance 1.0, optimization 3.0, cut-off 95%). Fourteen out of the 26 *C. parapsilosis* isolates are placed in one of the clusters, with a clustering rate of 53.8%. The largest cluster is genotype 1 with four isolates. It is followed by genotype 8, and genotype 13 clusters with three isolates, while genotype 7 and genotype 9 cluster with two isolates. (Figure 1)

Table 1. Age, gender, median length of hospital stays and accompanying diseases of the patients

	***C. parapsilosis* (n=26)**	***C. glabrata* (n=16)**	**p-value**
Gender Male Female	 17(65%) 9 (35%)	 9 (56%) 7 (44%)	 0.553[1]
Age (year)	74 (65-83)	65 (56-74)	0.036[2]
Median length of hospital stays (days)	35 (21-80)	28 (19-35)	0.109[1]
Malignancy (%)	8 (31%)	5 (31%)	0.973[2]
Hypertension (%)	6 (23%)	3 (19%)	13
Cardiovascular disease (%)	3 (12%)	6 (38%)	0.062[3]
Lung disease (%)	2 (8%)	0 (0%)	0.516[3]
Diabetes mellitus (%)	2 (8%)	0 (0%)	0.516[3]

Mann-Whitney U test was used, chi-square test was used, Fisher's exact test was used
C.: Candida
[1]: Mann Whitney U test was used
[2]: Chi-square test was used
[3]: Fisher Exact test was used

Among 16 *C. glabrata* isolates, 11 different genotypes were detected. The clustered isolates were collected into four clusters (tolerance 1.0, optimization 3.0, cut-off 95%). 9 out of the 16 *C. glabrata* isolates are placed in one of the clusters, with a clustering rate of 56.2%. The largest cluster is the genotype 1 with three isolates. It is followed by genotype 5, genotype 6, and genotype 7, each forming clusters with two isolates (Figure 2).

Discussion

Long ICU stays, long-term systemic antibiotic use, underlying immunosuppressive diseases, and old age are the main candidemia risk factors (1). In this study, 96% of the patients isolated with *C. parapsilosis* and 88% of the patients isolated with *C. glabrata* were in the ICU. The number of patients over 60 years of age with isolated *C. parapsilosis* was 21 (81%). It was found to be 11 (69%) in *C. glabrata* patients. Various studies, similar to our study (13-15), have reported that *Candida* infections are more common in older patients, those in intensive care, and those with underlying diseases. It is known that the incidence of *C. parapsilosis* strains decreases with age, while the incidence of *C. glabrata* strains increases with age (16). However, in the current study, the median age of the patients with *C. parapsilosis* was significantly higher than that of patients with *C. glabrata* (p=0.036). The reason for this different result may be because of the examination of a certain period and the limited number of patients.

Antifungal resistance is a major problem in the treatment of candidemia. Fluconazole is usually used in the treatment of candidemia as well as other invasive *Candida* infections (17). Although *C. parapsilosis* isolates are usually reported as susceptible to fluconazole, recent studies have documented an increase in fluconazole-resistant *C. parapsilosis* isolates (18). Pfaller et al. (19), in their study examining the activity of fluconazole against 20.788 invasive *Candida* spp. isolates collected from 39 countries between 1997 and 2016, reported that 3.9% of *C. parapsilosis* isolates were resistant to fluconazole. The rate of fluconazole resistance was reported as 26.4% in *C. parapsilosis* isolates in a recent study from our country. In the current study, 35% of *C. parasilosis* isolates were found to be resistant to fluconazole. However, in a study published by Caggiano et al. (20) in 2024, the fluconazole

Table 2. MIC range, MIC_{50}, MIC_{90} and Geometric mean values of *C. parapsilosis* and *C. glabrata* strains

	MIC range	**MIC_{50}**	**MIC_{90}**	**Geometric mean**
C. parapsilosis (n=26)	0.25->256	2	16	2.75
C. glabrata (n=16)	8->256	8	>256	17.44

C.: Candida, MIC: Minimum inhibitory concentration

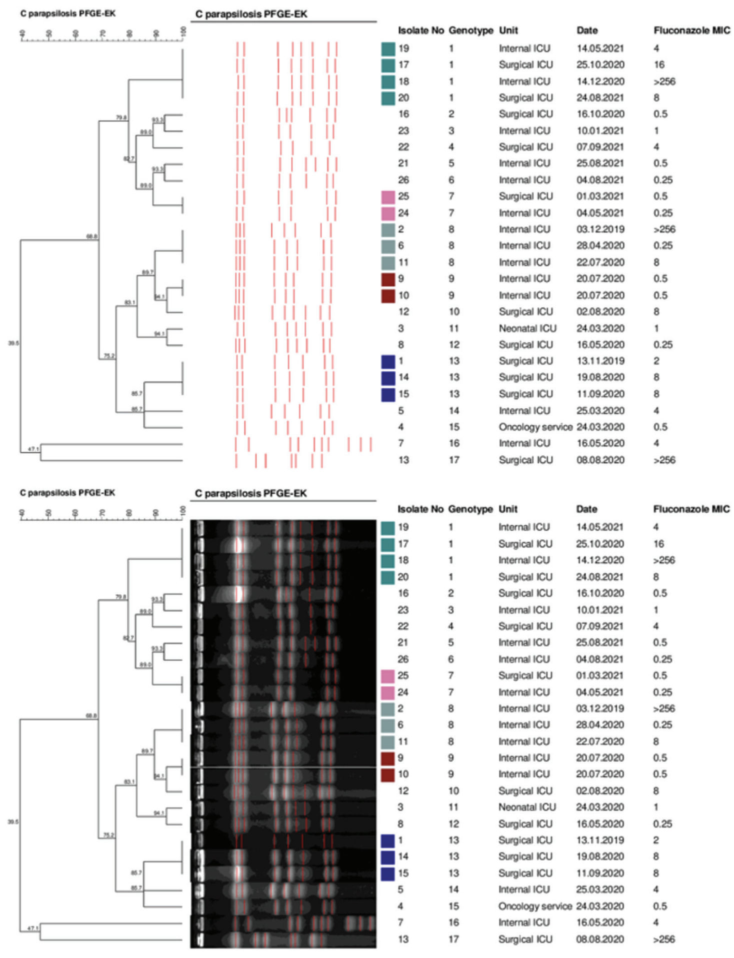

Figure 1. Dendogram showing clustering of *C. parapsilosis* strains
ICU: Intensive care unit, C.: Candida, PFGE: Pulsed-field gel electrophoresis MIC: Minimum inhibitory concentration

resistance rate in *C. parapsilosis* strains was reported as 82%. In the study by Misas et al. (4), while the fluconazole resistance rate of *C. parapsilosis* strains was found to be 17% in 2020, this rate became 34% in 2021. All these results show that the fluconazole resistance rate of *C. parapsilosis* strains has been increasing recently. This makes empirical drug selection and treatment of these strains quite difficult.

In a review study conducted by Beardsley et al., (21) fluconazole resistance rates in *C. glabrata* strains were found to range from 0% to 48% in 52 studies. In 16 of these, the fluconazole resistance rate was found to be above 10% (21). Castanheira et al. (22) have collected 1846 bloodstream isolates from 31 countries. They have documented that 11.9% of *C. glabrata* isolates were resistant to fluconazole. Yu et al. (13) reported a 6% fluconazole resistance rate in *C. glabrata* strains causing bloodstream infections. In this study, 81% of *C. glabrata* isolates were susceptible-dose dependent (S-DD) to fluconazole, while 19% were resistant. The incidence of *C. glabrata* strains and resistance rates to azole-echinocandin antifungals used in first-line therapy are increasing worldwide. Strategies need to be evaluated and implemented to prevent infection from occurring.

Candidemia mostly originates from endogenous *Candida* spp., but it can also be acquired. The pulsed-field gel electrophoresis (PFGE) plays an important role in tracking the source of candidemia, differentiating isolates, and conducting epidemiological investigations (23). In this study, we used PFGE to investigate the genetic relatedness among both the *C. parapsilosis* and *C. glabrata* isolates from candidemia.

Pulsed-field gel electrophoresis results of 26 *C. parapsilosis* isolates revealed the presence of 17 different genotypes. Fourteen of the 26 *C. parapsilosis* isolates were grouped into 5 clusters and one major group. All clustered isolates are from ICU patients. The largest cluster includes genotype 1, with 4 isolates. Three of these genotype 1 isolates were fluconazole resistant, while the other one was S-DD. The fact that all of these patients were treated

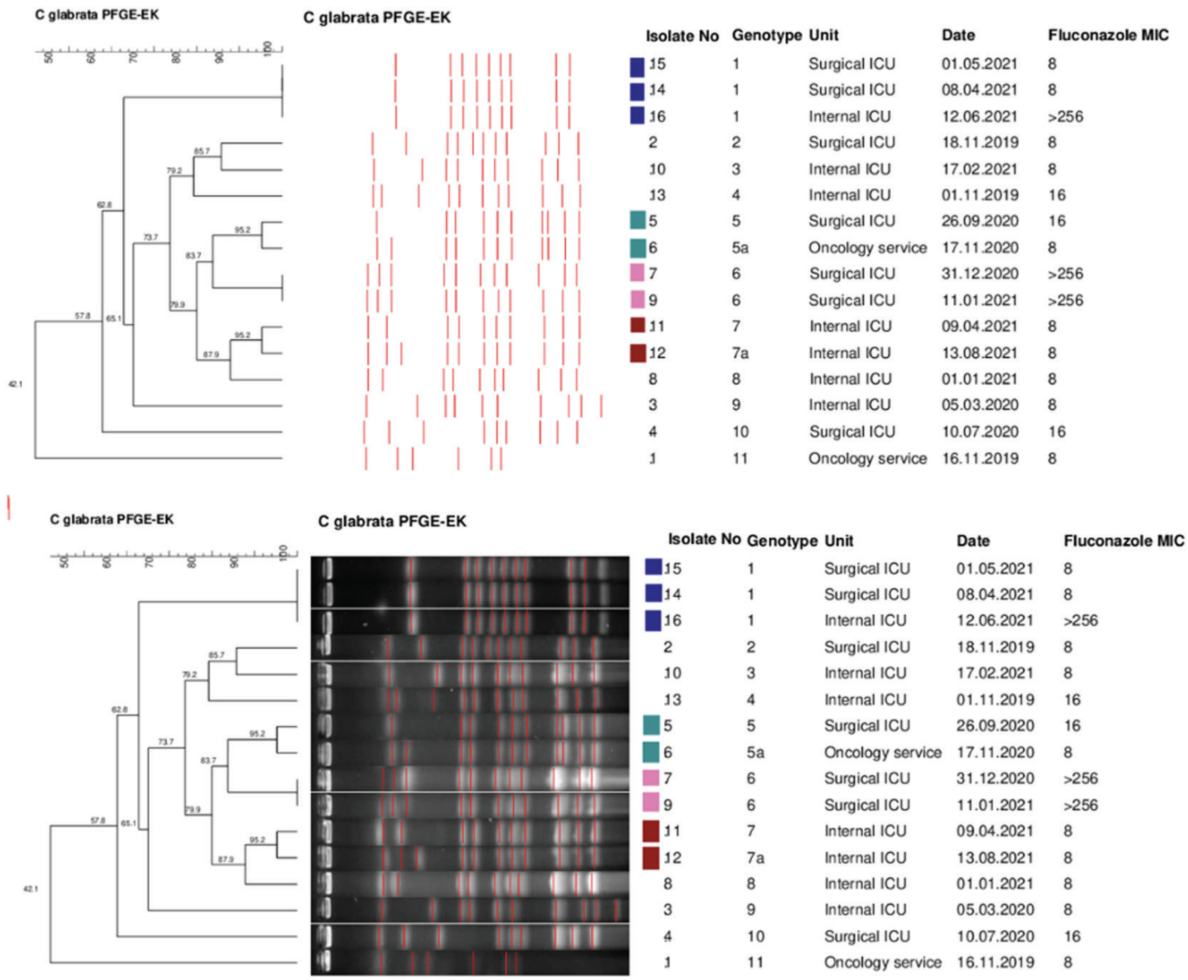

Figure 2. Dendogram showing clustering of *C. glabrata* strains
ICU: Intensive care unit, C.: Candida, PFGE: Pulsed-field gel electrophoresis, MIC: Minimum inhibitory concentration

in the same ICU and none of them were susceptible to fluconazole suggested that these strains originated from the same source. Pulcrano et al. (24) have analyzed 19 strains of *C. parapsilosis* isolated from the blood cultures of neonates by PFGE. They have reported a high degree of relatedness between the isolates. In their study, in which Caggiano et al. (20) examined 50 *C. parapsilosis* strains phylogenetically, it was found that fluconazole-resistant strains were similar to environmental strains. It was concluded that these strains may have been transmitted from the hospital environment. Another remarkable result was that all patients in genotype 13, the second largest cluster with 3 patients, had malignancy. In addition, the patients in this cluster were in the same ICU. Results suggest that these strains may have been transmitted from patient to patient within the hospital.

Pulsed-field gel electrophoresis results indicated that 9 of 16 *C. glabrata* isolates were clonally related with a clustering rate of 56.2%. All these isolates were recovered from the ICU except one. Two of the three fluconazole-resistant *C. glabrata* isolates belonged to genotype 6. Hwang et al. (8) examined 79 *C. glabrata* strains that caused infection in blood and found two different clusters in the same hospital. Similar to our study, they revealed that these strains were transmitted between patients treated within the hospital setting.

Study Limitations

The limitations of the current study are that the number of strains examined is small. This is because the study covers strains that grow within a certain period of time. Additionally, susceptibility testing could only be performed for fluconazole. Sensitivity testing against other antifungal drugs could not be performed. Despite these limitations, determining the genotypes of *Candida* strains and finding a cluster among them are the strengths of our study.

Conclusion

Our data indicated the possibility of nosocomial transmission of *C. parapsilosis* and *C. glabrata* among ICU patients in our hospital. Infection control policies such as hand washing, use of protective equipment, and personnel training should be strictly applied in our hospital to prevent cross-transmission. Antimicrobial resistance surveillance is crucial to prevent the spread of resistant isolates.

Ethics

Ethics Committee Approval: The study, ethics committee approval was obtained from Tokat Gaziosmanpasa University Faculty of Medicine Clinical Research Ethics Committee (approval no.: 23-KAEK-027, date: 16.02.2023)

Informed Consent: Written consent was obtained from all participants and the study complied with the human rights declaration.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.S.T., Concept: A.A., G.Y., Design: A.A., G.Y., B.O., Data Collection or Processing: E.S.T., Analysis or Interpretation: E.S.T., G.Y., B.O., Literature Search: A.A., G.Y., Writing: A.A., G.Y.

Conflict of Interest: No conflicts of interest were declared by the authors.

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