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Genotyping of *Candida albicans* Strains Obtained from Oropharyngeal Candidiasis Patients Based on ABC and RPS Typing Systems

Saeid Amanloo [1], Farzad Katiraee [2,*], Zahra Jahanshiri [3], Mojtaba Didehdar [4], Jamshid Mohammadi [5] and Zohreh Alibabaei [5]

[1]Department of Parasitology and Mycology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
[3]Department of Mycology, Pasteur Institute of Iran, Tehran, Iran
[4]Department of Parasitology and Mycology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
[5]Department of Entomology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

[*]*Corresponding author*: Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran. Email: katiraee_f@yahoo.com

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Abstract

Background: *Candida albicanss* has been introduced as one of the most common causes of nosocomial infections. Molecular typing methods are powerful tools in epidemiology to investigate the infection source of candidiasis, identify the transmission routes, and control the measures.
Objectives: This study aimed for genotyping *C. albicans* species isolated from oral cavities of the non-HIV patients who suffer from oropharyngeal candidiasis via combined ABC and repeat sequences (RPS) typing systems.
Methods: In this study, 31 DNA samples of clinical isolates of *C. albicans* were evaluated in terms of 25s ribosomal DNA region sequence or ABC typing, and ALT repeats numbers within RPS. DNA was amplified in two separate reactions, and the PCR products were electrophoresed to identify the genotypes of the isolates. Based on the band's pattern, phylogenetic analysis was conducted by UPGMA, and the discriminatory power of ABC and RPS typing was measured by Simpson's index of diversity.
Results: Genotype A with (14 isolates, 45.2%) were the most frequent and followed by genotype B (10 isolates, 32.3%) and Genotype C (7 isolates, 22.6%), respectively. In addition, genotype 3 with 25 isolates (80.6%) were the most prevalent, followed by genotype 2/3 (4 isolates, 12.9%) and genotype 3/4 (2 isolates, 6.5%) respectively. No significant relationship was found between the obtained genotypes and drug-resistant isolates ($P < 0.05$).
Conclusions: This study showed that 25s rDNA and RPS typing is a quick, simple, and cost-effective method with average discriminatory power and good reproducibility for *C. albicans* genotyping. It can be used for the epidemiology of *C. albicans* infections.

Keywords: *Candida albicans*, Candidiasis, Genotyping, Repetitive Sequence

1. Background

The incidence of *Candida*-induced infections has increased worldwide with high mortality rates in immunocompromised patients. These highly-prevalent yeasts, as a symbiotic microorganism and opportunistic pathogen, are responsible for a wide range of superficial, cutaneous, mucosal, and systemic infections (1). Recently, *Candida* has been introduced as the sixth most common cause of nosocomial infections (2). *Candida* species consists of a heterogeneous group of yeasts among which *Candida albicans* are known as the most prevalent species isolated from patients and healthy individuals (3-5). Despite the recent increase of non-*albicans* infections such as *C. glabrata*, *C. tropicalis*, the results of several studies indicate that over 80% of human infections caused by *Candida* species are associated with *C. albicans*. Studies have demonstrated that fungemia is associated with non-*albicans Candida* species (6-8). Moreover, mucocutaneous candidiasis is one of the most common fungal infections with *C. albicans* as its main pathogen. However, in recent decades non-*albicans* infections have increased (9, 10).

Currently, different typing methods are used to study population structure and species biodiversity, identify infection source and host-parasite relationship, determine and control the drug-resistant strains, and investigate the genetic link between the strains in epidemiologic studies. The most common typing methods are based on molecular advances, DNA fingerprinting, and genotyping. Each method should be evaluated in terms of ease of performance, reproducibility, discriminatory power, and inter-

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pretation (1). With high discriminatory power, genotyping methods are powerful tools in epidemiology to investigate the infection source of candidiasis, identify the transmission routes, and control measures (11, 12).

Ribosomal sequencing, and examining PCR products of 25s rDNA region have been frequently used in genotyping of *C. albicans* (13). In this method, based on the electrophoresis banding pattern, *C. albicans* is divided into several genotypes, including A, B, C, D, and E, among which genotype A has been reported as the dominant genotype in different regions (11, 13, 14). A study has reported that the combined analysis of 25s rDNA and repeated sequences (RPS) could increase the discriminatory power of *C. albicans* genotyping (10). In this method, a combination of microsatellite markers is used in strain typing within different chromosomes (15). Studies indicate that *C. albicans* consists of RPS in all its chromosomes except chromosome 3 (16-18). Each RPS region has a tandem short repeating unit, such as 172 bp known as ALT (16, 19, 20). The number of alternative lengthening of telomerase (ALT) repetitions in RPS differs in each chromosome, leading to diversity in RPSs fragment size (21). Consequently, subunits can be identified, which is an intriguing target for *C. albicans* genotyping (20). Based on the size difference of RPS and the number of copies of ALT, *C. albicans* is divided into four groups: Aa, Ab, Ac, and Ad. Moreover, it has been confirmed that genotype D is associated with *C. dubliniensis* (22, 23).

2. Objectives

This study aims for genotyping and identifying the population structure of *C. albicans* species isolated from oral cavities of the patients suffering oropharyngeal candidiasis lesions based on ABC and RPS typing systems.

3. Methods

In this study, 31 DNA samples of clinical isolates of *C. albicans* were collected from patients with oropharyngeal candidiasis who went to Cancer Institute of Tehran, Tehran, Iran. Then, they were evaluated in terms of ABC and RPS genotyping systems (24). The pair primers of CA-INT-L and CA-INT-R were used.to determine the genotyping of *C. albicans* based on 25s rDNA region sequence. Besides, pair primers of ASDcF and pCSCR were utilized to determine ALT repetitions within RPS (17, 23). Table 1 shows the nucleotide sequence of primers and molecular size of the PCR product (11). DNA was amplified in a reaction mixture (25 μL) containing 12.5 μL of master mix (SinaClon Bioscience Co., Karaj, Iran), 1 μL (4 ng) of DNA template, 1.5 μL of a forward primer (5 mM), 1.5 μL of a reverse primer (5 mM), and 8.5 μL of distilled water. The PCR cycle parameters were as follows: initial denaturation at 97°C for 7 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 40 sec and final extension 72°C for 5 min. All reaction mixtures were amplified using a thermal cycler (SimpliAmp; Applied biosystem; Cat No: A24811). Electrophoresis of the PCR product was performed using 1.5% agarose gel in TBE buffer. Band pattern of electrophoresis agarose gel was converted to a zero-one matrix.

3.1. Phylogenetic Analysis

Electrophoresis band pattern data was converted to zero-one matrix. Then, phylogenetic analysis was conducted by the unweighted pair group method with arithmetic averages (UPGMA) via an online tool (www.genoms.urv.cat/upgma) applied to zero-one matrix data. The discriminatory power of ABC and RPS typing was measured by Simpson's index of diversity (25), which demonstrates the capacity of a typing method to differentiate between species. To calculate the discriminatory power, an online tool (http://insilico.ehu.eus/) was used.

4. Results

The PCR product using 25s rDNA primer was obtained of 450 bp for genotype A, 840 bp for genotype B, and the two bands of 840 bp and 450 bp for genotype C. Genotype A with 14 isolates (45.2%) were the most frequent followed by genotype B (10 isolates, 32.3%) and genotype C (7 isolates, 22.6%) respectively. Also, the banding pattern of RPS typing showed that genotype 3 was the most prevalent (25 isolates, 80.6%), followed by genotype 2/3 (4 isolates, 12.9%) and genotype 3/4 (2 isolates, 6.5%) respectively. The frequency of genotypes in the combination of the ABC and RPS typing is shown in Table 2.

UPGMA dendrogram was constructed based on the combination of ABC and RPS typing system data using online tool (www.genoms.urv.cat/upgma), as shown in Figure 1. The discriminatory power of ABC and RPS typing system using Simpson's index of diversity was conducted through an online tool (http://insilico.ehu.eus/) (25). The results showed that the 31 clinical isolates of *C. albicans* were classified into 7 distinct genotypes, indicating a discriminatory power index of 0.7634.

5. Discussion

In this study, the ABC and RPS typing of *C. albicans* clinical isolates were carried out based on electrophoresis pattern of polymorphism. Since the ABC typing method cannot fully distinguish between strains, the fragment analysis was used via the combined microsatellite markers of

Table 1. Characteristics of Primers for the 25s rDNA, ALT Region, and Expected Bands

Primers	Sequence	Target	Expected amplicon size (bp)
CA-INT-L	5'-ATAAGGGAAGTCGGCAAAATAGATCCG-3'	25s rDNA	A-450; B-840; C-450, 840; D-1040; E-1080
CA-INT-R	5'-CTTGGCTGTGGTTTCGCTAGATAGTAGAT-3'		
ASDcF	5'-TGATGAACCACATGTGCTACAAAG-3'	RPS	1- 526; 2- 698; 3- 870; 4- 1042; 5- 1214; 6- 1396
pCSCR	5'-CGCCTCTATTGGTCGAGCAGTAGTC-3'		

Table 2. The Frequency of Genotypes *Candida albicans* Clinical Isolates in the Combination of the ABC and RPS Typing System, Based on the Result Genotype A3 is the Most Prevalent Genotype

ABC Typing	RPS Typing		
	Genotype 3 (%)	Genotype 2/3 (%)	Genotype 3/4 (%)
Type A	13 (41.9)	1 (3.2)	0 (0.0)
Type B	6 (19.35)	2 (6.5)	2 (6.5)
Type C	6 (19.35)	1 (3.2)	0 (0.0)

ABC and RPS typing system for more successful typing of *C. albicans*. RPS typing results of 25s rDNA region indicated that genotype A was the dominant genotype with 14 isolates (45.2%) followed by genotypes B and C with 10 (32.3%) and 7 (22.6%) isolates, respectively. The study results were in agreement with various reports from all over the world; genotype A has been reported as the dominant genotype (10, 11, 13, 23, 24, 26). Dalvand et al. (2018) reported that out of 27 *C. albicans* strains isolated from animal cases, 11, 6, 5, and 5 cases were recognized as genotypes A (40.8%), E (22.2%), B (18.5%), and C (18.5%), respectively. Iwata et al. (2006) reported that out of 179 strains of *C. albicans*, 92 isolates (51.4%) of genotype A, 49 isolates (27.3%) of genotype C and 38 isolates (21.2%) of genotype B were obtained (10).

Tamai et al. (2014) investigated *C. albicans* strains isolated from AIDS patients via ABC typing, and reported genotype A as the predominant genotype with 66% of frequency, followed by genotype B and genotype C with 24% and 10% respectively (26). The ratio of genotypes A, B, and C is not the same in different studies due to the clinical specimens, geographical areas, and different population of patients. In addition, the band pattern obtained from RPS (ALT) typing indicated that genotype 3 had the highest frequency with 25 isolates (80.6%) followed by genotypes 2/3 and 3/4 with 4 (12.9%) and 2 (6.5%) isolates, respectively. These results are also in agreement with the previous findings (10, 11). Some studies have reported that in RPS typing of *C. albicans*, genotype 3, and 3/4 are the predominant clinical isolates (10, 11).

It has been reported that RPS diagnostic and typing method based on the number of sequence repetitions of RPS (ALT) can be used not only for *C. albicans* genotyping but also for the identification and differentiation of *C. albicans* from *C. stellatoidea* and *C. dubliniensis* (10, 18, 22). *C. stellatoidea* and *C. dubliniensis* are known as pathogenic species that are closely related to *C. albicans* (18). however *C. stellatoidea* and *C. dubliniensis* were not isolated in this study. The RPS typing system has been introduced as a simple, quick, and efficient method for *C. albicans* genotyping (10, 11, 20). Unlike some methods, such as RAPD-PCR, this method has a proper reproducibility rarely affected by laboratory conditions (27). The study of Iwata et al. in 2006 showed that the RPS typing system was more appropriate than PFGE for the differentiation of *C. albicans* strains (10). However, discriminatory power (D.P) of samples indicated that 31 clinical isolates of *C. albicans* via RPS typing system were divided into 7 distinct genotypes with a D.P of 0.7634, while MLST genotyping of these samples (28) showed that 31 clinical isolates of *C. albicans* were classified into 29 different genotypes with D.P of 0.9957, indicating a much higher discriminatory power compared to RPS typing system.

MLST is a powerful method in the genotyping of *Candida* species with high discriminatory power and clear results (29-32). MLST is the only genotyping method, which allows global surveillance and comparison of *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. dubliniensis,* and *C. krusei* genotypes in a central web-based database. Thus, the possibility of epidemiological studies at the international level is provided. However, the high cost of nucleotide sequencing precludes public use of this method. In general, despite their deficiencies used in small-scale epidemiological studies, qualitative methods such as RAPD, PFGE, and RPS are affordable and time-saving, particularly for invasive nosocomial candidiasis with low sample size. Therefore, the typing method should be selected based on the purpose of study and facilities (33).

5.1. Conclusions

This study showed that 25s rDNA and RPS typing is a quick, simple, and cost-effective method with average discriminatory power for *C. albicans* genotyping and managing *C. albicans* infections.

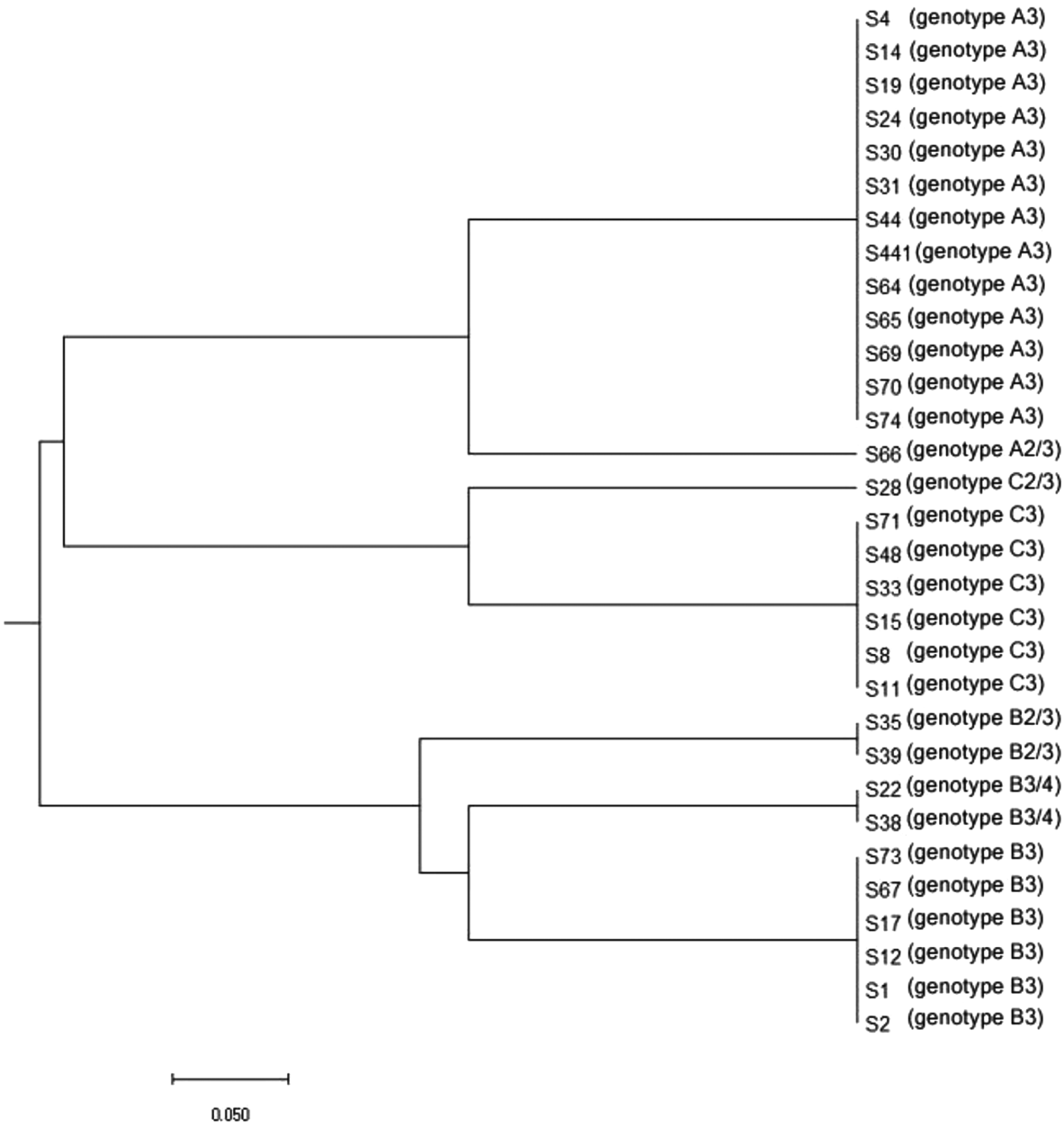

Figure 1. Dendrogram shows the genetic relationships between 31 isolates of *Candida albicans*. The dendrogram was constructed by zero-one matrix data from the combinations of ABC and RPS typing system and using the UPGMA method.

Footnotes

Authors' Contribution: Study concept and design: Farzad Katiraee and Saeid Amanloo; acquisition of data: Saeid Amanloo, Zahra Jahanshiri, Jamshid Mohammadi, and Zohreh Alibabaei; analysis and interpretation of data: Farzad Katiraee, Saeid Amanloo, and Mojtaba Didehdar; drafting of the manuscript: Saeid Amanloo and Farzad Katiraee.

Conflict of Interests: The authors declared no conflict of interest.

Ethical Approval: All procedures performed in studies including human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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