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ORIGINAL ARTICLE

Mating type and genotyping of *Candida albicans* isolated from different samples

Abed Karimpour[1], Maral Gharaghani[2], Amir Kamal Hardani[3], Hadis Jafarian[4], Ali Zarei Mahmoudabadi[1,5*]

[1]Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
[3]Department of Pediatrics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[4]Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[5]Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

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ABSTRACT

Background and Objectives: *Candida albicans* is a common opportunistic pathogen. Genotyping based on the 25S rDNA and mating type locus (MTL) allows for epidemiological and genetic profiling. This study aimed to characterize the genotypes and mating types of *C. albicans* isolates from various clinical sources in Iran.
Materials and Methods: Ninety-four isolates from clinical samples (saliva, urine, vaginal swabs, and skin scrapings) were cultured on CHROMagar Candida and identified by standard phenotypic methods. Genotyping was performed using CAINT primers, and mating type analysis was conducted using MTLa1 and MTLα1 primers.
Results: In this study, 94 isolates of *C. albicans* from various sources were analyzed. Genotype A was the most frequent (65%), followed by genotypes C (24.5%), B (9.6%), and D (1.1%). Most isolates (97.9%) were heterozygous at the MTL locus, only two isolates homozygous (α/α).
Conclusion: Genotype A and MTL-heterozygous strains were predominant among *C. albicans* isolates, suggesting a consistent molecular pattern across different clinical sources and regions.

Keywords: *Candida albicans*; Genotyping; Mating type; Homozygosity; Heterozygosity

INTRODUCTION

Candida albicans is the most clinically relevant species among the *Candida* genus (1). It belongs to the *C. albicans* complex, which also includes *C. africana* and *C. dubliniensis. Candida albicans* is a diploid yeast with eight heterozygous chromosomes, found in warm-blooded animals and environmental resources (2, 3). It is a common commensal fungus that colonizes the oropharyngeal cavity, gastrointestinal and vaginal tract, and the skin of healthy individuals (4). However, *C. albicans* can cause a wide

*Corresponding author: Ali Zarei Mahmoudabadi, Ph.D, Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Tel: +98-6133330074 Fax: +98-6133332036 Email: zarei40@hotmail.com

variety of infections due to changes in host conditions, such as alterations in host immunity, stress, resident microbiota, diabetes, etc. (5).

Among the available genotyping methods (6), the ABC system based on 25S rDNA is widely used due to its simplicity, cost-effectiveness, and ability to distinguish closely related species like *C. dubliniensis.* ABC genotyping is a PCR-based method that categorizes *C. albicans* into five genotypes: A, B, C, D, and E (7). Most studies have identified genotype A as the most common genotype among *C. albicans* isolates, but the frequency of other genotypes has varied in different studies (8). In a study by Mahmoodi et al., genotype A and genotype C were the most common types, among blood and vaginal isolates respectively (9). In another study by Shinobu et al., genotype A of *C. albicans* was the most common in vulvovaginal isolates (10). It is believed that these differences in genotype distribution may be influenced by multiple factors, including geographic location, isolate resources, and patient populations (11, 12).

Recent studies have revealed the capacity of *C. albicans* for sexual reproduction. The mating process in *C. albicans* was discovered through the identification of a mating-type-like (*MTL*) locus (13) located on chromosome 5 (14, 15). The MTL contains mating type genes and plays a crucial role in the mating process. In mating-incompetent a/α cells, two of the mating type genes, MTLa1 and MTLα2, encode components of the a1-α2 corepressor that suppresses mating and switching (16). Most *C. albicans* strains are heterozygous in this locus, but mating occurs in hemi- or homozygous strains (17). In one study, *MTL* analysis of 220 *C. albicans* isolates showed that the majority of strains (approximately 97%) were *MTL* heterozygous, while only 3% were *MTL* homozygous (14).

The present study aimed to characterize the genotypes and mating types of *C. albicans* complex isolates recovered from diverse clinical specimens in Iran, in order to contribute to the understanding of molecular epidemiology and strain distribution.

MATERIALS AND METHODS

Isolate collection and identification. A total of 94 *C. albicans* isolates were analyzed in this study. The isolates were collected from different anatomical sources, including the oral cavity, urine, vaginal discharge, and skin scrapings, in three Iranian cities: Yasuj, Shiraz, and Ahvaz. Oral swabs and saliva samples from healthy individuals were cultured on CHROMagar™ Candida plates (CHROMagar, France) and incubated at 35°C for 5-7 days. Green colonies presumptively identified as *C. albicans* were subcultured on Sabouraud Dextrose Agar (SDA; Liofilchem, Canada) and incubated for 24 hours at 35°C. Similarly, 50 µL of urine samples (≥10 mL per patient) were inoculated on CHROMagar Candida and incubated under the same conditions.

Phenotypic identification of *C. albicans* was confirmed by: Germ tube formation in fresh human serum, chlamydoconidia production on Cornmeal agar supplemented with 1% Tween 80 (HIMEDIA, India; Merck, Germany) and Growth at 42-45°C (8). Additionally, 10 isolates from patients with vaginal candidiasis and 10 isolates from patients with recurrent vaginal candidiasis were obtained from a previously published study by Gharaghani et al. (18). These isolates had been previously characterized morphologically and molecularly and were stored at room temperature prior to reactivation. Isolates were randomly selected from stocks. In total, the isolate pool included: 20 vaginal isolates (18), 13 skin isolates and 61 oral and urinary isolates collected directly in the present study. All procedures involving human samples were approved by the institutional ethics committee of Ahvaz Jundishapur University of Medical Sciences (Protocol No. OG-0204).

DNA extraction. Genomic DNA was extracted following the protocol of Looke et al. (19). Briefly, a fresh colony was suspended in 100 µL of 0.2 M lithium acetate (Central Drug House) and 1% SDS, (Cinna Gen, Iran) incubated at 70°C for 5 minutes. Then, 300 µL of 96% ethanol was added, followed by brief vortexing and centrifugation at 15,000 rpm for 2 minutes. The pellet was washed with 300 µL of 70% ethanol, centrifuged again, air-dried, and resuspended in 100 µL of sterile distilled water and the tubes were stored at -20°C. DNA quality and concentration were assessed by spectrophotometry (260/280 nm ratio) and 1% agarose gel electrophoresis (Cinna Gen, Iran).

ABC genotyping. Genotyping was performed using the ABC typing method described by Sawadogo et al. (7) targeting the 25S rDNA region with primers

Table 1. Used primers and their nucleotide sequences.

Primer	Nucleotide Sequences (5′-3′)	Reference
CA-INT-L	ATAAGGGAAGTCGGCAAAATAGATCCGTAA	(7)
CA-INT-R	CCTTGGCTGTGGTTTCGCTAGATAGTAGAT	(7)
MTLa1-F	TAAGAATGAAGACAACGAGG	(8)
MTLa1-R	CGTGTTTTTCTGCTATCAATTCC	(8)
MTLα1-F	TACATTCTGGTCGCGATGCTC	(8)
MTLα1-R	GTAATCCAAAGCCTCGCATAA	(8)

CA-INT-L and CA-INT-R (Table 1). The PCR mixture (25 µL total volume) included: 12.5 µL Master Mix RED (Ampliqon, Denmark), 0.5 µM of each primer, 2 µL genomic DNA and Nuclease-free water. PCR cycling conditions were: Initial denaturation: 97°C for 7 minutes, 35 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 40 sec and final extension at 72°C for 5 minutes. PCR products were transferred to a 1.5% agarose gel using TBE buffer for 40 minutes. Band sizes were interpreted as follows: 450 bp: Genotype A, 840 bp: Genotype B, 450 + 840 bp: Genotype C, 1040 bp: Genotype D and 1080 bp: Genotype E Genotypes D and E are typically associated with *C. dubliniensis.* Positive controls (typed *C. albicans* strains) and negative controls (nuclease-free water) were included in each run.

Mating type determination. Mating type was assessed by multiplex PCR using MTLa1 and MTLα1 primer sets as described by Jafarian et al. (8) (Table 1). Each 25 µL PCR reaction contained: Master Mix RED with 1.5 mM $MgCl_2$, 0.5 µM of each primer, 2 µL of genomic DNA and Nuclease-free water. PCR amplification conditions: Initial denaturation: 94°C for 10 minutes, 30 cycles of 94°C for 60 sec, 57°C for 45 sec, 72°C for 45 sec and Final extension: 72°C for 7 minutes. The PCR products were separated on 1.5% agarose gel in TBE buffer. Mating types were determined as: 535 bp band: MTLa homozygous, 423 bp band: MTLα homozygous and Both bands: MTLa/α heterozygous.

Statistical analysis. Statistical analysis was used to report frequencies and percentages of genotypes and mating types. Chi-square tests were applied to assess the association between genotypes, sample sources, and geographic origin using Social Science Statistics (https://www.socscistatistics.com/). A p-value < 0.05 was considered statistically significant.

RESULTS

In this study, 94 isolates of *C. albicans* from various sources were analyzed. Of these 36 isolates from Yasuj included samples of saliva, vaginal, recurrent vaginal, and urine. Additionally, 15 isolates from Shiraz, consisting of saliva and urine samples, were examined. Furthermore, 43 isolates of *C. albicans* were obtained from Ahvaz, including skin scraping, saliva, and urine samples. Our findings revealed that type A *C. albicans* had the highest frequency with 61 (65%) isolates, making it the dominant genotype among all isolates. This was followed by *C. albicans* genotype C with 23 (24.6%) isolates and then *C. albicans* genotype B with 9 (9.6%) isolates identified as the third type. Only 1 (1.1%) isolate was found to be correlated with genotype D (Fig. 1 and Table 2).

As shown in Table 2, genotype A was the most common genotype in oral isolates from Ahvaz and Yasuj. In contrast, genotype C was the most common in oral isolates from Shiraz, followed by genotype A. Additionally, genotypes B, A, and C were the most common in urinary *C. albicans* isolates from Yasuj,

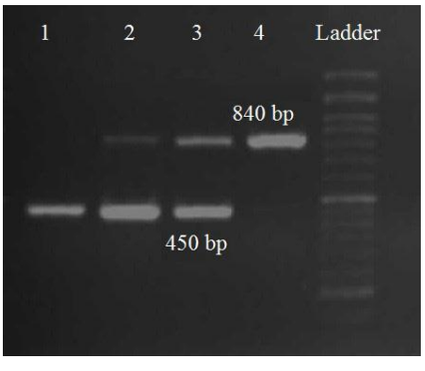

Fig. 1. PCR amplification of the 25S rDNA region showing genotyping patterns. Lane M: 100 bp DNA ladder; Lane 1: genotype A (450 bp); Lanes 2–3: genotype C (450 bp and 840 bp); Lane 4: genotype B (840 bp).

Table 2. Results of genotyping and mating type of tested isolates.

City	Sample sources	Isolates no.	Genotypes				Mating type	
			A	B	C	D	aα	αα
Yasuj	Oral cavity	6	5 (13.9%)	0 (0.0%)	1 (2.78%)	0 (0.0%)	6 (16.7%)	0 (0.0%)
	Urine	10	3 (8.3%)	5 (13.9%)	2 (5.6%)	0 (0.0%)	10 (27.8%)	0 (0.0%)
	Vaginal	20	14 (38.9%)	2 (5.6%)	4 (11.1%)	0 (0.0%)	20 (55.6%)	0 (0.0%)
	Total	36	22 (61.1%)	7 (19.4%)	7 (19.4%)	0 (0.0%)	36 (100%)	0 (0.0%)
Shiraz	Oral cavity	7	3 (20%)	0 (0.0%)	4 (26.7%)	0 (0.0%)	7 (46.7%)	0 (0.0%)
	Urine	8	5 (33.3%)	1 (6.7%)	2 (13.3%)	0 (0.0%)	8 (53.3%)	0 (0.0%)
	Total	15	8 (53.3%)	1 (6.7%)	6 (40%)	0 (0.0%)	15 (100%)	0 (0.0%)
Ahvaz	Oral cavity	22	14 (32.6%)	1 (2.3%)	6 (14%)	1 (2.3%)	20 (46.5%)	1 (2.3%)
	Urine	8	6 (14%)	0 (0.0%)	2 (4.7%)	0 (0.0%)	9 (20.9%)	0 (0.0%)
	Skin scarping	13	11 (25.6%)	0 (0.0%)	2 (4.7%)	0 (0.0%)	12 (27.9%)	1 (2.3%)
	Total	43	31 (72.1%)	1 (2.3%)	10 (23.4)	1 (2.3%)	41 (95.6%)	2 (4.7%)
Total		94	61 (64.9%)	9 (9.6%)	23 (24.5%)	1 (1.%)	92 (97.9%)	2 (2.1%)

aα, Heterozygous; αα, Homozygous (type a)

respectively. In comparison, the most common genotypes among isolates from Shiraz and Ahvaz were genotypes A followed by genotypes C. Overall, the frequency of genotypes among isolates from Ahvaz and Shiraz was similar, genotype A > genotype C > genotype B, while in Yasuj the rank was different: genotype A > genotype B / C. This study found no significant relationship between genotypes and different cities (p=0.068). There was also no significant difference when comparing genotypes with the sampling source (p=0.206). Mating types of *C. albicans* were investigated using the multiplex PCR method. Out of 94 *C. albicans* isolates, only 2 (2.1%) were homozygotes, both of which were α types. The remaining 92 (97.9%) isolates were heterozygous and showed both bands (Fig. 2 and Table 2).

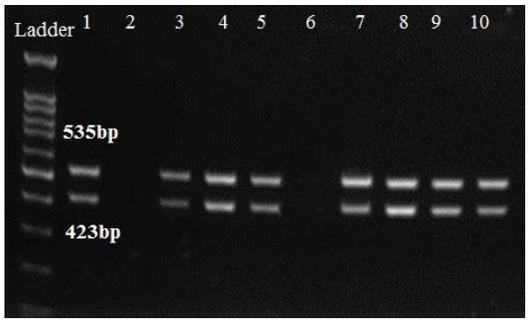

Fig. 2. Multiplex PCR for mating type determination. Lane M: 100 bp DNA ladder; Lanes 1, 3-5, 7-8: heterozygous a/α strains (bands at 535 bp and 423 bp); Lanes 2 and 6: no amplification detected.

DISCUSSION

Molecular typing of an infectious agent is crucial for epidemiological studies and the development of appropriate infection control strategies (20). In a study conducted in Malaysia, genotype A was found to be most abundant, followed by genotype B, and then genotype C. While similar to our study, in terms of genotype A being the most prevalent, unlike our findings, genotype B was more common than genotype C (21). Another study in Africa reported that genotype A was the most prevalent in 85% of isolates, followed by genotype B and then genotype C (7).

In this particular study, unlike ours, genotype B was more abundant than genotype C. Genotype D was the least frequent in our study, with only one isolate (1.06%) observed, and genotype E was not detected. Similarly, a study by Mahmoudi et al. in Shiraz, which examined 40 isolates of *C. albicans*, did not find genotypes (9). Likewise, in a study by Karakhan et al. in Ankara, genotypes D and E were absent, with genotype A being the most common, followed by genotypes B and C (22). In a study on animal samples, genotype E was significantly prevalent. A study by Dalvand et al. in 2018 in Tabriz examined 27 isolates of *C. albicans* from various animal samples. In this study, genotype E was the second most abundant after genotype A, with a frequency of approximately 22% compared to genotype A, which had a frequency of 40% (23).

Previous studies on the mating process in *C. al-*

bicans indicate that two homozygous organisms are involved, resulting in the formation of heterozygous organisms that are unable to mate (13). Therefore, a higher frequency of heterozygous organisms is expected in the population. In this study, the mating type of *C. albicans* was determined using MTLa and MTLa primers. Only 2 (2.1%) isolates were found to be homozygous (aa), while 92 (97.9%) were heterozygous (aa). In a study by Legard et al., it was reported that 108 out of the 120 samples examined were heterozygous strains, with only 12 samples being homozygous (24). Similarly, Jafarian et al. found that most of the *C. albicans* isolates were MTL-heterozygous (69.9%). Three isolates (4%) were homozygous for MTLa, and 3 isolates (4%) were homozygous for MTLα. All MTL-homozygous isolates belong to genotype A (8).

Limitations. The limitation of this study was that other PCR typing were not used for typing *C. albicans.* Moreover, the subgroup sizes of each province were limit.

CONCLUSION

In our study, four genotypes of the *C. albicans* complex were identified. Genotype A was the most common, accounting for 64.9% (61 isolates). However, genotype B was more prevalent among oral and urine isolates of *C. albicans* from Shiraz and Yasuj. Additionally, heterozygous strains were the most common mate type, representing 97.9% of the isolates. The correlation of antifungal susceptibility and different genotypes of *C. albicans* are interesting subjects for future studies.

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