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Genotyping of clinical isolates of *Candida glabrata* from Iran by multilocus sequence typing and determination of population structure and drug resistance profile

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Abstract

Candida glabrata is often the second most common causative agent for candidiasis following *Candida albicans*. Despite the importance of *C. glabrata* infections, few epidemiological studies have been conducted on this issue. The goal of this study was genotyping of clinical isolates of *C. glabrata* by multilocus sequence typing (MLST) technique for determination of the endemic prevalent genotypes and any association between isolation source and drug resistance. A total of 50 *C. glabrata* clinical isolates from Iran were analyzed by MLST and tested for *in-vitro* susceptibilities to amphotericin-B, caspofungin, fluconazole, and voriconazole according to the Clinical Laboratory Standards Institute (CLSI) M27-A4 document guidelines. Among these isolates, 16 distinct STs were identified, indicating a discriminatory power index of 0.9029. The three major sequence types (STs) were ST-59, ST-74, and ST-7 with 10, 8, and 7 isolates, respectively. Furthermore, a total of 11 new sequences were found, to which no allele numbers were assigned in the MLST database. All the isolates were susceptible to amphotericin B and caspofungin. Fluconazole resistance was shown in four isolates. Also, a sole isolate was voriconazole resistant. This study shows that the population structure of *C. glabrata* in Iran consists of groups closely related to the global database as well as to some new clonal clusters and STs. Regarding the high prevalence of 11 new sequences found in this study, it can be concluded that, these new alleles are among the endemic genotypes of Iran. The genotypes or STs were independent of drug susceptibility and anatomic sources.

Key words: *Candida glabrata*, candidiasis, multilocus sequence typing, genotyping, drug susceptibility, Iran.

Introduction

Candida species include a heterogeneous group of ubiquitous yeasts. These organisms are part of the normal flora of humans, rarely causing superficial or systemic infections.[1] Although *C. albicans* is the most common pathogenic yeast of humans, the incidence of non-*albicans* infections is increasing.[2] Likewise, the prevalence of *C. glabrata* infections has been increasing, and today is the second most common cause of candidiasis in the United States.[2–4] *Candida glabrata* infections are associated with high mortality rates,[5] and there is a tendency to rapidly developing resistance to azole antifungal agents, especially fluconazole.[6,7] Studies to comprehend the epidemiology and population structure of clinical isolates of *C. glabrata* are required.

Multilocus sequence typing (MLST) has emerged as a reliable, reproducible, and sensitive technique both for the detection of strains and establishing population structure in a number of *Candida* species.[8] At present, MLST schemes have been published for five of the pathogenic *Candida* species: *C. albicans*,[9,10] *C. dubliniensis*,[11] *C. glabrata*,[12] *C. krusei*,[13] and *C. tropicalis*.[14] On the other hand, MLST could not be used for *C. parapsilosis* because of the paucity of allelic polymorphisms in this species.[15] This approach provides a definition of population structures within a species and can reveal differences in geographical origins, anatomical sources, and other properties among clades or clonal complexes.[9,12,16–18] An MLST system with six housekeeping genes on separate chromosomes was developed for *C. glabrata*,[12] and two central internet databases were established for registration and analysis of *C. glabrata* MLST data from any global source (http://cglabrata.mlst.net and http://pubmlst.org).

Thus, considering the importance of genotyping to determine the source of infection, the geographical distribution and drug susceptibility, MLST was applied in this study for typing clinically relevant strains of *C. glabrata* with the aim of determining the prevalence of endemic genotypes and any association between isolation source and drug resistance.

Methods

Isolates

A total of 50 *C. glabrata* clinical isolates from clinical laboratories in Tehran-Iran were used in this study. Each strain was collected from a different patient. Table 1 summarizes the details of the tabulation of isolates tested. Prior to examination, all isolates were stored in glycerol at −20°C. The isolates were identified phenotypically by conventional biochemical methods, including API 20C AUX (API *Candida*) and CHROMagar®*Candida* (CHROMagar®, Paris, France) and confirmed by polymerase chain reaction (PCR) technique using *CGL1/CGL2 C. glabrata* specific primers, *C. glabrata* ATCC 90030 as the positive control, and *C. albicans* ATCC 10231 as the negative control.[19]

DNA extraction, PCR amplification, and sequencing

Fungal DNA was extracted using the conventional phenol-chloroform extraction method previously described by Müller et al.[20] The oligonucleotide primers used for MLST analysis had been described previously,[12] as shown in Table 2. PCRs were performed in a 50 μl volume containing 10 ng of genomic DNA, 0.2 μM each primer, and Sinaclon PCR master mix as described by the manufacturer (SinaClon BioScience Co., Karaj, Iran). Reaction conditions were as previously described for each individual primer set.[12] PCR products were purified using a gel extraction kit as described by the manufacturer (Kardan Co., Zanjan, Iran). Sequencing reactions were performed using BigDye terminator technology (ABI, Foster City, CA) with an ABI Prism 3730 (ABI series) DNA sequencer. All loci were sequenced in both forward and reverse directions with the same primers as used for the PCRs.

Data analysis

Nucleotide sequences were defined by alignment of forward and reverse sequences using MEGA software, 5.2 version, and polymorphic sites were confirmed by visual examination of the chromatograms. To assign allele numbers and STs, sequences were compared to the *C. glabrata* MLST database (http://cglabrata.mlst.net). For each gene, distinct alleles were identified and numbered using the database program (http://cglabrata.mlst.net). All new alleles were submitted to the *C. glabrata* database (http://pubmlst.org) and new allele numbers assigned to them. The alleles at each of the six loci of a strain's allelic profile was considered a unique sequence type (ST) or genotype.

The discriminatory power was measured by Simpson's index of diversity,[21] which calculates the probability of any pair of isolates to have different genotypes. For MLST the genotypes were based on distinct STs. The estimation for the possibility of selective pressure at each of the loci was computed by the ratio of nonsynonymous to synonymous nucleotide substitutions (dN/dS) calculated by the Nei and Gojobori.[22]

Statistical analysis was performed using SPSS, version 23, using cross tabs in the descriptive statistics based on Pearson's χ^2 to determine significant correlations between anatomical sources and drug resistance among the genotypes and clades. The results were considered significant for a $P < .05$.

Table 1. Clinical features, antifungal susceptibility and gnenotypes of *C. glabrata* isolates tested by MLST*.

Strain no.	Source	MICs (μg/ml)**				Genotype						ST
		FCZ	VCZ	CASP	AmB	FKS	LEU2	NMT1	TRP1	UGP1	URA3	
1	Pharynx	0.5	0.125	0.0313	0.0313	20	13	22	9	3	2	46
2	Vagina	4	0.016	0.0313	0.0313	7	13	17	9	3	19	59
3	Toenail	4	0.5	0.25	0.0625	20	13	22	9	3	2	46
4	Groin	32	1	0.016	0.125	**50**	16	9	4	13	**50**	**75**
5	Vagina	4	0.0625	0.016	0.0313	20	13	22	9	3	2	46
6	Blood	8	0.5	0.0313	0.25	5	7	8	7	3	6	3
7	Blood	64	0.25	0.0313	0.125	7	13	17	9	3	19	59
8	Blood	8	0.0313	0.016	0.0313	3	4	4	3	3	4	7
9	Blood	32	0.0625	0.0625	0.0313	**51**	7	**50**	**50**	**50**	9	**74**
10	Urine	8	0.25	0.0625	0.125	5	7	8	7	3	6	3
11	Urine	8	0.0313	0.0313	0.0625	7	13	17	9	3	19	59
12	Blood	4	0.125	0.016	0.25	7	13	17	9	3	19	59
13	Blood	2	0.125	0.0625	0.25	**50**	16	9	4	13	**50**	**75**
14	Blood	1	0.0625	0.25	1	7	13	17	9	3	19	59
15	Blood	2	0.016	0.0625	0.125	19	**50**	1	**51**	**51**	**51**	**76**
16	Blood	2	0.016	0.016	0.0313	7	13	17	9	3	19	59
17	Blood	4	0.0313	0.016	0.125	7	13	17	9	3	19	59
18	Blood	8	0.016	0.0313	0.0625	**51**	7	**50**	**50**	**50**	9	**74**
19	Blood	2	0.0313	0.0313	0.25	**51**	7	**50**	**50**	**50**	9	**74**
20	Urine	1	0.016	0.0313	0.125	7	13	17	9	3	19	59
21	Stool	32	0.25	0.0313	0.25	3	4	4	3	3	4	7
22	Sputum	8	0.25	0.0313	0.25	7	13	17	9	3	19	59
23	Vagina	2	0.016	0.016	0.125	**50**	16	9	4	13	**50**	**75**
24	Blood	1	0.016	0.0313	0.5	3	12	22	2	3	4	56
25	Blood	1	0.016	0.0313	0.25	3	12	22	2	3	4	56
26	Blood	2	0.016	0.0313	0.25	3	12	22	2	3	4	56
27	Blood	2	0.016	0.016	0.25	**51**	7	**50**	**50**	**50**	9	**74**
28	Vagina	1	0.0313	0.016	0.0313	**51**	7	**50**	**50**	**50**	9	**74**
29	Vagina	4	0.016	0.016	0.0313	**52**	7	**50**	4	**50**	9	**77**
30	Blood	2	0.016	0.008	0.125	3	4	4	3	3	4	7
31	Urine	32	1	0.008	0.0625	7	5	6	12	1	8	22
32	Urine	64	2	0.008	0.125	3	4	4	3	3	4	7
33	Vagina	1	0.25	0.008	0.25	5	7	8	7	3	6	3
34	Urine	1	0.5	0.0313	0.25	**51**	7	**50**	**50**	**50**	9	**74**
35	Urine	1	0.125	0.008	0.0313	**51**	7	**50**	**50**	**50**	9	**74**
36	Urine	4	1	0.016	0.25	3	4	4	3	3	4	7
37	Urine	64	1	0.008	0.0625	3	12	22	2	3	4	56
38	Urine	8	2	0.016	0.5	3	4	4	3	3	4	7
39	Urine	2	0.25	0.016	0.125	**50**	7	9	4	13	**50**	**78**
40	Urine	1	0.5	0.0313	0.125	7	13	17	4	3	19	**71**
41	Urine	32	2	0.0313	0.25	5	7	8	7	3	6	3
42	Urine	2	1	0.016	0.5	5	7	17	7	3	6	**72**
43	Vagina	64	4	0.016	0.125	**52**	7	**50**	**50**	**50**	9	**79**
44	BAL	2	1	0.0625	0.0625	3	4	4	3	3	4	7
45	Blood	1	1	0.016	0.125	**50**	16	9	4	13	**50**	**75**
46	Blood	2	0.25	0.016	0.25	**51**	7	**50**	**50**	**50**	9	**74**
47	Pharynx	4	0.5	0.0313	0.5	7	13	17	9	3	19	59
48	Blood	1	0.25	0.0313	0.25	7	13	8	9	3	19	**73**
49	Blood	0.5	1	0.008	0.25	8	5	3	5	1	1	15
50	Blood	4	1	0.008	0.0313	3	12	22	2	3	4	56

* ST-71, ST-72 and ST-73 are novel combinations of existing alleles of database. ST-74, ST-75, ST-76, ST-77, ST-78 and ST-79 are combinations of new alleles, with new allele numbers and new STs numbered in bold face type.

** AmB, amphotericin B; CASP, caspofungin; FCZ, fluconazole, VCZ, Voriconazole.

Table 2. General features of *C. glabrata* MLST loci.

Locus	GenBank accession no.	Primer sequence (5′–3′_)	Sequence target size (bp)	No. of variable sites	Percentage of variable sites	No. of alleles	Discriminatory ratio	dN/dS ratio*
FKS	AF229171	GTCAAATGCCACAACAACAACCT AGCACTTCAGCAGCGTCTTCAG	589	9	1.53	9	1	0.115
LEU2	U90626	TTTCTTGTATCCTCCCATTGTTCA ATAGGTAAAGGTGGGTTGTGTTGC	512	8	1.56	7	0.87	0.143
NMT1	AF073886	GCCGGTGTGGTGTTGCCTGCTC CGTTACTGCGGTGCTCGGTGTCG	607	16	2.63	9	0.56	0.101
TRP1	U31471	AATTGTTCCAGCGTTTTTGT GACCAGTCCAGCTCTTTCAC	419	11	2.62	9	0.82	0.190
UGP1	AB037186	TTTCAACACCGACAAGGACACAGA TCGGACTTCACTAGCAGCAAATCA	616	6	0.97	5	0.83	0.478
URA3	L13661	AGCGAATTGTTGAAGTTGGTTGA AATTCGGTTGTAAGATGATGTTGC	602	11	1.83	9	0.82	0.218

* Nonsynonymous to synonymous substitution ratio.

Phylogenetic analysis by unweighted pair group method with arithmetic averages (UPGMA) and neighbor-joining algorithms based on p-distance were conducted using MEGA, version 5.2, applied to concatenated sequence data. The value of the cluster nodes was determined by bootstrapping with 1,000 randomizations.

STs were also analyzed using the eBURST package (available at http://eburst.mlst.net/) to determine possible relationships between isolates, with the default group definition of 5/6 shared alleles. The output is a display of the most parsimonious patterns of descent of each ST from the ancestral type.[14]

Antifungal susceptibility testing

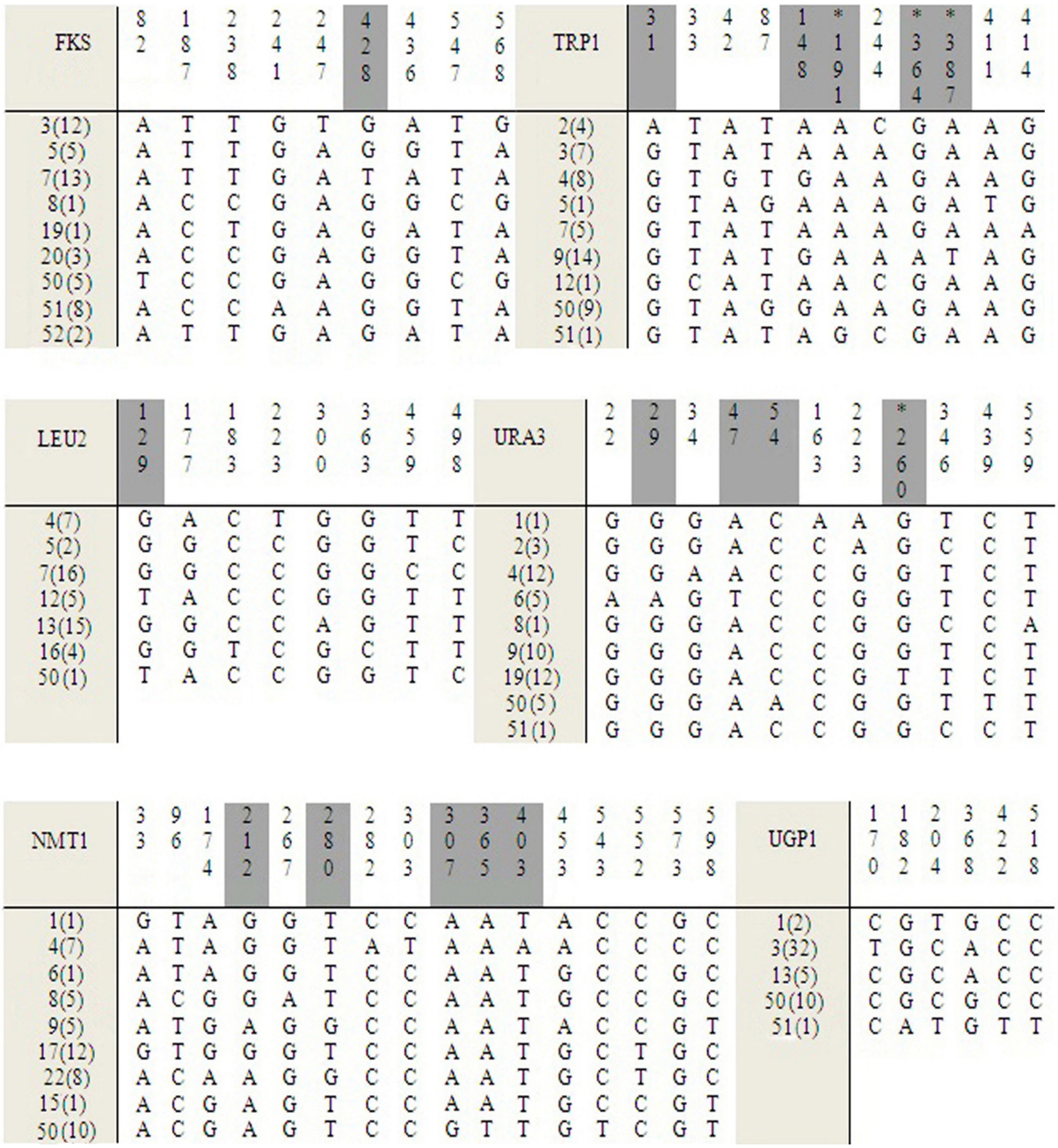
Isolates were tested for *in vitro* susceptibilities to amphotericin-B (AmB), caspofungin (CASP), fluconazole (FCZ) and voriconazole (VCZ) as described by the Clinical Laboratory Standards Institute (CLSI) M27-A4 document guidelines.[23] *Candida albicans* ATCC 10231 and *C. glabrata* ATCC 90030 were used as quality control standards. MIC results were read visually following 24 h of incubation as the lowest concentration of drug that caused a meaningful reduction (≥90% inhibition for AmB and ≥50% inhibition for azoles and CASP) of growth compared with control levels.[23]

Results

A total of 50 clinical isolates of *C. glabrata* were examined by MLST technique. As shown in Table 1, 16 distinct STs were identified, indicating a discriminatory power index of 0.9029. The six sequenced loci resulted in 3,345 combined base pairs. The number of polymorphic sites per locus ranged between 6 (0.97%, *UGP1*) and 16 (2.63%, *NMT1*), as shown in Table 2. For all sequences, the most informative site was *NMT1*, with 16 distinct alleles in the total collection, and the least informative site was *UGP1*, with only six distinct alleles. The number of genotypes identified for each of the six loci investigated ranged from five (*UGP1*) to nine (*FKS*, *NMT1*, *TRP1*, *URA3*) genotypes per locus, as shown in Table 2. Among the six fragments sequenced, *FKS* gave the highest discriminatory ratio, yielding nine different genotypes from only nine polymorphic sites, followed by *LEU2* (seven alleles from eight variable sites), *UGP1* (five alleles from six variable sites), *TRP1* and *URA3* (9 alleles from 11 variable sites) and *NMT1* (9 alleles from 16 variable sites).

A total of 61 nucleotide sites (1.82%) among all six genes combined were found to be polymorphic, as shown in Figure 1. The majority of these variable nucleotide sites had been identified previously. In this study, a total of 11 new sequences were found, to which no allele numbers were assigned in the MLST database (http://cglabrata.mlst.net). However, five sequences of them were previously registered in Iran in GenBank (www.ncbi.nlm.nih.gov/genbank/), with accession numbers: KT763098, KT763119, KT763199, KT763239, and KT763279. All sequences, including new sequences generated in this study, have been deposited in the GenBank database under accession numbers KX187005 to KX187304 by us. The variable nucleotide sites newly identified in this study are highlighted in Figure 1.

The ratio of nonsynonymous (change of amino acid) to synonymous (no change of amino acid) nucleotide substitutions (dN/dS) was below 1 for all six loci, as shown in Table 2. The 61 nucleotide polymorphic sites among the six gene fragments resulted in 16 nonsynonymous



FKS	82	187	238	241	247	428	436	547	568
3(12)	A	T	T	G	T	G	A	T	G
5(5)	A	T	T	G	A	G	G	T	A
7(13)	A	T	T	G	A	T	A	T	A
8(1)	A	C	C	G	A	G	G	C	G
19(1)	A	C	T	G	A	G	A	T	A
20(3)	A	C	C	G	A	G	G	T	A
50(5)	T	C	C	G	A	G	G	C	G
51(8)	A	C	C	A	A	G	G	T	A
52(2)	A	T	T	G	A	G	A	T	A

TRP1	31	33	42	87	148	*191	244	*364	*387	411	414
2(4)	A	T	A	T	A	A	C	G	A	A	G
3(7)	G	T	A	T	A	A	A	G	A	A	G
4(8)	G	T	G	T	G	A	A	G	A	A	G
5(1)	G	T	A	G	A	A	A	G	A	T	G
7(5)	G	T	A	T	A	A	A	G	A	A	A
9(14)	G	T	A	T	G	A	A	A	T	A	G
12(1)	G	C	A	T	A	A	C	G	A	A	G
50(9)	G	T	A	G	G	A	A	G	A	A	G
51(1)	G	T	A	T	A	G	C	G	A	A	G

LEU2	129	177	183	223	300	363	459	498
4(7)	G	A	C	T	G	G	T	T
5(2)	G	G	C	C	G	G	T	C
7(16)	G	G	C	C	G	G	C	C
12(5)	T	A	C	C	G	G	T	T
13(15)	G	G	C	C	A	G	T	T
16(4)	G	G	T	C	G	C	T	T
50(1)	T	A	C	C	G	G	T	C

URA3	22	*229	334	447	554	163	223	*260	346	439	559
1(1)	G	G	G	A	C	A	A	G	T	C	T
2(3)	G	G	G	A	C	C	A	G	C	C	T
4(12)	G	G	A	A	C	C	G	G	T	C	T
6(5)	A	A	G	T	C	C	G	G	T	C	T
8(1)	G	G	G	A	C	C	G	G	C	C	A
9(10)	G	G	G	A	C	C	G	G	T	C	T
19(12)	G	G	G	A	C	C	G	T	T	C	T
50(5)	G	G	G	A	A	C	G	G	T	T	T
51(1)	G	G	G	A	C	C	G	G	C	C	T

NMT1	33	96	174	*212	267	*280	282	303	*307	*365	*403	453	543	552	573	598
1(1)	G	T	A	G	G	T	C	C	A	A	T	A	C	C	G	C
4(7)	A	T	A	G	G	T	A	T	A	A	A	A	C	C	C	C
6(1)	A	T	A	G	G	T	C	C	A	A	T	G	C	C	G	C
8(5)	A	C	G	G	A	T	C	C	A	A	T	G	C	C	G	C
9(5)	A	T	G	A	G	G	C	C	A	A	T	A	C	C	G	T
17(12)	G	T	G	G	G	T	C	C	A	A	T	G	C	T	G	C
22(8)	A	C	A	A	G	G	C	C	A	A	T	G	C	T	G	C
15(1)	A	C	G	A	G	T	C	C	A	A	T	G	C	C	G	T
50(10)	A	C	G	A	G	T	C	C	G	T	T	G	T	C	G	T

UGP1	171	182	204	248	262	518
1(2)	C	G	T	G	C	C
3(32)	T	G	C	A	C	C
13(5)	C	G	C	A	C	C
50(10)	C	G	C	G	C	C
51(1)	C	A	T	G	T	T

Figure 1. Polymorphic nucleotide sites of the six loci used in the MLST scheme and alleles identified at each locus. The numbers in parentheses represent the number of isolates with that allele. The numbers in the vertical format represent the positions of the variable nucleotides relative to the fragment sequenced. The highlighted nucleotide positions are nonsynonymous nucleotide substitutions. Where nucleotide positions were newly identified as variable in this study, the number has been marked with an asterisk.

changes in amino acids encoding. Nonsynonymous nucleotide substitutions that were newly identified in this study; include a T→G first position nonsynonymous transition (Ser→Ala) at nucleotide position 260 for the *URA3* locus. In *TRP1*, a G→A second position nonsynonymous transition (Gly→Asp) at nucleotide position 191, A→G first position nonsynonymous transition (Asn→Asp) at nucleotide position 364 and T→A third position nonsynonymous transition (His→Gln) at nucleotide position 387.

Among the STs that were found in this study, ST-59, ST-74, and ST-7 were the most prevalent. These three STs together represented 50% of the entire study population. A total of nine new STs were identified in this study; these STs were obtained in two different types. The first type was

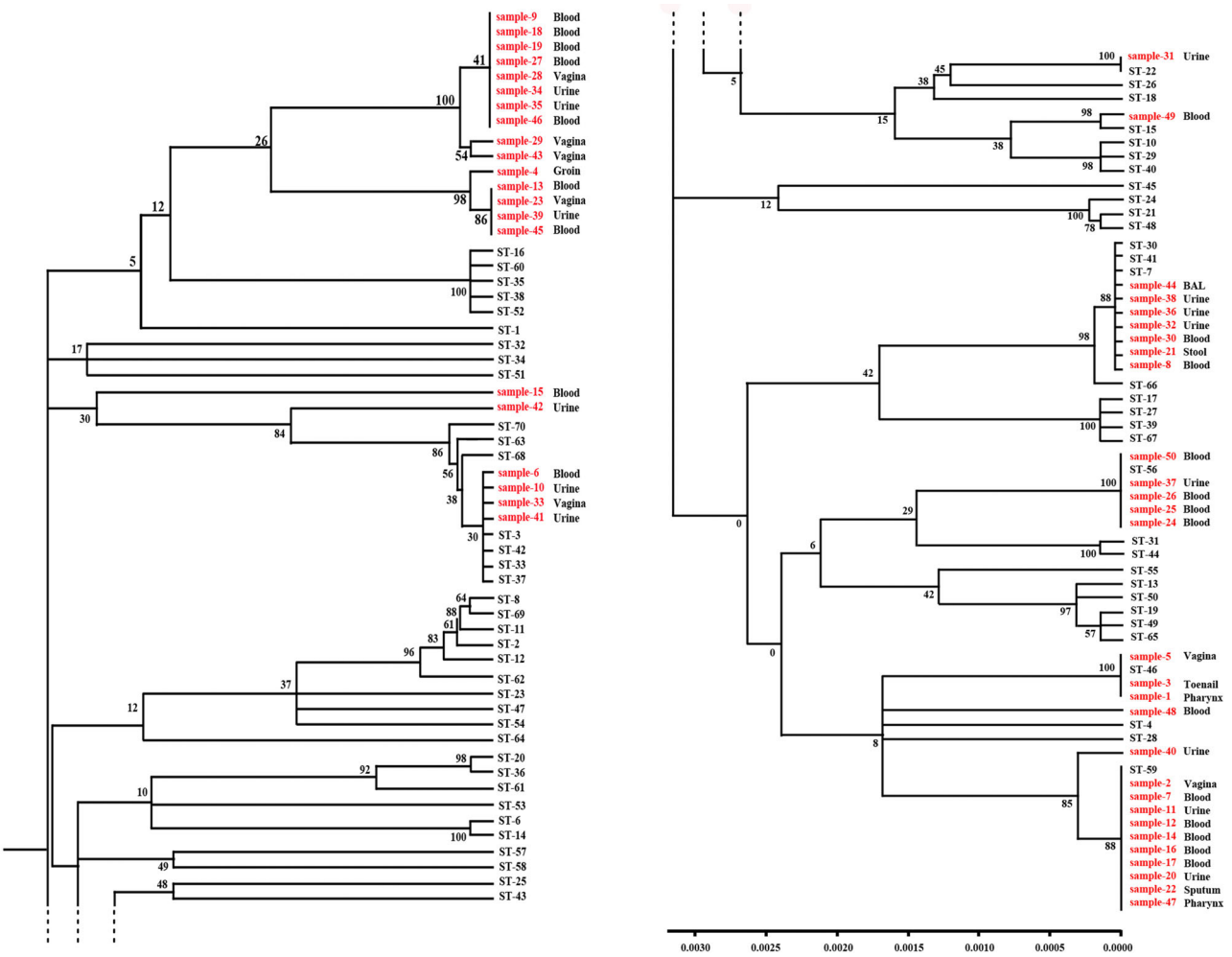


Figure 2. Dendrogram shows the genetic relationships between 50 isolates of *C. glabrata*. The dendrogram was constructed by concatenated sequences from combinations of six MLST loci and using the neighbor-joining method. Numbers within the tree indicate the bootstrap values for the cluster nodes.

the result of a new combination of previously identified alleles, and the second type was a result of a combination of new alleles that were found in this study, as shown in Table 1.

A neighbor-joining dendrogram was constructed based on the concatenated sequences, as shown in Figure 2. Estimation of the value of the nodes was done by bootstrapping with 1,000 randomizations. In the neighbor-joining dendrogram, bootstrap values of cluster nodes were generally less than 50%, except for very closely -related isolates. The eBURST analysis of the set of 50 *C. glabrata* isolates by combining all isolate profiles in the database revealed 15 groups (Figure 3).

FCZ resistance (MIC $\geq$ 64 μg/ml)[23] was shown in four isolates in the total collection (Table 1). These resistant isolates were distributed among four STs, although this distribution was not statistically significant. One isolate was VCZ resistant (MIC $\geq$ 4 μg/ml).[24] None of the isolates in the total population had high MIC values for CASP (MIC $\geq$ 0.5 μg/ml).[23] In the same manner all isolates were within the range considered clinically susceptible for AmB (MIC $\geq$ 1 μg/ml).[23]

According to Fisher's exact test results, no significant differences were observed in the distribution of drug-resistant isolates among the genotypes and clades (i.e., for which $P < .05$). However, a significant relationship was found between *FKS* ($P = .033$) and *LEU2* ($P = .018$) with FCZ resistance.

Discussion

This study was designed to determine the prevalence of endemic genotypes of *C. glabrata* clinical isolates and any possible association between isolation source and drug resistance. In this study, a total of 11 new sequences were

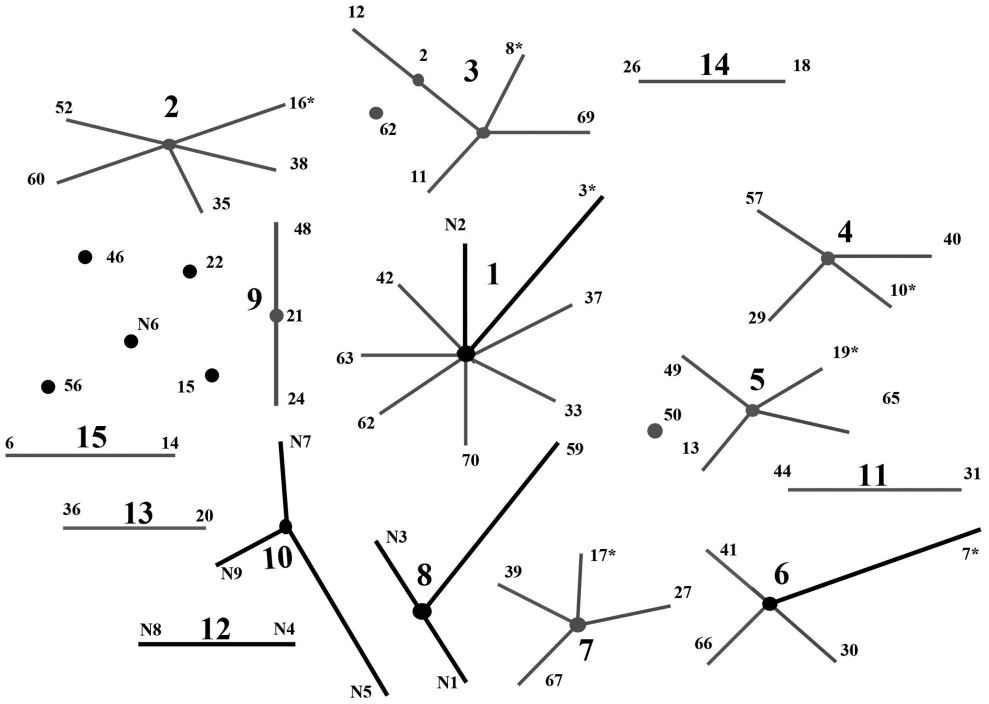


Figure 3. Modified eBURST snapshot for 50 clinical isolate of *C. glabrata* along with all isolate profiles in the database. Our isolates are displayed with black lines, and database collection with gray lines. The length of each line is proportional to the number of isolates with the same ST. Where a putative ancestral ST was determined for a cluster, the ST number has been marked with an asterisk. Each group of related isolates is numbered in boldface type.

found. Given the high prevalence of these new sequences, it can be concluded that these new alleles are among the endemic genotypes of Iran. These new polymorphic sites in the *TRP1* and *URA3* were nonsynonymous, as shown in Figure 1. The ratio of nonsynonymous to synonymous nucleotide substitutions (dN/dS) was below 1 for all six loci, suggesting that the genes were under neutralizing selective pressure.

The MLST for *C. albicans*[9] revealed different ranges for the percentage of variable sites (1.5 to 4.0%) compared to *C. glabrata* (1.5 to 3.5%). Therefore, the percentage of polymorphic sites in our MLST scheme was 1.82% (61 of 3,345 nucleotides), which revealed relatively low percentage of variable sites compared to similar studies, due to the small sample size and limitations related to sample collection, namely, the unequal distribution of anatomical and geographical sources of isolates.

The discriminatory powers calculated from MLST databases according to Hunter's formula,[21] were 99.9% for *C. albicans* ($n = 1{,}594$), 99.8% for *C.* krusei ($n = 134$), and 99.9% for C. tropicalis ($n = 270$).[25] For *C. dubliniensis* the equivalent published value was 90.9% ($n = 50$).[11] In this study, discriminatory power for *C. glabrata* MLST was 90.3%. Compared to other studies excluding *C. dubliniensis* results,[8,11,14,17,25] the discriminatory power of *C. glabrata* MLST was relatively low. This is probably due to the haploid nature of *C. glabrata*, which is in contrast to diploid genome and heterozygosity at the tested loci of other *Candida* species.[26]

The eBURST algorithm deposits all related isolates into clonal complexes with the default group definition of 5/6 shared alleles, where possible, predicts the founding, or ancestral ST of each complex. All clusters were constructed using the neighbor-joining method correlated with eBURST data. However, comparison of the clustering in the neighbor- joining dendrogram with the eBURST groups showed isolates in groups 4 and 12; 6 and 7; 5 and 11; and also 13 and 15. Despite differences, the groups were mixed with each other by low bootstrap values, also several eBURST singletons dispersed within the various groups. Unlike the eBURST analysis, isolates with little diversity in the concatenated sequences were considered as similar by neighbor- joining analysis, even if the polymorphic sites were located on separate fragments.

We compared the data from Iranian isolates with global data on http://cglabrata.mlst.net to check for global geographical relationship and epidemiology. ST-59, the most prevalent isolate, has been reported only from Taiwan.[27] Also, ST-46 and ST-56 have been reported only from the United States.[27] ST-3 and ST-7, taken together, represented

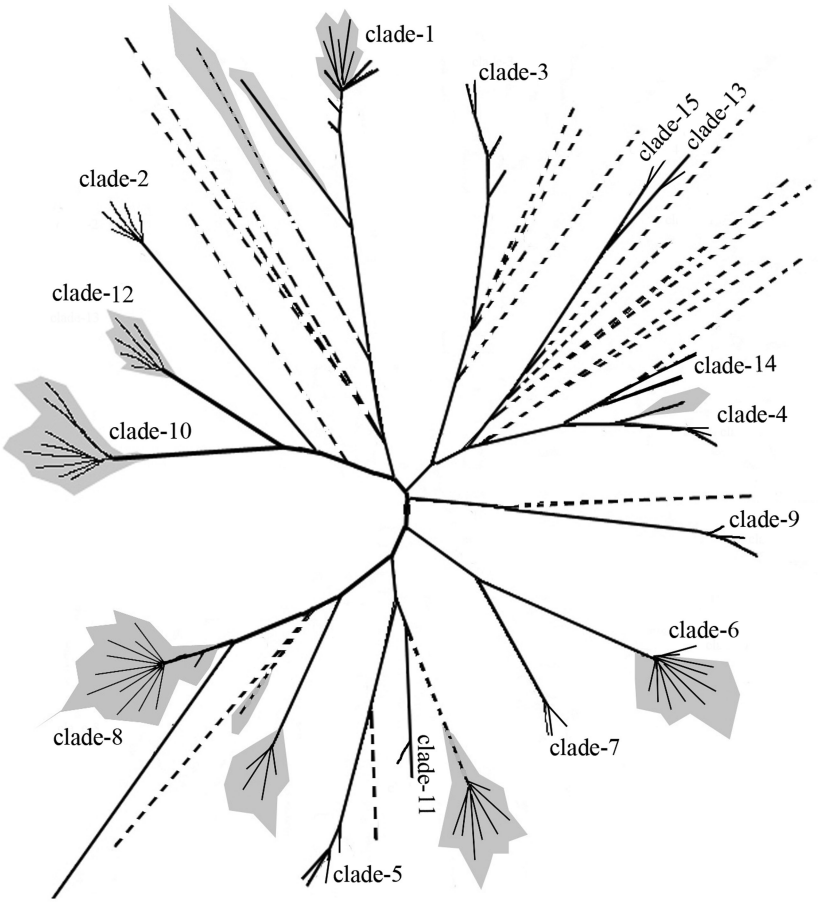


Figure 4. Population structure of 50 clinical isolates of *C. glabrata* along with all isolate profiles in the database, as determined by p-distance in a neighbor-joining analysis. The clade numbers correspond to those in Fig. 2; branches to singleton isolates without clade are shown with dashed lines.

22% of the entire study population and are scattered in the whole world and have been reported from Europe, America, and Asia.[12,27,28,29] As shown in Figure 4, the population structure of *C. glabrata* in Iran consists of clonal groups closely related to those in other countries even though there are some new clonal clusters and STs.

In the present study, the sequence types (STs) were independent of both anatomic sources and drug resistance. When these isolates were analyzed for their drug resistance, no correlation between reduced susceptibility and the MLST genotype was found, which is in accordance with the previous reports.[12,17,26,30] Like some previous reports, we found no association between different anatomical sources of isolates and STs or groups.[31–34] In contrast, Dhieb et al.[35] showed that *C. glabrata* population structures were associated with geographical distribution and antifungal drug resistance. Other studies have shown a significant association between clades and anatomical source of the isolates for *C. glabrata*[8] and *C. albicans*.[17] However, it is notable that in our study, the number of isolates for each anatomical site was too small to allow a reliable statistical analysis of isolate properties by clade.

In this study, we collected all samples from patients with candidiasis. There were no isolates from normal flora strains in healthy people. Lott et al.[36] reported that *C. glabrata* isolates from bloodstream infections were genetically indistinguishable from normal flora strains, indicating that pathogenicity may be related to disease-associated and commensal strains. Therefore, it is recommended that more comprehensive studies be performed to evaluate and compare the two groups of pathogenic and non-pathogenic genotypes of *C. glabrata*.

In epidemiological and phylogenetic studies, MLST technique allows a standardization of molecular typing data as well as the application of this technology as a reference in future studies.[37] With an increased use of this technique, more comprehensive database could be established. On the other hand, global epidemiological studies will be facilitated by collaborative surveillance and comparison of the genotypes with a central database.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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