Medical Mycology, 2015, 53, 828–836
doi: 10.1093/mmy/myv065
Advance Access Publication Date: 1 September 2015
Original Article

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Molecular epidemiology of invasive *Candida albicans* at a tertiary hospital in northern Taiwan from 2003 to 2011

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Received 24 March 2015; Revised 21 June 2015; Accepted 22 June 2015

Abstract

Candida albicans is a common cause of bloodstream fungal infections in hospitalized patients. To investigate its epidemiology, multilocus sequence typing (MLST) was performed on 285 *C. albicans* bloodstream isolates from patients in Chang Gung Memorial Hospital at Linkou (CGMHL), Taiwan from 2003 to 2011. Among these isolates, the three major diploid sequence types (DSTs) were 693, 659, and 443 with 19, 16, and 13 isolates, respectively. The 179 DSTs were classified into 16 clades by unweighted pair-group method using arithmetic averages (UPGMA). The major ones were clades 1, 4, 3, and 17 (54, 49, 31, and 31 isolates, respectively). Further analyses with eBURST clustered the 285 isolates into 28 clonal complexes (CC). The most common complexes were CC8, CC20, and CC9. DST 693 that had the highest number of isolates was determined to be the cluster founder of CC20, which belonged to clade 3. So far, 33 isolates worldwide including 29 from Taiwan and 4 from Korea, are CC20, suggesting that CC20 is an Asian cluster. Two fluconazole-resistant isolates belonging to CC12 and CC19 were detected. All other CGMHL isolates were susceptible to 5-flucytosine, amphotericin B, anidulfungin, caspofungin, fluconazole, itraconazole, micafungin, posaconazole, and voriconazole. However, CC20 isolates exhibited significantly lower susceptibility to fluconazole. In conclusion, the 285 CGMHL *C. albicans* isolates displayed geographically clustering with

Asian isolates, and most of them are susceptible to common antifungal drugs. Isolates of DST 693, a Taiwanese major genotype belonging to MLST clade 3, were more resistant to fluconazole than other isolates.

Key words: *Candida albicans*, candidemia, multilocus sequence typing (MLST), antifungal susceptibility test.

Introduction

Candida albicans is an organism of the normal gut flora in humans. It is also an opportunistic pathogen and is the fourth most common cause of nosocomial bloodstream infections with a mortality rate of 30–60% [1–5]. The risk factors of candidemia include invasive surgeries such as dialysis [6], implantation of central venous catheter [7], diabetes [6], burns [8], human immunodeficiency virus (HIV) infections, immunosuppression due to chemotherapy [9], and use of steroid drugs or broad-spectrum antibiotics [7].

The increasing frequency of invasive candidiasis and its serious outcome demand more epidemiological studies. A well-accepted method for typing *C. albicans* isolates is the multilocus sequence typing (MLST) [10–12]. It is based on nucleotide sequence variations within the 300- to 400-bp internal regions of seven housekeeping genes, including *AAT1a*, *ACC1*, *ADP1*, *MPIb*, *SYA1*, *VPS13*, and *ZWF1b*. A sequence variation in each locus is assigned an allele number. Combination of allele numbers of these seven genes constitutes a unique diploid sequence type (DST) of a *C. albicans* isolate. Because MLST analysis relies only on nucleotide sequencing, the information about *C. albicans* isolates can be exchanged around the world through a global database (http://calbicans.mlst.net) [12,13]. At least 2400 DSTs have been recorded in the *C. albicans* MLST database.

UPGMA (unweighted pair-group method using arithmetic averages) is a method that can be used to determine the phylogenetic relationship among *C. albicans* isolates [11,14]. Using this method, Gong et al. has recently classified 1500 *C. albicans* isolates into 18 distinct clades [14]. Clade 1 isolates distribute globally, whereas isolates from the Pacific Rim cluster mostly in clades 14 and 17. Clade 1 isolates in general have a higher acid phosphatase activity and are less susceptible to 5-fluorocytosine [11,15] and more salt tolerant [16]. Another method called eBURST (electronic Based upon Related Sequence Types) allows determination of patterns of evolutionary descent by grouping isolates that differ at one or two of the seven MLST alleles into clonal clusters [17].

In this study, we determined the DSTs of 285 *C. albicans* isolates causing bloodstream infections in Chang Gung Memorial Hospital at Linkou (CGMHL) and investigated the epidemiology of the isolates using both UPGMA and eBURST. We also determined antifungal susceptibility of these isolates. Results showed that isolates of DST 693/clonal complex 20/clade 3 are more resistant to fluconazole than other isolates.

Materials and Methods

Candida Albicans Isolates

A total of 285 bloodstream infection isolates from CGMHL obtained between 2003 and 2011, including all 72 from pediatric patients and 213 randomly selected from 1098 archived adult ICU isolates, were investigated in this study (Supplementary Table). Each isolate was collected only once from a patient within the hospital admission. All isolates were identified by MALDI-TOF mass spectrometry and germ tube formation methods or CHROMagar Candida (BD).

Multilocus Sequence Typing

MLST of *C. albicans* isolates were performed as described by Bougnoux et al. [12,13] A portion of each of *AAT1a*, *ACC1*, *ADP1*, *MPIb*, *SYA1*, *VPS13*, and *ZWF1b* genes was amplified by polymerase chain reaction (PCR), and the resulting PCR products were sequenced. Each nucleotide sequence thus generated was compared to those in the *C. albicans* MLST database (http://calbicans.mlst.net/) to obtain an allele number. Any sequence that does not match with any of the preexisting sequences was given a new allele number. The combination of the seven allele numbers defined a unique DST of an isolate.

UPGMA Analysis

To determine phylogenetic relatedness, DSTs of the 285 CGMHL isolates and 996 isolates with known clades retrieved from the *C. albicans* MLST database were analyzed by UPGMA as described previously [11,14]. Briefly, the sequences of each housekeeping gene of each isolate were aligned to reveal polymorphic bases. The seven MLST polymorphic sequences from each isolate were then concatenated into a single sequence. Each base of the combined sequence was rewritten with two letters representing a homozygous or heterozygous diploid sequence. The genetic relatedness of the transformed sequences were analyzed by

the software MEGA version 6 to generate a dendrogram [18].

eBURST Analysis

The relationships among the 285 CGMHL isolates and all 2448 isolates in the MLST database (date accessed 10.01.14) were determined by eBURST (http://eburst.mlst.net/). Based on the seven allele numbers of each isolate, eBURST placed related isolates into a clonal complex (CC) and predicted the ancestral DST of each CC by calculating the frequency of each DST genotype. The results of eBURST were displayed as the most parsimonious pattern of each descent of the ancestral DST type.

Antifungal Susceptibility Testing

A commercially available dried colorimetric microdilution panel (Sensititre YeastOne, TREK Diagnostic Systems) was used for susceptibility testing of *C. albicans* isolates to 5-flucytosine, amphotericin B, anidulafungin, caspofungin, fluconazole, itraconazole, micafungin, posaconazole, and voriconazole. Briefly, *C. albicans* isolates ($1.5–8 \times 10^2$ cfu) were seeded in YeastOne medium containing antifungal agents and incubated at 35°C without CO_2 for 24 hours. The minimum inhibition concentration (MIC) of each antifungal agent was determined according to the guideline provided by the kit. The clinical breakpoints for sensitive, intermediate, and resistant isolates and epidemiological cutoff values for wild-type and non-wild-type isolates for the antifungal agents were referenced to those of Pfaller et al. [19] MIC_{50} and MIC_{90} values of *C. albicans* isolates against each antifungal agent were also calculated. Isolates with a fluconazole MICs $> = 0.5$ μg/ml were defined as having a lower fluconazole susceptibility, that is, more resistant to fluconazole.

Statistical analysis

The χ^2 and Fisher exact tests were performed to compare genotype distributions and antibiotic susceptibility. A *P*-distance $< .05$ was considered significant. Prism 5.0 software (GraphPad, San Diego) was used for the analysis.

Results

Multilocus Sequencing Typing

Of the 285 isolates, 172 isolates (60.4%) were assigned 68 previously defined DSTs, and the other 113 isolates were assigned 110 new DSTs (DST 2427–2555) (Supplementary Table). The 213 isolates from adult patients were assigned 142 DSTs, and the 72 isolates from pediatric patients were assigned 53 different DSTs. Among the 213 isolates from adults, the most prevalent clones were DST 659 (combination alleles 11, 26, 6, 4, 34, 60, and 119 for *AAT1a*, *ACC1*, *ADP1*, *MPIb*, *SYA1*, *VPS13*, and *ZWF1b*, respectively), DST 693 (combination alleles 1, 7, 15, 6, 61, 105, and 112), DST 443 (combination alleles 59, 5, 21, 2, 80, 108, and 15), and DST 766 (combination alleles 23, 3, 5, 3, 57, 100, and 6). Among the 72 isolates from children, the most dominant clones were DST693, DST 1849 (combination alleles 5, 5, 5, 9, 2, 6, and 5), DST 365 (combination alleles 55, 14, 4, 3, 6, 45, and 15), DST 443, and DST 659. Overall, the three most prevalent DST clones were DST 693 (19 isolates; 6.7%), DST 659 (16 isolates; 5.6%), and DST 443 (13 isolates; 4.6%) (Supplementary Table).

MLST clade distribution of CGMHL *C. albicans* isolates

To compare the genotypes of the CGMHL isolates to those of global isolates with previously reported MLST clades, UPGMA phylogenetic analyses were performed. The MLST clade distribution of all *C. albicans* isolates is shown in Figure 1 and Table 1, and the detail of MLST genotyping is shown in the Supplementary Table. The most common clades were clade 1 (54 isolates, 18.9%), clade 4 (49 isolates, 17.2%), clade 3 (31 isolates, 10.9%), clade 17 (31 isolates, 10.9%), and clade 16 (29 isolates, 10.2%). Among the 113 isolates with newly assigned DSTs, the most common clades were clade 1 (25 isolates, 22.1%), clade 17 (15 isolates, 13.3%), clades 4 and 16 (12 isolates each, 10.6%) (Table 1). Within the same clade, the isolates from this study were clustered together, especially those in clades 3, 4, and 11 (Fig. 1).

eBURST Clonal Clustering of CGMHL *C. albicans* Isolates

To determine whether the CGMHL isolates that clustered together within the same clade belonged to a specific genotype, eBURST analysis was performed. The 285 CGMHL isolates were clustered in 28 clonal complexes (CCs) (Supplementary Table), and 195 (55.8%) isolates belonged to 6 CCs, including CC1, CC8, CC9, CC15, CC17, and CC20 (Table 2). The most common cluster was CC8 (45 isolates, 15.8%), followed by CC20 (27 isolates, 9.5%), and CC9 (25 isolates, 8.8%). The predicted clonal founders of CC8 and CC20 were DST 659 and DST 693 (Table 2), respectively. They were also the most dominant DSTs in this study. The majority of isolates of clades 3, 4, 11, 16, and 17 were clustered in CC20 (27/31,

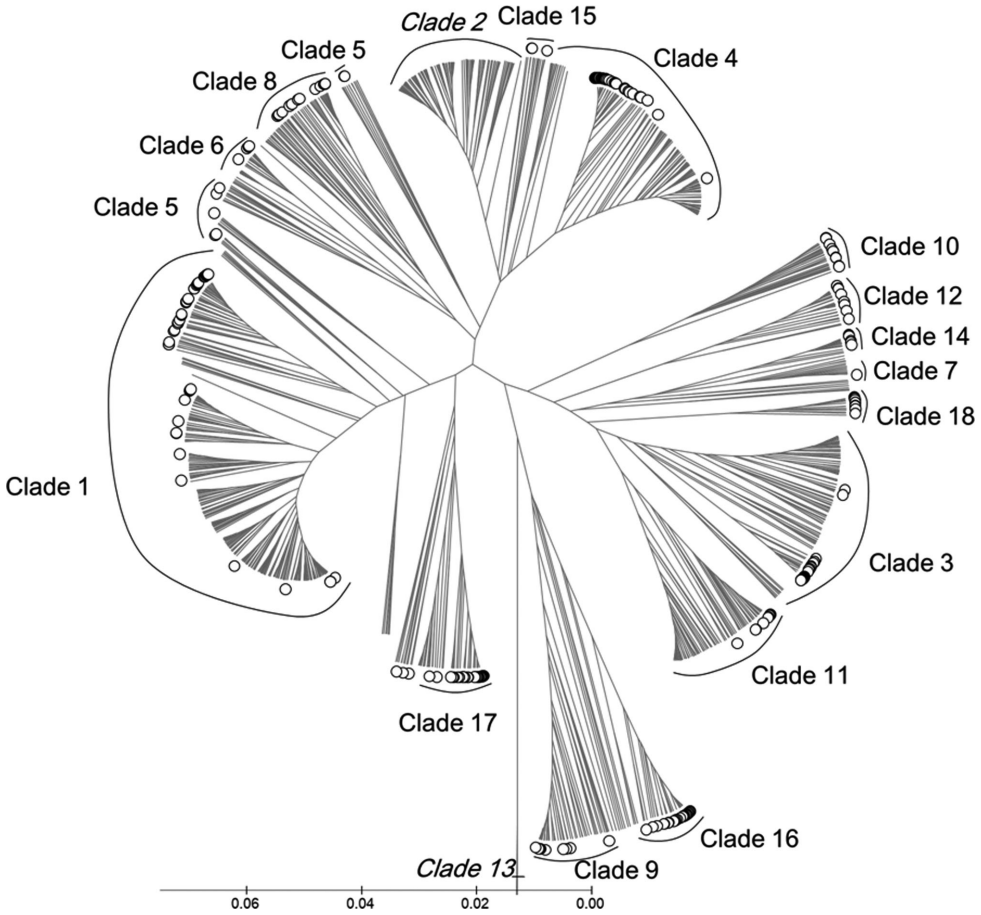

Figure 1. MLST analysis of CGMHL *C. albicans* isolates. Nucleotide sequences of 7 housekeeping genes of 285 CGMHL isolates and 996 reference strains retrieved from the MLST database were analyzed by UPGMA. Clade numbers were assigned as described previously [11,29]. Clades 2 and 13 (in italic letters) contained no CGMHL isolates. Open circles represent CGMHL isolates. The scale bar indicates *P*-distance.

87.1%), CC8 (45/49, 91.8%), CC10 (10/11, 90.9%), CC15 (24/29, 82.8%), and CC17 (23/31, 74.2%), respectively (Table 3).

Antifungal Susceptibility Testing of CGMHL *C. albicans* Isolates

The mean MIC_{50} of the 285 CGMHL isolates against 5-flucytosine, amphotericin B, anidulafungin, caspofungin, fluconazole, itraconazole, micafungin, posaconazole, and voriconazole were 0.06 μg/ml, 0.5 μg/ml, 0.06 μg/ml, 0.06 μg/ml, 0.5 μg/ml, 0.03 μg/ml, 0.008 μg/ml, 0.015 μg/ml, and 0.008 μg/ml, respectively. The mean MIC_{90} of the 285 CGMHL isolates against these antifungal agents were 0.12 μg/ml, 0.5 μg/ml, 0.06 μg/ml, 0.06 μg/ml, 0.5 μg/ml, 0.06 μg/ml, 0.008 μg/ml, 0.03 μg/ml, and 0.008 μg/ml, respectively. Two fluconazole resistant isolates C001 (DST 1933/CC19/clade 14) and D034 (DST 1363/CC12/clade 10) from adult patients were detected (Supplementary Table). The remaining isolates were susceptible to all nine drugs tested. Most MIC_{50} and MIC_{90} values of the antifungals tested in CGMHL isolates are similar to that reported in a global survey conducted in 2013 [20]. The MIC_{50} and MIC_{90} of micafungin in CGMHL isolates (0.008 μg/ml and 0.008 μg/ml, respectively) is lower than that in the survey (0.015 μg/ml and 0.03 μg/ml, respectively), but that of fluconazole (MIC_{50} = 0.5 μg/ml and MIC_{90} = 0.5 μg/ml) and caspofungin (MIC_{50} = 0.06 μg/ml and MIC_{90} = 0.06 μg/ml) in this study are higher than the survey (fluconazole MIC_{50} = 0.12 μg/ml and MIC_{90} = 0.25 μg/ml; caspofungin MIC_{50} = 0.03 μg/ml and MIC_{90} = 0.03 μg/ml). One hundred and fifty isolates (52.6%) were found to be less susceptible to fluconazole (MIC > = 0.5 μg/ml), including all CC10 isolates and 85.2% of CC20 isolates (Table 4 and Supplementary Table).

Discussion

In the current study, the epidemiology of 285 bloodstream isolates of *C. albicans* was investigated. Based on

Table 1. Clade distribution of isolates from CGMHL and other areas.

	No. of Isolates from CGMHL		No. of Isolates (%)[#]			
Clade	Known DST	New DST	CGMHL	Asia[†]	Global (after 2000)	Global (before 2000)
1	29	25	54 (18.9)	53 (31)**	249 (29.9)***	156 (44.1)***
2	0	0	0 (0)	3 (1.8)	111 (13.3)***	35 (9.9)***
3	25	6	31 (10.9)	8 (4.7)*	67 (8.0)	31 (8.8)
4	37	12	49 (17.2)	15 (8.8)*	127 (15.2)	33 (9.3)*
5	2	5	7 (2.5)	4 (2.3)	12 (1.4)	6 (1.7)
6	2	2	4 (1.4)	5 (2.9)	20 (2.4)	13 (3.7)
7	1	0	1 (0.4)	1 (0.6)	7 (0.8)	7 (2.0)
8	7	8	15 (5.3)	11 (6.4)	29 (3.5)	9 (2.5)
9	5	5	10 (3.5)	7 (4.1)	35 (4.2)	8 (2.3)
10	5	6	11 (3.9)	2 (1.2)	9 (1.1)**	4 (1.1)*
11	9	2	11(3.9)	7 (4.1)	74 (8.9)**	16 (4.5)
12	6	3	9 (3.2)	5 (2.9)	15 (1.8)	9 (2.5)
13	0	0	0 (0)	1 (0.6)	3 (0.4)	1 (0.3)
14	5	2	7 (2.5)	11 (6.4)*	13 (1.6)	1 (0.3)*
15	1	2	3 (1.1)	4 (2.3)	10 (1.2)	2 (0.6)
16	17	12	29 (10.2)	6 (3.5)*	7 (0.8)***	2 (0.6)***
17	16	15	31 (10.9)	14 (8.2)	23 (2.8)***	7 (2.0)***
18	5	5	10 (3.5)	–	–	–
Singlet	0	3	3 (1.1)	14 (8.2)***	22 (2.6)	14 (4.0)***
Total	172	113	285 (100)	171 (100)	825 (100)	362 (100)

[#]Number of Asian and global isolates were reported by Odds et al. [11]; *$P < .05$; **$P < .01$; ***$P < .001$.
[†]More than 95% of Asia isolates were collected after year 2000.

nucleotide sequence variations in the seven housekeeping genes, 172 isolates were assigned 68 previously known DSTs; the other 113 (39.6%) isolates were assigned 110 new DSTs. The number of variable bases of each of the seven housekeeping genes was 20 for *VPS13*; 18 for *MPIb*; 16 each for *ADP1*, *SYA1* and *ZWF1b*; 10 for *AAT1a,* and 6 for *ACC1*. The number of genotypes (alleles) of each gene was 57 for *VPS13*, 39 for *ZWF1b*, 38 for *SYA1*, 34 for *AAT1a*, 28 for *MPIb*, 21 for *ACC1*, and 19 for *ADP1*. Of the seven genes used for MLST, *ACC1* and *AAT1a* showed the highest typing efficiency, distinguishing 3.50 (21 genotypes divided by 6 variable bases) and 3.40 (34 genotypes divided by 10 variable bases) genotypes per polymorphism, respectively. These two genes were also found to have the best discriminating power by Bougnoux et al. [21]

The 285 CGMHL and 996 MLST reference isolates were classified into 18 clades by UPGMA. The CGMHL isolates (open circles in Fig. 1) were clustered in all MLST clades except clades 2 and 13. Isolates in these two clades are mostly found in Europe and Africa, and clade 13 was previously recognized as *Candida africana* by phenotying [11,22]. Clades 1–4 and 11 have been evidenced the most consistent during rapid expansion of the database, and clades 3 and 11 have been shown to be very close to each other [22]. The uneven distribution of the CGMHL isolates in clades 3, 4, and 11 (Fig. 1) suggests a close phylogenetic association of CGMHL isolates within the same clade. The population of CGMHL isolates in clade 1 and singlets (isolates that could not be classified into any clade by UPGMA with a cutoff value of $P = .04$) was much smaller but that in clade 16 was significantly bigger than that of the global isolates published in 2007 [11]. The populations of CGMHL isolates in clades 3 and 4 were bigger than those of other Asian isolates, but not those of global isolates collected since 2000 (Table 1). In contrast, the number of CGMHL isolates in clade 17 was higher than that of global (both before and after 2000) but not of other Asian isolates.

UPGMA measures the *P*-distance of polymorphic nucleotide sequences. Although it provides a simple view of phylogenetic relationship of the isolates, some minor clades were altered when the isolate number increased [22]. Therefore, eBURST, another powerful algorism to reveal the genetic relationship of isolates, was also used in this study. Results showed that the percentages of CC8, CC20, CC15, and CC17 CGMHL isolates (15.8%, 9.5%, 8.4%, and 8.1%, respectively) were significantly higher than those of

Table 2. eBURST clonal distribution of *C. albicans* isolates.

Clonal Complex	Predicted Founder	No. of DSTs in CGMHL Isolates	No. of Isolates (%)[#]		
			CGMHL	Asia[††]	Global[†]
1	69	10	15 (5.3)	26 (7.2)	600 (24.6)***
2	124	1	1 (0.4)	10 (2.8)*	248 (10.2)***
3	155	0	0 (0)	2 (0.6)	221 (9.1)***
4	344	2	2 (0.7)	6 (1.7)	87 (3.6)*
5	538	0	0 (0)	0 (0)	71 (2.9)**
6	299	7	9 (3.2)	13 (3.6)	54 (2.2)
7	735	6	7 (2.5)	4 (1.1)	55 (2.3)
8	659	18	45 (15.8)	21 (5.8)***	38 (1.6)***
9	766	12	25 (8.8)	19 (5.3)	35 (1.4)***
10	461	6	10 (3.5)	4 (1.1)	39 (1.6)*
11	409	4	4 (1.4)	0 (0)*	38 (1.6)
12	304	5	8 (2.8)	2 (0.6)*	23 (0.9)*
13	727	6	7 (2.5)	17 (4.7)	20 (0.8)*
14	365	2	5(1.8)	11 (3)	35 (1.4)
15	669	12	24(8.4)	2 (0.6)***	8 (0.3)***
16	90	0	0(0)	4 (1.1)	24 (1)
17	443	10	23(8.1)	6 (1.7)***	9 (0.4)***
18	840	1	1(0.4)	2 (0.6)	17 (0.7)
19	439	4	6(2.1)	12 (3.3)	14 (0.6)*
20	693	7	27(9.5)	6 (1.7)***	9 (0.4)***
Others	–	65	66(23.2)	194 (53.7)	790 (32.4)
Total		178	285(100)	361 (100)	2435 (100)

[#]The difference between the distributions of the CGMHL group and Asia or Global groups were calculated by $\chi 2$ and Fisher exact tests.
*$P < .05$; **$P < .01$; ***$P < .001$.
[†]Global isolates includes all isolates in the MLST database (date accessed 10.01.14).
[††]Asian isolates include those from China, Hong Kong, Japan, Korea/South Korea, Malaysia, and Taiwan recorded in the MLST database (date accessed 10.01.14).

other Asian (5.8%, 1.7%, 0.6%, and 1.7%) and global isolates (1.6%, 0.4%, 0.3%, and 0.4%), suggesting an expansion of CC8, CC20, CC15, and CC17 isolates in CGMHL (Table 2).

Results of this study also showed that the CGMHL isolates in the same eBURST clonal complexes were grouped together in the same UPGMA clades (Table 3). Thus, there was a good correlation between UPGMA grouping and eBURST clustering. DST 659 was the predicted founder of the largest CC in the CGMHL isolates. DST 659 was determined to be CC11 (*i.e.*, 11th largest eBURST cluster) by Odds et al. [11], but was determined to be CC8 (*i.e.*, 8th largest eBURST cluster) in this study (Supplementary Table). In addition, DST 693, which was previously classified as a member of CC35 [11], was determined to be CC20 in this study (Supplementary Table). Both DST 659 and DST 693 clusters were greatly expanded during 2000–2011. So far, thirty-three isolates including 29 from Taiwan and 4 from Korea, were classified by eBURST as CC20 (Table 5), suggesting that CC20 is an Asian cluster, which constitutes MLST clade 3. Interestingly, when comparing with CC4, another clade 3 cluster, CC20 isolates showed a significant expansion in north Taiwan.

Among the 285 isolates characterized, only two fluconazole resistant isolates were detected. This result is consistent with the previous report that most *C. albicans* isolates causing invasive infections are susceptible to antifungal drugs [3,23–26]. This low rate of fluconazole resistance may be explained by fewer patients having prior fluconazole treatments in Taiwan [27]. No significant antifungal susceptibility trend of the 9 drugs was observed during 2003–2011 (data not shown). It is worth noting that most (15/19, 78.9%) DST 693 isolates and CC20 cluster isolates showed a lower susceptibility to fluconazole. DST 693 is predominant in the CGMHL isolates. It was first discovered (MIC = 0.25 μg/ml) in Taiwan from the sputum of an AIDS patient in 1996 [11,28]. It is likely that isolates of DST 693 have been in existence in Taiwan for years and have gained some anti-fluconazole activity since then. The decrease in fluconazole sensitivity may benefit DST 693 or CC20 isolates than other clade 3 isolates during prophylactic fluconazole

Table 3. Correlation between UPGMA grouping and eBURST clustering of CGMHL isolates.

UPGMA Clade (No. of Isolates)	eBURST Clonal Complex (No. of Isolates)
Clade 1 (54)	CC1 (15), CC9 (25), CC50 (2), Singleton (12)
Clade 2 (0)	–
Clade 3 (31)	CC4 (2), CC20 (27), Singleton (2)
Clade 4 (49)	CC2 (1), CC8 (45), Singleton (3)
Clade 5 (7)	CC33 (1), CC41 (1), CC79 (2), Singleton (3)
Clade 6 (4)	CC11 (4)
Clade 7 (1)	CC18 (1)
Clade 8 (15)	CC14 (5), CC23 (1), CC32 (2), CC68 (1), Singleton (6)
Clade 9 (10)	CC7 (7), Singleton (3)
Clade 10 (11)	CC12 (8), Singleton (3)
Clade 11 (11)	CC10 (10), Singleton (1)
Clade 12 (9)	CC6 (9)
Clade 13 (0)	–
Clade 14 (7)	CC19 (6), Singleton (1)
Clade 15 (3)	CC25 (1), CC59 (2)
Clade 16 (29)	CC15 (24), Singleton (5)
Clade 17 (31)	CC17 (23), CC22 (1), CC31 (3), Singleton (4)
Clade 18 (10)	CC13 (7), Singleton (3)

Table 4. CGMHL *C. albicans* isolates with lower fluconazole susceptibility.

Clonal Complex	Predicted Founder	No. of Isolates	No. of Isolates with MIC > = 0.5 (%)[#]
1	69	15	5 (33.3)
6	299	9	2 (22.2)
7	735	7	3 (42.9)
8	659	45	27 (60.0)
9	766	25	15 (60.0)
10	461	10	10 (100)**
11	409	4	4 (100)
12	304	8	4 (50.0)
13	727	7	4 (57.1)
14	365	5	0 (0)*
15	669	24	5 (20.8)**
17	443	23	13 (56.5)
19	439	6	2 (33.3)
20	693	27	23 (85.2)***
31	466	3	1 (33.3)
Others[†]	–	67	32 (47.8)
Total	–	285	150 (52.6)

[#]The difference in the population of isolates with MIC >= 0.5 between the indicated clonal complex and others was calculated by X^2 and Fisher exact tests.
*$P < .05$; **$P < .01$; ***$P < .001$.
[†]Others are the isolates belonging to singletons or those in the CCs with less than 3 isolates.

treatment, and that probably makes CC20 expansion in north Taiwan.

Acknowledgments

We appreciate the assistance of Professor Frank C. Odds in assigning new allele numbers and DSTs of the CGMHL isolates. We thank Dr. Chao-Hung Lee for editing the manuscript and Dr. Hsin-Fu Liu for helpful discussions in phylogenetic analyses. This work was supported by grants from Chang Gung Memorial Hospital (CMRPG3B1302, CMRPG3D1241, CMRPG3D1611) in Taiwan.

Declaration of interest

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

Table 5. DST and geographic distribution of eBURST clonal complex 20 isolates.

DST	No. of Isolate	MLST ID	Strain	Country	Reference
674	1	923	P06	Taiwan	Ref. [28]
693 (clonal founder)	20	944	P20–1	Taiwan	Ref. [28]
		CGMHL	C006, C040, C049, C070, C072, C105, C109, C122, C163, D027, D038, D048, P004, P020, P043, P059, P061, P067, P068	Taiwan	This Study
1563	3	1872	C6355	South Korea	MLST database
		CGMHL	C066, C082	Taiwan	This Study
1564	2	1873	C4502	South Korea	MLST database
		CGMHL	C141	Taiwan	This Study
1796	3	2019	C1098	Korea	MLST database
		CGMHL	C031, C096	Taiwan	This Study
2006	1	2115	NICU_6643	Korea	MLST database
2433	1	CGMHL	C022	Taiwan	This Study
2437	1	CGMHL	C038	Taiwan	This Study
2451	1	CGMHL	C085	Taiwan	This Study

Supplementary material

Supplementary material is available at *Medical Mycology* online (http://www.mmy.oxfordjournals.org/).

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