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Association of multilocus sequence typing, *MSH2* gene mutations, and antifungal resistance in *Candida glabrata*: implications for clinical outcomes in Chinese hospitals

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Abstract

Background *Candida glabrata* is the second most common cause of invasive candidiasis worldwide. In this study, we determined the clinical characteristics and drug sensitivity of *C. glabrata* isolates and investigated the associations between *MSH2* gene mutations, sequence types (ST), and drug resistance.

Methods A total of 154 *C. glabrata* isolates were collected from patients being treated in three hospitals in China. The antifungal sensitivity of the strains was assessed using the broth microdilution method. Multilocus sequence typing (MLST) was also performed, followed by *MSH2* sequencing. The clinical features and outcomes of *C. glabrata* infection were analysed for a total of 49 strains, which were collected from patients with invasive *Candida* infection at Longhua Hospital.

Results All 154 isolates were found to be susceptible to amphotericin, 5-fluorocytosine, anidulafungin, caspofungin, and micafungin, whereas 11.7% were fluconazole-resistant, 18.8% were itraconazole non-wild type, and 35.7% were voriconazole non-wild type. ST7 (62.34%) was the most common ST genotype, followed by ST10 (16.88%) and ST15 (7.79%). The total azole resistance rates for all isolates, ST7, ST10, and other STs were 36.4, 42.7, 34.6, and 18.8%, respectively. The ST7 and ST10 isolates were characterised by a higher drug resistance rate than the other minor ST isolates. Moreover, 59.09% of isolates had one or more *MSH2* non-synonymous mutations, with V239L being the most commonly detected mutation. The frequency of *MSH2* mutations was significantly higher in azole-resistant isolates than in other isolates, whereas P6L or L87P mutations were associated with the highest azole resistance rates of up to 87.5% and 80%, respectively. Our results indicated that ST7 and ST15 are independent predictors of mortality caused by *C. glabrata* infection and revealed a higher 30-day mortality in patients infected with these strains than in those infected with other ST isolates.

Conclusions Our findings revealed the relationships between MLST, *MSH2* gene mutations, and drug resistance in the common pathogenic fungus *C. glabrata*, and thereby enabled us to identify strains that are associated with higher

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rates of mortality. These findings will contribute to enhancing our understanding of the pathogenesis of *C. glabrata* infection.

Keywords *Candida glabrata*, MLST, *MSH2*, Drug sensitivity, Mortality, Sequence type, Risk factors

Background

Candida albicans is a well-known pathogen responsible for candidiasis. However, the relevance of *non-albicans Candida* species in pathogenicity has increased [1]. Notably, *Candida glabrata* has emerged as the second most frequent cause of invasive *Candida* infections (ICI) across the globe. Epidemiological studies have shown that *C. glabrata* is the predominant non-*albicans Candida* species in China [2]. The mortality of patients infected with *C. glabrata* has increased, with that of patients with a bloodstream infection being approximately 20% [3], whereas that of patients in intensive care units can be as high as 40–50% [4].

Azoles, such as fluconazole, voriconazole, and itraconazole, are frontline antifungal agents that inhibit sterol biosynthesis by targeting Erg11, a lanosterol demethylase enzyme. Despite their widespread use, an increasing prevalence of azole resistance has been observed, particularly among patients with a history of azole exposure. This resistance is a significant concern, as it limits the efficacy of what was once a first-line therapy [5, 6].

Understanding the clinical characteristics of candidiasis and the sequence types (STs) of *Candida* based on multilocus sequence typing (MLST) analysis will aid in investigating disease prevalence. The genotypes of different *Candida* species in different regions have been established to influence the prevalence of other *Candida* species, and it has been demonstrated that the ST genotype is linked to fluconazole resistance [7, 8]. Consequently, epidemiological investigations that seek to determine the distribution of *Candida* in hospitals and identify the *Candida* genotypes, when possible, are essential for promoting timely clinical treatment and preventing disease development. Different STs may be associated with *MSH2* alleles, suggesting variations in virulence and resistance factors among STs. DNA mismatch repair (MMR) systems maintain DNA replication fidelity and have been identified in various organisms. Defects in these systems are associated with elevated rates of gene mutation and can lead to gene instability and acquired resistance to fluconazole [9]. *MSH2* is a nuclear MMR gene, the mutation of which drives multiple drug resistance in *C. glabrata*, with strains harbouring specific missense mutations showing high resistance in vitro [10].

At present, however, the relationships among *MSH2* gene mutations, STs, and fluconazole resistance in *C. glabrata* have yet to be sufficiently established. In this study, we sought to determine whether *C. glabrata* isolates with different MLST genotypes exhibit different clinical characteristics, *MSH2* gene mutations, and fluconazole resistance. The findings of this study will make a valuable contribution to the clinical treatment of *Candida* infections.

Methods

Strains and culture conditions

A total of 154 *C. glabrata* strains were collected from 154 patients being treated in three hospitals in China. Among these, 55 *C. glabrata* strains were collected from patients at Longhua Hospital, 47 strains from patients at Renji Hospital, and 52 strains from patients at Dongfang Hospital. Strains were primarily isolated as part of previous studies [11, 12]. Species identification was performed using matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry. All strains were maintained on a yeast-peptone-dextrose medium at 30 ℃, as previously described [13].

Antifungal sensitivity testing

Antifungal sensitivity was assessed using the broth microdilution method, based on Clinical and Laboratory Standards Institute (CLSI) guidelines (document M27-A3) [14]. Susceptibility breakpoints were evaluated according to the CLSI M27M44S guidelines [15], where available, and epidemiological cut-off values were interpreted according to CLSI M60 [16], CLSI M59 [17], and CLSI M27-S3 [18].

Multilocus sequence typing (MLST) and phylogenetic analysis

The isolates were subjected to MLST using two previously described methods [19], and subsequently used for *MSH2* extraction. The polymerase chain reaction products of six housekeeping gene loci, namely, *FKS*, *NMT1*, *UGP1*, *LEU2*, *TRP*, and *URA3*, were subjected to bidirectional sequencing, and the combination of the alleles of these six loci was defined as an MLST genotype or ST based on references to the MLST database (http://pubmlst.org/C.glabrata). Phylogenetic analysis was performed using the Mega software and visualised using the online tool Evolview [20].

MSH2 sequencing

Isolates were incubated overnight in RPMI medium at 37 ℃. Cell suspensions were adjusted to 5×10^7 cells/mL with phosphate-buffered saline buffer, and the supernatants were collected via centrifugation at 3,000 × *g* for

5 min. Total DNA was isolated using a Yeast DNAiso Reagent Kit (TaKaRa, Shiga, Japan) according to the manufacturer's instructions. *MSH2* was amplified and sequenced commercially by Sangon Biotech Company. Gene sequences were compared with those of the *C. glabrata* ATCC2001 reference genome. Nucleotide sequences were analysed using Clustal X.

Clinical data analysis

The clinical information used in this study, including demographics, department of admission, underlying diseases, risk factors, and outcomes, was retrospectively collected from 49 patients with ICI who had been admitted to Longhua Hospital in Shanghai, China, between January 2018 and December 2022. The diagnosis of ICI is based on host factors, clinical features, and mycological evidence, as previously suggested [21]. The case definition often includes the isolation of *Candida* species from normally sterile sites, such as blood, deep tissue biopsies, or body fluids, along with evidence of invasive infection, such as histopathological findings or specific biomarkers in the context of appropriate clinical signs and symptoms.

Statistical analysis

Statistical analyses were performed using SPSS (version 24.0). Fisher's exact probability method was used to analyse unqualified data, and the chi-square test was used to analyse the composition ratio. The statistical significance level was set at a P-value <0.05. Biologically plausible variables with a P-value <0.1 obtained from the univariate analyses were included in a multiple logistic regression model [22].

Results

Antifungal susceptibility and MLST genotypes of *C. Glabrata*

The susceptibility to antifungal agents and non-synonymous *MSH2* mutations according to the MLST genotype are listed in Table S1 and Fig. 1. All isolates were found to be susceptible to amphotericin, 5-fluorocytosine, anidulafungin, caspofungin, and micafungin, whereas 11.7% (18/154) were resistant to fluconazole, 18.8% (29/154) were itraconazole non-wild type, and 35.7% (55/154) were voriconazole non-wild type. The total azole resistance rate for all isolates was 36.4% (56/154).

The allelic profiles of six housekeeping gene loci (*FKS*,*LEU2*,*NMT1*,*TRP1*,*UGP1*, and *URA3*) were examined based on MLST typing, the findings of which revealed the occurrence of 13 ST genotypes (ST3, 7, 10, 15, 19, 22, 26, 43, 45, 55, 82, 83, and 182), all of which have previously been described. Among these, ST7 (96/154, 62.34%) was identified as the most commonly occurring ST, followed by ST10 (26/154, 16.88%) and ST15 (12/154, 7.79%), which collectively accounted for 87.01% of all *C. glabrata* isolates collected from the three hospitals.

With respect to antifungal susceptibility, we found that 15.63% (15/96) of the ST7 isolates and 11.54% (3/26) of the ST10 isolates were resistant to fluconazole; 21.88% (21/96) of the ST7 isolates and 26.92% (7/26) of the ST10 isolates were itraconazole non-wild type; and 41.67% (40/96) of the ST7 isolates and 34.62% (9/26) of the ST10 isolates were voriconazole non-wild type. None of the remaining 32 ST isolates was resistant to fluconazole, whereas 3.13% (1/32) and 18.75% (6/32) were itraconazole and voriconazole non-wild type, respectively. The total azole resistance rates for ST7, ST10, and other STs were 42.7% (41/96), 34.6% (9/26), and 18.8% (6/32), respectively.

Although we detected no significant difference between the ST7 and ST10 isolates with respect to their resistance to azole, ST7 was found to differ significantly from the other minor ST isolates with regards to fluconazole, itraconazole, and voriconazole resistance ($P=0.0173$, 0.0149, and 0.0193, respectively). Similarly, ST10 differed significantly from the other minor ST isolates in terms of fluconazole and itraconazole resistance ($P=0.0485$ and 0.0090, respectively).

Antifungal susceptibility and *MSH2* mutations in *C. Glabrata* isolates

MSH2 was amplified from all 154 detected isolates, of which 123 (79.87%) were characterised by synonymous or non-synonymous nucleotide substitutions in *MSH2*, and 31 (20.13%) had no mutations (Table S1 and Fig. 1). Furthermore, 91/123 isolates (73.98%) had one or more non-synonymous mutations, among which 47.23% (43/91), 39.56% (36/91), and 35.16% (32/91) harboured V239L, L359S, and S431P mutations, respectively.

Comparisons among the *MSH2* alleles revealed that the frequency of non-synonymous mutations was significantly higher in fluconazole-resistant isolates (83.3%, 15/18) than in fluconazole-susceptible isolates (56.62%, 77/136; $P=0.0299$), and in voriconazole non-wild-type isolates (72.73%, 40/55) than in voriconazole wild-type isolates (52.53%, 52/99; $P=0.0143$).

Among the different *MSH2* mutations, isolates with the P6L and L87P mutations showed the highest azole resistance of up to 87.5% (7/8) and 80% (8/10), respectively. Moreover, among the azole-resistant isolates, four *MSH2* mutations (P6L, T50L, L87P, and A385S) were detected only in the voriconazole non-wild-type isolates.

MLST genotypes and *MSH2* mutations in *C. Glabrata*

Of the 96 ST7 isolates (15 fluconazole-resistant and 81 fluconazole-susceptible), 64 (13 fluconazole-resistant and 51 fluconazole-susceptible) harboured ten *MSH2* mutations. The most frequent mutation appeared to

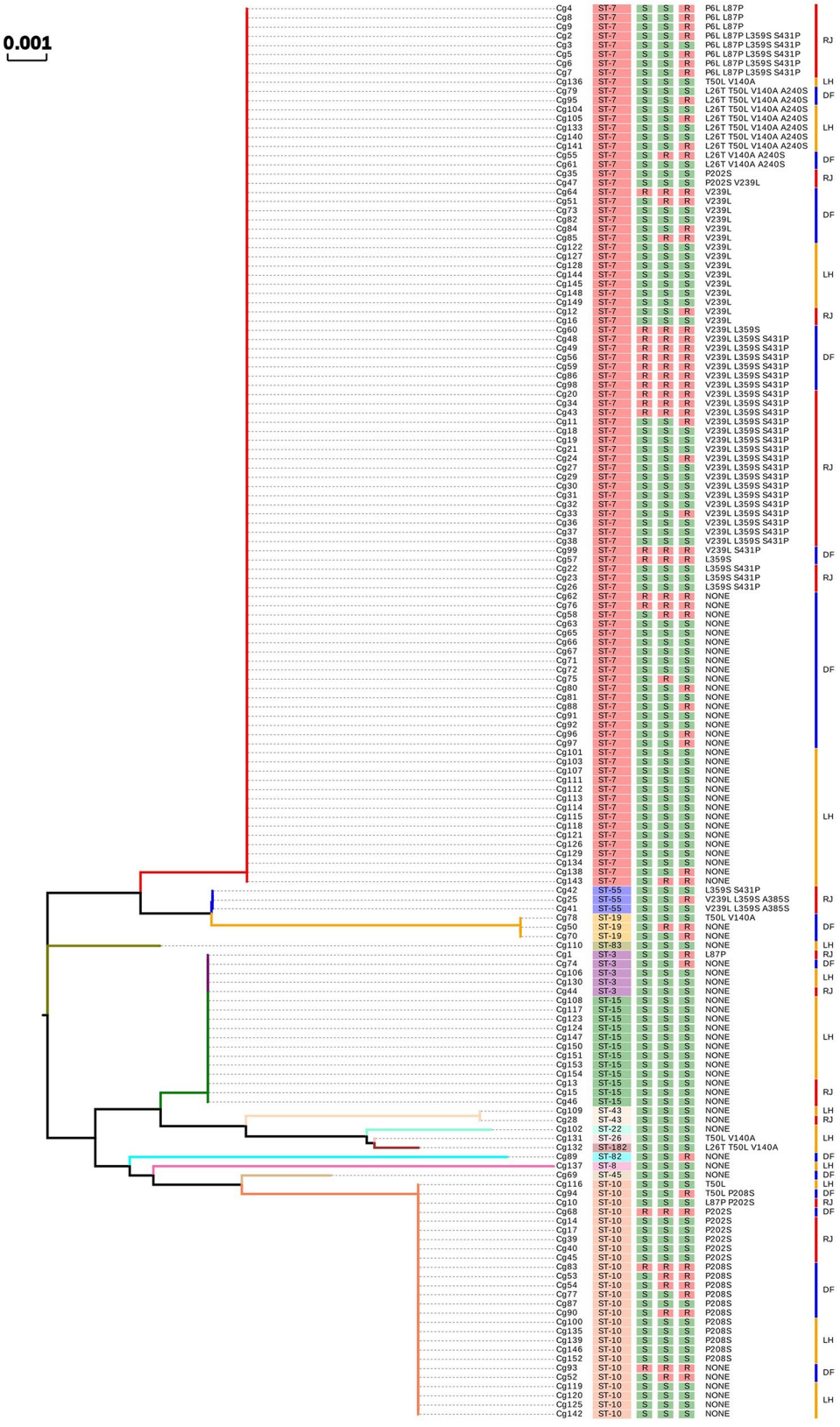

Fig. 1 MLST phylogenetic tree of the 154 *Candida glabrata* isolates

be the V239L mutation, which occurred in 41 of the 64 ST7 isolates, among which 26 isolates also harboured one or more of the following mutations: P202S, L359S, and S431P. Furthermore, eight of the 64 ST7 isolates harboured P6L/L87P mutations, among which five harboured two additional mutations (L359S and S431P). A further ten ST7 isolates harboured the V140A mutation and one or more of the following additional mutations: L26T, T50L, and A240S.

Among the 26 ST10 isolates (3 fluconazole-resistant and 23 fluconazole-susceptible), 20 (3 fluconazole-resistant and 17 fluconazole-susceptible) harboured four mutations. Of these latter isolates, seven harboured the P202S mutation, one of which harboured an additional L87P mutation; 12 isolates harboured the P208S mutation, one of which harboured an additional T50L mutation; and one isolate harboured only the T50L mutation.

Among the five ST3 isolates (with no resistance to fluconazole), only one harboured the L87P mutation. All three ST55 isolates harboured L359S/A385S mutations, and two showed additional V239L mutations. In addition, one of the three ST19 isolates, one ST26 isolate, and one ST182 isolate possessed T50L/V140A mutations, with the ST182 isolate harbouring an additional L26T mutation. In contrast, we detected no mutations in any of the ST15 (12), ST43 (2), ST8 (1), ST22 (1), ST45 (1), ST82 (1), or ST83 (1) isolates.

Overall, our findings revealed a close correlation between *MSH2* and ST genotypes, with the P6L, A240S, and S431P mutations being identified only in the ST7 isolates, whereas P208S and A385S were detected only in the ST10 and ST55 isolates, respectively. Although we detected no significant difference between the ST7 and ST10 isolates with respect to the percentage of *MSH2* mutations, the percentage of mutations in the ST7 isolates (66.67%, 64/96) differed significantly from that in the other ST isolates (24.24%; $P<0.001$). Similarly, the percentage of mutations in the ST10 isolates (76.92%, 20/26) differed significantly from that in the other ST isolates (24.24%; $P<0.001$).

Clinical characteristics and mortality rates of patients with *C. Glabrata* infection

For each of the 49 patients with ICI assessed in this study, we also analysed the clinical features and outcomes of *C. glabrata* infection. Table 1 summarises the clinical characteristics of the 49 patients infected with different *C. glabrata* STs. Among these patients, a large proportion (44.9%) was aged between 71 and 84 years, and 57.1% were males. Furthermore, 57.1% of the patients were admitted to a medical ward, and the use of antibiotics (89.8%) was identified as the most common risk factor. Most patients had comorbid lung disease (73.5%) or hypertension (69.4%).

The mortality rate among the patients was 30.6%. Patients infected with ST15, ST7, and ST10 isolates had overall mortality rates of 66.7%, 38.5%, and 10.0%, respectively. No patients infected with other ST genotypes died. To better understand the impact of different factors on patient clinical outcomes, we divided the patients into three groups based on mortality: ST7, ST15, and other ST isolates. In terms of clinical outcomes, we detected no significant differences with respect to age, sex, or clinical status among the different groups. However, significant differences were observed in department distribution and 30- and 120-day mortality rates among the three groups. Multivariate logistic regression analysis indicated that an ST7 or ST15 genotype was significantly associated with mortality (Table 2). Kaplan–Meier survival analysis revealed a significant difference in the 30-day mortality between patients infected with ST7 or ST15 isolates and those patients infected with other ST isolates (Fig. 2). However, we observed no significant differences with regards to clinical features, including mortality rate, associated with the *MSH2* mutation type or azole resistance.

Discussion

MLST analysis can help gain an understanding of the molecular characteristics of epidemics and facilitate clinical investigation. In this study, we identified ST7 as the dominant genotype (96/154) among the *C. glabrata* strains isolated from patients being treated in three hospitals in China. ST7 has been reported as the most common *C. glabrata* genotype in East Asian countries, including China, Korea, and Japan [8, 12, 23]. In contrast, ST3 has been identified as the predominant genotype in Australia and the United States [7, 24, 25], thereby highlighting the geographical differences in the distribution of MLST genotypes. Although other studies have reported ST3 to be the second most common *C. glabrata* genotype in China [26], in the present study, we detected only five ST3 isolates among the 154 *C. glabrata* isolates examined. Other common genotypes (ST10 and ST15) identified in this study have been previously detected in several other countries [27, 28].

Although previous studies have attempted to establish an association between specific genotypes and resistance traits, only few have demonstrated an association between certain STs and *C. glabrata* resistance to azole drugs. For example, ST7 isolates from Japan and China have been found to have comparatively higher fluconazole resistance [12, 23], whereas among *C. glabrata* isolates from Tanzania, those with the ST18 genotype were associated with a susceptibility to fluconazole [29]. Among the 154 *C. glabrata* isolates assessed in the present study, those with the ST7 and ST10 genotypes were found to be characterised by higher resistance to azole than the other less commonly encountered ST isolates,

Table 1 Clinical characteristics of *Candida glabrata* isolates of different sequence types (STs)

Patient characteristic	Total	ST7	ST15	Other	*P*-value
	n = 49	*n* = 26	*n* = 6	*n* = 17	
Age (years)					0.517
≤ 70	11 (22.4)	6 (23.1)	1 (16.7)	4 (23.5)	
71–84	22 (44.9)	10 (38.5)	2 (33.3)	10 (58.8)	
≥ 85	16 (32.7)	10 (38.5)	3 (50)	3 (17.6)	
Sex (n,%)					0.328
Male	28 (57.1)	17 (65.4)	2 (33.3)	9 (52.9)	
Female	21 (42.9)	9 (34.6)	4 (66.7)	8 (47.1)	
Department					0.086
ICU	16 (32.7)	8 (30.8)	4 (66.7)	4 (23.5)	
Medical wards	28 (57.1)	17 (65.4)	2 (33.3)	9 (52.9)	
Surgical wards	5 (10.2)	1 (3.8)	0 (0)	4 (23.5)	
Underlying diseases (n,%)					
Lung disease	36 (73.5)	18 (69.2)	4 (66.7)	14 (82.4)	0.586
Hypertension	34 (69.4)	16 (61.5)	4 (66.7)	14 (82.4)	0.346
Cardiovascular	26 (53.1)	14 (53.8)	2 (33.3)	10 (58.8)	0.557
Cerebral infarction	23 (46.9)	10 (38.5)	4 (66.7)	9 (52.9)	0.380
Sepsis	16 (32.7)	8 (30.8)	3 (50)	5 (29.4)	0.624
Diabetes	17 (34.7)	8 (30.8)	1 (16.7)	8 (47.1)	0.336
Kidney disease	18 (36.7)	11 (42.3)	1 (16.7)	6 (35.3)	0.496
Digestive tract disease	9 (18.4)	5 (19.2)	1 (16.7)	3 (17.6)	0.985
Tumour	12 (24.5)	7 (26.9)	4 (66.7)	1 (5.9)	**0.011**
Urinary tract infection	12 (24.5)	7 (26.9)	1 (16.7)	4 (23.5)	0.865
Liver disease	6 (12.2)	2 (7.7)	1 (16.7)	3 (17.6)	0.585
Gallbladder disease	5 (10.2)	3 (11.5)	2 (33.3)	0 (0)	0.064
Multiple organ injury	3 (6.1)	3 (11.5)	0 (0)	0 (0)	0.243
Immune system diseases	2 (4.1)	2 (7.7)	0 (0)	0 (0)	0.398
Risk factors (n,%)					
Use of antibiotics	44 (89.8)	24 (92.3)	4 (66.7)	16 (94.1)	0.133
Presence of CVC	27 (55.1)	13 (50)	6 (100)	8 (47.1)	0.061
Urine catheter	25 (51)	11 (42.3)	5 (83.3)	9 (52.9)	0.190
Previous surgery	26 (53.1)	12 (46.2)	5 (83.3)	9 (52.9)	0.259
Stomach tube	20 (40.8)	8 (30.8)	5 (83.3)	7 (41.2)	0.062
Total parenteral nutrition	13 (26.5)	8 (30.8)	3 (50)	2 (11.8)	0.147
Immunosuppressant use	11 (22.4)	6 (23.1)	3 (50)	2 (11.8)	0.154
Outcome (n,%)					
7-day all-cause mortality	5 (10.2)	4 (15.4)	1 (16.7)	0 (0.0)	0.227
30-day all-cause mortality	10 (20.4)	8 (30.8)	2 (33.3)	0 (0.0)	**0.035**
120-day all-cause mortality	15 (30.6)	10 (38.5)	4 (66.7)	1 (5.9)	**0.010**

Bold indicates significant relationships ($P < 0.05$)

thereby indicating an association between MLST genotype and antifungal resistance.

MSH2 mutations contribute to microevolution and population diversity and induce specific polymorphisms. In this study, we found that 59.09% (91/154) of *C. glabrata* isolates harboured non-synonymous *MSH2* mutations, the most common of which was V239L, which we identified in 47.25% (43/91) of the isolates that harboured *MSH2* mutations, and is consistent with previous findings [8, 10, 14, 30, 31]. Certain mutations in this gene are believed to impair DNA repair, thereby leading to higher mutation rates that may promote the emergence of drug-resistant lineages. In this study, the percentage of *MSH2* mutation in isolates with fluconazole resistance was significantly higher than that in fluconazole-susceptible isolates, which is again consistent with the findings of previous studies [9]. This phenomenon was also observed in the voriconazole non-wild-type isolates. Moreover, isolates with P6L and L87P mutations were found to be characterised by a high resistance to voriconazole, thus indicating an association between certain *MSH2* gene mutations and azole resistance.

Mutations in *PDR1* have been associated with increased azole resistance in clinical isolates and petite

Table 2 Multivariate logistic regression analysis of risk factors for *Candida glabrata*-associated mortality

Characteristics	Total	Univariate analysis		Multivariate analysis	
	(*N*)	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex					
Male	28	Reference			
Female	21	0.563 (0.158–1.997)	0.374		
Age					
<=70	11	Reference		Reference	
>70	38	5.833 (0.674–50.496)	0.109	11.469 (0.671–195.906)	0.092
Department					
Non-ICU	33	Reference		Reference	
ICU	16	9.333 (2.327–37.442)	0.002	8.254 (0.780–87.330)	0.079
Presence of CVC					
0	22	Reference		Reference	
1	27	5.067 (1.207–21.276)	0.027	0.191 (0.004–9.237)	0.403
Urine catheter					
0	24	Reference		Reference	
1	25	6.462 (1.528–27.324)	0.011	24.261 (0.472–1247.889)	0.113
Stomach tube					
0	29	Reference		Reference	
1	20	4.800 (1.305–17.657)	0.018	0.313 (0.009–10.625)	0.518
Total parenteral nutrition					
0	36	Reference		Reference	
1	13	6.629 (1.652–26.590)	0.008	3.206 (0.405–25.414)	0.27
MLST					
other ST	17	Reference		Reference	
ST15	6	32.000 (2.287–447.824)	0.01	53.594 (1.484–1934.991)	0.03
ST7	26	10.000 (1.143–87.518)	0.037	21.798 (1.460–325.500)	0.025

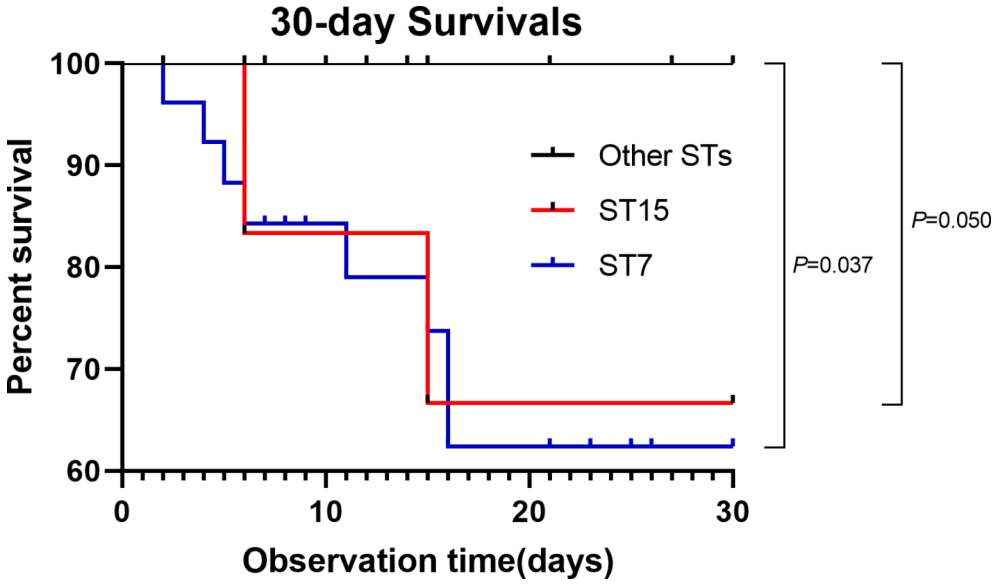

Fig. 2 Survival analysis of patients with *Candida glabrata* infection. Patients were grouped according to the MLST genotype (ST7, ST15, or other STs) and outcome at death or day 30

mutants of *C. glabrata* [32, 33]. *PDR1* mediates the expression of several genes involved in drug efflux, which can lead to reduced intracellular concentrations of antifungal drugs and thus resistance. In our previously study [11], four *PDR1* missense mutations (A848V, G348C, D876N, and N764D) were found in azole-resistant isolates. The results suggested that efflux pumps in azole-resistant isolates exhibit more potent effects than those

in azole-susceptible isolates, which is consistent with the overexpression of *CDR1* and *CDR2*, two genes regulated by *PDR1*. The relationship between *MSH2* and *PDR1* mutations in *C. glabrata* has been the subject of research aimed at understanding the mechanisms of antifungal resistance in this species. The link between *MSH2* and *PDR1* mutations may lie in the fact that both genes are involved in the development of resistance to antifungal drugs. In the present study, we did not identify any novel *PDR1* mutations or a significant correlation between the *MSH2* and *PDR1* genes (Table S2). This absence of a detectable link may be attributed to the limited number of azole-resistant strains that we examined. Although the direct interaction between these two genes has not been well established, it is plausible that a strain with a defective *MSH2* gene may have a higher mutation rate than other strains, which increases the likelihood of developing mutations in other genes, such as *PDR1*, that confer resistance. Further research is necessary to elucidate the exact mechanisms by which these mutations interact and contribute to multidrug resistance in *C. glabrata*.

The findings of recent studies have also revealed that *MSH2* gene mutations are relevant to STs. Hou et al., for example, have reported that all the ST7 isolates they assessed carried the V239L mutation, whereas all ST10 isolates harboured P208S/N890I mutations [10]. Similar observations have been reported by Byun et al., with the exception that the V239L mutation was also identified in isolates with other STs [8]. Bordallo et al. also found that the V239L mutation was prominent in ST7 and other STs, whereas ST145 isolates harboured P208S/N890I mutations [30]. In the present study, the V239L mutation was present in 42.7% (41/96) of the ST7 isolates and 66.7% (2/3) of the ST55 isolates, whereas the P208S mutation appeared to occur exclusively in the ST10 isolates, and P6L, A240S, and S431P mutations occurred only in the ST7 isolates. We also observed a correlation between ST55 and the presence of A385S mutations, whereas the ST15 isolates carried the wild-type *MSH2* gene. Furthermore, Healey et al. have reported an association between ST16 isolates and E231G/L269F mutations [34]; however, we were unable to detect ST16 isolates or the presence of E231G/L269F in other STs. Collectively, these findings indicate a link between certain STs and *MSH2* mutations.

A further important contribution of this study is that we report the clinical characteristics and outcomes of patients infected with specific MLST genotypes. Notably, higher mortality was observed in patients with *Candida* infections due to the ST7 or ST15 isolates. With the exception of tumours, the patients infected with either of these STs or other ST isolates were found to be characterised by similar clinical features. However, multivariate logistic regression analysis revealed that tumours had no significant influence on the mortality attributed to *C. glabrata* infection. Furthermore, our results revealed that ST7 and ST15 were independent predictors of mortality. Compared with the other detected ST genotypes, we recorded higher 30-day mortalities in patients infected with ST7 and ST15 isolates. The findings of other studies have similarly indicated a relationship between ST7 and ST3 infections and poor outcomes [8]. To the best of our knowledge, this study is the first to report that ST7 and ST15 genotypes are associated with higher mortality in patients with *Candida* infections in China. Although it is difficult to attribute mortality solely to *Candida* infection, the association between the aforementioned two STs and increased mortality may explain our results. Further studies, including those utilising a larger sample size, are warranted to assess the impact of clinical infections with ST7 or ST15 isolates.

Conclusions

The clinical outcomes of *Candida* infections are influenced by the fungal strain and host status. In this regard, our findings of high mortalities attributable to *C. glabrata* infection, particularly in patients infected with the ST7 and ST15 isolates, tend to indicate that the pathogenic potential of *C. glabrata* is due, at least in part, to the MLST genotype. However, we detected no direct correlation between the ST genotype and azole resistance. ST has been established to be strongly correlated with geographical distribution and *MSH2* mutations, and plays a directive role in *C. glabrata* infections in the same geographical location. Consequently, it is necessary to determine the virulence characteristics of *C. glabrata* with unique MLST genotypes. Further studies on the major MLST genotypes and the molecular characteristics of *C. glabrata* isolates will provide additional insights into the clinical outcomes of patients with *C. glabrata* infections and antifungal resistance.

Abbreviations

MLST	Multilocus Sequence Typing
ICI	Invasive Candida Infection
ST	Sequence Type
MMR	Mismatch Repair
CLSI	Clinical and Laboratory Standards Institute

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions

GZ performed the investigations and prepared the original draft. YC performed the investigations and contributed to the methodology adopted in the study. JC conducted the formal analysis. DY conceptualized the study

and was a major contributor for reviewing and editing the manuscript. All authors read and agreed to the published version of the manuscript. GZ and YC contributed equally to this study.

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Data availability
The nucleotide sequences of strains analysed in this study have been deposited in the NCBI database under the accession number PRJNA1130670.

Declarations

Ethical approval
The authors confirm that the ethical policies of the journal, as noted on the journal's author guideline page, have been adhered to. This retrospective study was approved by the Medical Research Ethics Committee of Longhua Hospital; individual data were collected anonymously and the requirement to obtain informed written consent was waived.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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