ORIGINAL ARTICLE

Diversity of the diploid sequence type of *Candida albicans* clinical isolates from a tertiary-care hospital in Mwanza, Tanzania

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Abstract

Geographical strain variations of *Candida albicans* causing different clinical conditions in susceptible individuals have been reported. In this study, the distribution of diploid sequence type of *C. albicans* was investigated in Mwanza, Tanzania. A total of 64 *C. albicans* were selected on the basis of their antifungal susceptibility patterns, followed by multilocus sequence typing (MLST) to establish the circulating sequence types (STs). Forty-eight MLST were obtained out of 64 isolates amounting to 75% population structure differences. Out of these STs, 27 (56.3%) were new diploid ST types. *C. albicans* isolates with new ST were more diverse than isolates with known STs (27/29, 93.1% vs. 21/35, 60%, p 0.002). In conclusion, *C. albicans* from clinical specimens were highly diverse, with more than half of the detected diploid ST not previously reported in the MLST database, thus confirming the genetic differences of *C. albicans* from different geographical regions.

Keywords: *C. albicans*, clinical isolates, diploid sequence type, isolate diversity, MLST
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Introduction

Candida albicans is the most frequently isolated fungal species from human as either commensal or pathogen [1]. The population structure difference is crucial in describing the epidemiology, genetic dynamics and diversity of *C. albicans*. Multilocus sequence typing (MLST) has a high discriminatory power and interlaboratory reproducibility in the detection of pathogen population structure differences [2]. The high discriminatory power of *C. albicans* MLST is associated with its ability to detect the polymorphic nucleotide sites in these genes [2–4]. The allelic difference combinations at different loci in MLST results in the development of unique sequence types (STs) which discriminate different strains [2].

Worldwide, *C. albicans* diploid sequence type (DST) 69 has been reported to be the leading cause of both invasive and noninvasive infection [5]. However, geographical variations of *C. albicans* genotypes causing different clinical conditions in susceptible individuals have been reported [6]. DST79, DST69 and DST155 have been found to be more common in Africa than other continents (https://pubmlst.org/calbicans/). Furthermore, the DST ST3 or ST16 have been associated with reduced susceptibility to azole antifungal [7,8]. As a result of limited data on ST of *C. albicans* causing different clinical conditions in Africa and the overuse of over-the-counter antifungal treatments [9], it is still not clear whether *Candida albicans* strains in Africa have local or international distributions.

This study was conducted to determine the distribution patterns of *C. albicans* STs causing different clinical conditions in Mwanza, Tanzania, using MLST.

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TABLE 1. Gene fragments of *Candida albicans* used for multilocus sequence typing

Gene	Primer sequence	Sequence bases	Gene product
AAT1a	F-5′-ACTCAAGCTAGATTTTTGGC-3′ R-5′-CAGCAACATGATTAGCCC-3′	373	Aspartate aminotransferase
AAC1	F-5′-GCAAGAGAAATTTTAATTCAATG-3′ R-5′-TTCATCAACATCATCCAAGTG-3′	407	Acetylcoenzyme A carboxylase
ADP1	F-5′-GAGCCAAGTATGAATGATTTG-3′ R-5′-TTGATCAACAAACCCGATAAT-3′	443	ATP-dependent permease
MP1b	F-5′-ACCAGAAATGGCCATTGC-3′ R-5′-GCAGCCATGCATTCAATTAT-3′	375	Mannose phosphate dehydrogenase
SYA1	F-5′- AGAAGAATTGTTGCTGTTACTG-3′ R-5′-GTTACCTTTACCACCAGCTTT-3′	391	Alanyl-RNA synthetase
VPS13	F-5′-TCGTTGAGAGATAATCGACTT-3′ R-5′-ACGGATGGATCTCCAGTCC-3′	403	Vacuolar protein sorting protein
ZWF1a	F-5′-GTTTCATTTGATCCTGAAGC-3′ R-5′-GCCATTGATAAGTACCTGGAT-3′ R-5′-AATTCGGTTGTAAGATGATGTTGC-3′	491	Glucose-6-phosphate dehydrogenase

Methods

Strain set

The current study used 64 archived *Candida albicans* isolates from a previous cross-sectional study that was conducted between January and December 2017 at the Bugando Medical Centre [10]. *C. albicans* isolated from patients with oral thrush (n = 9), vaginal candidiasis (11), candiduria (29), esophageal candidiasis (12) and blood candidiasis (3) were included in this study. The selected isolates were previously tested for susceptibility to fluconazole, voriconazole and posaconazole (Discovery Fine Chemicals, Bournemouth, UK), using European Committee on Antimicrobial Susceptibility Testing (EUCAST) minimum inhibitory concentration broth microdilution [10]. The study included all *C. albicans* with reduced susceptibility to azole antifungal agents. The reduced susceptibility to azole antifungal was based on the increase in epidemiologic cutoff value for azole antifungal agents compared to values in the literature, as documented in a previous study [10].

Five *C. albicans* isolates had increased MIC values on the initial testing using broth microdilution; however, only two isolates were confirmed by repeating broth microdilution and Etest.

MLST procedure

Frozen isolates were subcultured on Sabouraud dextrose agar (Oxoid, Basingstoke, UK) and aerobically incubated at 37°C for 24 hours. Genomic DNA was extracted by the phenol–chloroform method as previously described [11]. The purity of the extract was tested using the NanoDrop

TABLE 2. Novel MLST sequence types

Sample no.	Isolate	DST	*AAT*	*ACC1a*	*ADP*	*MP1b*	*SYA*	*VPS*	*ZWF*
1	V066	3540	33	7	32	26	2	61	295
2	V023	3542	3	3	5	3	57	10	218
3	V056	3543	2	3	5	9	2	20	5
4	V059	3544	8	14	8	3	7	3	8
5	V060	3545	55	7	8	3	6	45	249
6	V064	3546	8	3	6	4	7	10	89
7	V075	3547	55	14	4	26	6	45	15
8	V077	3548	33	7	32	3	2	61	48
9	V092	3549	55	14	4	2	6	45	12
10	V094	3550	8	3	5	9	2	6	5
11	V103	3551	80	14	4	3	24	45	15
12	V105	3552	43	14	8	4	74	45	89
13	V106	3553	58	7	6	6	7	60	8
14	V107	3554	11	26	6	4	7	10	119
15	V108	3555	8	14	8	2	7	3	8
16	V109	3556	35	4	4	63	4	24	4
17	V111	3557	39	3	6	3	6	45	15
18	V112[a]	3558	35	3	6	3	6	45	249
19	V117	3539	8	14	8	4	238	3	8
20	V146	3559	4	7	16	7	194	19	14
21	V158	3560	55	14	4	3	2	45	15
22	V180	3561	8	14	8	4	7	10	89
23	V210	3562	47	3	52	28	18	30	6
24	V268[a]	3563	2	5	5	2	6	6	5
25	V336	3564	21	3	25	9	38	65	12
26	V347	3565	2	3	5	2	2	34	247
27	V386	3566	55	7	6	3	3	45	12

DST, diploid sequence type; MLST, multilocus sequence typing.
[a]Two isolates had similar sequence type for each.

TABLE 3. Antifungal susceptibility pattern of *Candida albicans* stratified by sequence type

Isolate ID	DST	Isolation year	Sample	Age (years)	FL	VR	PO	CA	MI	5-FC
V006	574	2016	High vaginal swab	25	0.25	0.004	0.016	0.125	0.063	0.031
V015	519	2016	High vaginal swab	30	4 (0.094)	0.125 (0.006)	4 (0.008)	0.125	0.008	0.5
V020	311	2016	High vaginal swab	29	0.25	0.25	0.016	0.063	0.5	0.031
V021	69	2016	High vaginal swab	30	0.25	0.004	0.016	0.063	0.016	0.25
V023	3542	2016	High vaginal swab	19	0.25	0.004	0.016	0.125	2	0.063
V048	69	2016	High vaginal swab	16	16 (0.125)	0.004 (0.008)	0.016 (0.012)	0.031	0.125	0.5
V051	79	2016	Urine	16	0.25	0.004	0.008	0.063	0.063	0.5
V056	3543	2016	Urine	23	0.25	0.125	0.016	0.008	0.031	0.25
V057	1624	2016	Urine	23	0.25	0.125	0.016	0.063	0.063	0.063
V059	3544	2016	Urine	21	0.25	0.004	0.016	0.031	0.031	0.25
V060	3545	2016	Urine	20	0.25	0.004	0.016	0.063	0.031	0.25
V061	52	2016	Urine	18	0.25	0.004	0.016	0.063	0.063	0.063
V064	3546	2016	Urine	20	0.25	0.004	0.016	0.008	0.125	0.125
V065	916	2016	Urine	22	0.25	0.004	0.016	0.004	0.063	0.125
V066	3540	2016	Urine	20	0.25	0.004	0.008	0.016	0.063	0.5
V067	3330	2016	Urine	20	8 (8.000)	0.016 (0.75)	0.063 (0.064)	0.008	0.063	0.125
V069	1755	2016	Urine	20	0.25	0.125	0.016	0.25	0.063	0.063
V070	1755	2016	Urine	20	0.25	0.125	0.016	0.25	0.063	0.063
V073	124	2016	Urine	9	0.25	0.125	0.016	0.125	0.063	0.063
V075	3547	2016	Urine	7	0.25	0.004	0.016	0.25	0.063	0.5
V077	3548	2016	Urine	53	0.25	0.004	0.016	0.25	0.063	0.063
V078	365	2016	Urine	9	0.25	0.125	0.031	0.125	0.063	0.125
V085	365	2016	Urine	53	0.25	0.004	0.016	0.25	0.063	0.063
V088	365	2016	Urine	47	0.25	0.004	0.016	0.25	0.063	0.063
V089	365	2016	Urine	53	0.25	0.063	0.016	0.25	0.063	0.125
V092	3549	2016	Urine	28	0.25	0.004	0.016	0.25	0.063	0.031
V094	3550	2016	Urine	21	0.25	0.004	0.016	0.125	0.063	0.125
V097	124	2016	Urine	35	0.25	0.031	0.004	0.25	0.063	0.063
V100	124	2016	Urine	31	0.25	0.125	0.016	0.125	0.031	0.063
V101	299	2016	Urine	25	0.25	0.004	0.016	0.25	0.125	0.063
V103	3551	2016	Urine	34	0.25	0.125	0.031	0.25	0.063	0.063
V105	3552	2017	Blood	3m	0.25	0.004	0.008	0.25	0.063	0.063
V106	3553	2017	Blood	1	0.25	0.125	0.031	0.125	0.031	0.063
V107	3554	2017	Blood	1	0.25	0.004	0.016	0.25	0.063	0.063
V108	3555	2017	Esophageal brush	83	0.5	0.004	0.031	0.25	0.063	0.063
V109	3556	2017	Esophageal brush	72	0.25	0.004	0.016	0.25	0.063	0.063
V111	3557	2017	Esophageal brush	54	0.25	0.125	0.063	0.125	0.063	0.063
V112	3558	2017	Esophageal brush	30	0.25	0.004	0.008	0.125	0.063	0.063
V114	759	2017	Esophageal brush	23	0.25	0.008	0.002	0.125	0.063	0.063
V115	759	2017	Esophageal brush	60	0.25	0.125	0.016	0.25	0.063	0.063
V116	927	2017	Esophageal brush	48	0.25	0.004	0.002	0.125	0.063	0.063
V117	3539	2016	Urine	20	0.25	0.004	0.002	0.25	0.063	0.063
V118	927	2016	Urine	27	0.25	0.004	0.002	0.25	0.031	0.031
V123	3126	2016	Urine	36	0.25	0.004	0.031	0.125	0.063	0.063
V138	124	2016	Oral swab	2	0.25	0.25	0.016	0.125	0.063	0.063
V143	1075	2016	Oral swab	8	256 (0.190)	32 (0.008)	0.125 (0.012)	0.004	0.125	0.063
V146	3559	2016	Oral swab	3	0.25	0.5	0.031	0.031	0.063	0.063
V158	3560	2016	Oral swab	2	0.25	0.25	0.016	0.125	0.063	0.125
V179	144	2017	Esophageal brush	59	0.25	0.004	0.016	0.125	0.063	0.5
V180	3561	2017	Esophageal brush	43	0.25	0.004	0.016	0.25	0.063	0.063
V181	3126	2017	Esophageal brush	82	0.25	0.004	0.031	0.5	0.063	0.063
V210	3562	2015	Oral swab	14	0.25	0.004	0.031	0.25	0.063	0.063
V227	69	2016	High vaginal swab	24	1 (4)	0.004 (0.094)	0.063 (0.25)	0.125	0.063	0.063
V268	3563	2016	High vaginal swab	24	0.25	0.004	0.016	0.125	0.016	0.5
V276	3563	2016	High vaginal swab	39	4	0.125	0.25	0.125	0.031	0.063
V278	2757	2016	High vaginal swab	31	0.25	0.004	0.016	0.125	0.016	0.125
V280	3126	2016	High vaginal swab	38	0.25	0.031	0.125	0.125	0.125	0.063
V294	3126	2015	Esophageal brush	51	0.25	0.25	0.016	0.25	0.031	0.063
V295	277	2015	Esophageal brush	52	0.25	32	0.25	0.004	0.031	1
V336	3564	2015	Oral swab	45	0.5	0.025	0.125	0.002	0.031	0.063
V347	3565	2015	Oral swab	4	1	16	4	0.5	0.125	0.5
V350	2716	2016	Oral swab	82	0.25	0.031	0.031	0.25	0.063	0.063
V386	3566	2015	Oral swab	14	0.25	0.004	0.016	0.25	0.063	0.125
V397	3558	2016	Urine	42	0.25	0.031	0.031	0.125	0.063	0.063

All values European Committee on Antimicrobial Susceptibility Testing (EUCAST) edef7.2, except those in parentheses (Etest; bioMérieux, Marcy l'Étoile, France).
CA, caspofungin; DST, diploid sequence type; FL, fluconazole; MI, micafungin; PO, posaconazole; VR, voriconazole.

spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific, Waltham, MA, USA). An extract with absorbance ratio of 1.8 (280/260) and above was considered to be pure DNA extract.

The scheme developed by Bougnoux et al. [2] was used for MLST. The amplification of the seven housekeeping genes was performed using primers listed in Table 1 [2]. The PCR was carried out in 45 µL volume containing Ampli Taq DNA polymerase 0.5 µL (5 U/µL), PCR buffer 5 µL, 0.5 µL deoxynucleoside triphosphate (dNTP) 0.1mM, 3 µL each of the primers (Qiagen, Hilden, German), 34 µL PCR water and 2 µL of DNA template [2].

PCR conditions were as follows: 95°C for 15 minutes for initial denaturation, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds, extension at 72°C for 2 minutes and final extension at 72°C for 10

minutes. To confirm amplification, 5 μL of amplicon was visualized on 1% agarose gel in a Tris–acetate EDTA buffer.

The DNA amplicon was purified using a Qiagen purification kit followed by DNA Sanger sequencing (Seqlab-Microsynth, Göttingen, Germany) using same primers listed in Table 1. Analysis of sequences was carried as described in *C. albicans* database (https://pubmlst.org/calbicans/). The maximum-likelihood tree was constructed based on concatenated DNA sequences of seven loci using Geneious 11 software (Biomatters, Auckland, New Zealand).

Ethical clearance for conducting this study was granted by a joint CUHAS/BMC research ethics and review committee (certificate no. CREC/280/2017).

Results

A total of 3953 bp were sequenced in seven loci of each nonrepetitive *Candida albicans* isolate (Table 1). Forty-eight STs were obtained, resulting in 75% population structure differences. A total of 27 new STs were detected in 29 *C. albicans* isolates. The 29 *C. albicans* isolates were from high vaginal swabs (n = 3, 10.3%), urine (n = 12, 41.4%), blood/esophageal brushes (n = 8, 27.6%) and oral swabs (n = 6, 20.7%) (Table 2). Of the 35 *C. albicans* with known STs, 21 diploid STs were assigned.

On the basis of the STs, the isolates with new STs were significantly more diverse than the *C. albicans* isolates with known STs (27/29, 93.1% vs. 21/35, 60%, p 0.002). The most frequently detected STs were DST124, DST365 and DST3126, each of which was observed in four isolates, followed by DST69, with three isolates (Table 3).

Discussion

The 64 *C. albicans* clinical isolates from different samples were typed in 48 distinct DST, indicating 75% diversity of these isolates. The observed high population structure difference could be explained by the heterozygosity of the diploid *C. albicans* genome, which contributes to a high rate of genetic exchange [4]. The high population structure difference observed in the current study is similar to what was observed previously by Wu *et* al. [12] among clinical *C. albicans* isolates from sputum and urine samples, and by Ge *et* al. [13] among *C.*

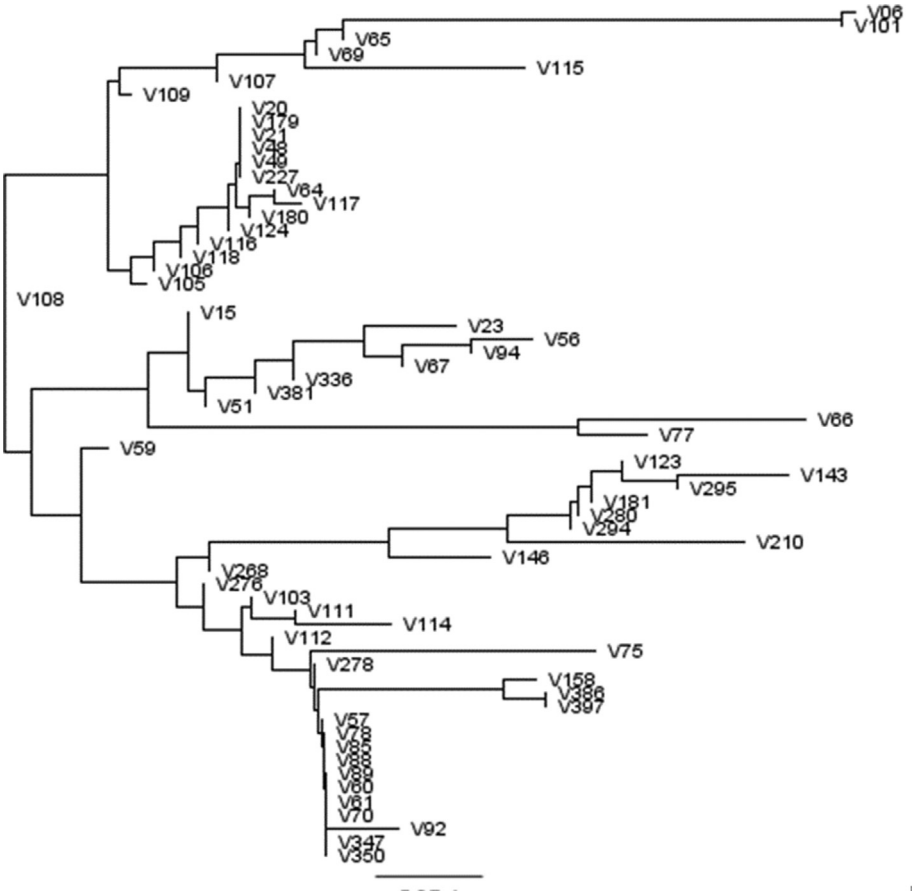

FIG. 1. *Candida albicans* genotype distributions. Maximum-likelihood tree was constructed based on concatenated DNA sequences of seven loci using Geneious R11 (Biomatters, Auckland, New Zealand) showing multilocus sequence typing–based phylogenetic relationship of all *C. albicans* isolates contained in study.

albicans isolated from urogenital samples in China. Furthermore, the observed difference is higher than that observed among *C. albicans* causing healthcare-associated bloodstream infections in United States [14]. The differences in the nature of the clinical samples and study setting explain the observed differences [15]. The current study involved *C. albicans* isolates from diverse specimens, which therefore has a greater likelihood of having diverse DSTs compared to the study from the United States, which studied *C. albicans* isolated from blood samples in the hospital setting.

As has been observed previously by different studies [2,8,16], DST69 was among the frequently observed STs in the current study. DST69 was also the most frequently detected genotype from noninvasive samples in Morocco (https://pubmlst.org/calbicans/). This observation confirms that DST69 is a globally distributed *C. albicans* genotype (Fig. 1).

A number of new DSTs were observed among *C. albicans* isolates in this study. Furthermore, it was observed that *C. albicans* with new DSTs were more diverse than those with known DST. This confirms that *Candida albicans* from different geographical location are likely to display distinct DSTs [7,8]. The variation also confirms the ability of *C. albicans* MLST systems to detect minor genetic changes [16,17]. The geographical variation of *C. albicans* has also been observed among isolates from other African countries (https://pubmlst.org/calbicans/). The observed high variations of genotypes in Tanzania is similar to previous studies in China which reported 79% and 82% new DST among *Candida albicans* studied [12,13].

Conclusions

C. albicans from clinical specimens were highly diverse, with a number of new DSTs that had not been previously reported in the MLST world database. This confirms the presence of genetic differences of *C. albicans* in different geographical regions. Because of the high diversity of genotypes, we need to develop robust point-of-care techniques to genotype *C. albicans* isolates, especially those causing invasive infection.

Conflict of interest

None declared.

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