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Oral *Candida albicans* strain diversity and maintenance in HIV positive women in South Africa

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ABSTRACT

Objective: This study investigated *C. albicans* strain diversity and maintenance in the oral cavity of HIV positive women over a 6 month period.
Study design: C. albicans strains were isolated from 17 HIV positive women at Charlotte Maxeke Academic Hospital, Johannesburg at 3 intervals over a 6 month period. Strains were genotyped using ABC and Multilocus Sequence Typing (MLST) techniques. In the MLST technique, for each strain, a Diploid Sequence Type (DST) number was obtained. Using cluster analysis, an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram and a matrix of strain similarities were generated. Strains were also compared to the previous South African isolates documented in the MLST database.
Results: Ninety four percent of women carried the same ABC genotype for 6 months. MLST technique, showed that ten women (58.8%) carried the same DST at 2 visits, while seven (41.2%) carried different DST at all visits. Further analysis showed that 64.7% of women were recolonised with different strains and 35.3% carried the same strains of *C. albicans* with heterozygosity. A total of 40 diploid sequence types were identified of which 27 DSTs were unique to this study group that were added to the MLST database. Most of the strains were closely related to previously isolated strains from South Africa.
Conclusion: Recolonization of the oral cavity with different strains and microevolution of the original strains of *C. albicans* can occur, which can be a potential problem for HIV patients, in whom highly virulent and drug resistant strains can emerge.

1. Introduction

Candida albicans is a commensal carried by 60% of healthy humans in their oral cavity and digestive system (Mahalingam et al., 2022; Shanmuga et al., 2022; Delavy et al., 2023). This carrier rate increases with predisposing factors such as HIV infection, immunosuppression, cancer treatment and the use of antibiotics (Patel, 2022). For example, in a South African study, up to 81% of HIV positive patients, and up to 57% of cancer patients carried *C. albicans* in their oral cavity, which is often responsible for the development of oral candidiasis (Lourenço et al., 2017; Patel et al., 2006). Oral candidiasis affects up to 90% of individuals with HIV (Leigh et al., 2004). With the introduction of Highly Active Antiretroviral Treatment (HAART) there has been a decrease in the prevalence of oral candidiasis (de Almeida et al., 2017). Unlike candidiasis, carrier rates are only slightly affected by HAART (Thompson et al., 2010; Owotade et al., 2013). In addition, a high proportion of HIV positive patients, particularly in developing countries, do not use HAART. According to WHO database, at the end of 2022, out of 39 million people living with HIV, two thirds reside within the WHO African region. Of this HIV positive population, only 76% was receiving antiretroviral therapy (WHO, 2023). Due to lack of funding and resources, 9.2 million HIV positive people are not receiving treatment (UNAIDS, 2023). This untreated population is still vulnerable to frequent *Candida* infections. In the oral cavity, although individuals tend to carry a single type of strain *C. albicans,* over time, this strain may undergo microevolution or microvariation leading to detection of a variety of closely related types (Odd, 2010). Nevertheless, limited studies have reported the acquisition of new strains (Tian et al., 2021;

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Table 1
Distribution of *C. albicans* genotypes (ABC genotyping) in HIV positive women over 6 months.

	C. albicans genotypes			
Patient	**Baseline**	**3 months**	**6 months**	**Comment**
003	A	A	A	Consistent
006	B	B	B	Consistent
010	A	A	A	Consistent
012	B	B	B	Consistent
027	C	C	C	Consistent
035	C	C	C	Consistent
041	A	A	A	Consistent
042	B	B	B	Consistent
045	B	B	B	Consistent
078	B	B	B	Consistent
082	A	A	B	Inconsistent
132	B	B	B	Consistent
140	B	B	B	Consistent
166	B	B	B	Consistent
173	B	B	B	Consistent
185	B	B	B	Consistent
193	A	A	A	Consistent

Samaranayake et al., 2003).

HIV infection is associated with compromised immunity and immune status may deteriorate in the course of the disease. The acquisition of strains carried by family members and people in close proximities may predispose HIV positive individuals to potentially severe *Candida* infection, as the acquired strains may be more virulent or resistant to antifungal agents compared to their own colonizing strains (Tian et al., 2021, Mothibe and Patel, 2017). Studies on *Candida* strain diversity and evolutionary changes over time in HIV positive patients are relatively uncommon (Millon et al., 2002; McCullough et al., 1994; Samaranayake et al., 2003), and to our knowledge, none in HIV positive women. In addition, in South Africa, HIV-related oral *Candida* carriage rate is also very high (Patel et al., 2006). Therefore, the present study examined the molecular epidemiology of *C. albicans* strains in HIV positive women over a 6 month period to detect the microevolution, heterozygosity, repeated strain acquisition and diversity using MLST genotyping. These molecular aspects are important in understanding the epidemiology, transmission and spread of *C. albicans* in this population.

2. Materials and methods

2.1. Study population and sample collection

The study investigated patients attending the HIV clinic at the Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa in 2011 - 2012. All 197 subjects were HIV-positive women who agreed to participate after signing a written consent form. Ethical clearance was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg. This study population has been thoroughly described elsewhere (Owotade et al., 2013; Owotade & Patel, 2014). Briefly, 117 women colonized with oral *Candida,* were asked to come for follow up visits. They were on antiretroviral therapy and the mean age was 38.3 ± 8.4 years. Only 17 women came for their follow up visits and carried *C. albicans* at all 3 visits (0, 3 and 6 months). Some women either missed visit/s or at ≤ 2 visits carried no *C. albicans* or only carried *non- C. albicans Candida* only, in their oral cavity. Oral rinse samples were collected, cultured on Chromagar and *C. albicans* was identified using the API 20 C technique. Genomic DNA was extracted from one randomly selected pure colony of *C. albicans* using a previously described technique with modifications (De Baere et al., 2002).

The extracted DNA samples of stored *C. albicans* for all the three visits were subjected to ABC genotyping (McCullough et al., 1999) and Multilocus Sequence Typing (MLST) technique (Bougnoux et al., 2002; Spampinato & Leonardi, 2013).

2.2. ABC genotyping

A 50 µl PCR mix was prepared using the primers described for the technique (McCullough et al., 1999). From the 2 primers CA-Int L (5′-ATAAGGGAAGTCGGCAAAATAGATCCG TAA-3′) and CA-Int R (5′-CCTTGGCTGTGGTTTCGCTAGATAGTAGAT-3′), 0.5 µl of each primer was added to the mix. The other components of the mix were 10 µl of Taq buffer, 10 µl of $MgCl_2$, 2 µl of dNTPs, 0.5 µl of Taq polymerase enzyme and 5 µl of DNA from respective samples and 21.5 µl of sterile distilled water. Amplification was carried out using the MJ Mini Personal Thermal Cycler® (BIO-RAD Hercules, CA, USA) as follows: initial denaturation, 95 °C for 2 min and subsequently 30 s, annealing 57 °C for 1 min, extension 72 °C for 2 min and the cycle repeated for 34 times with final temperature of 72 °C for 10 min. Agarose gel electrophoresis was used to demonstrate the PCR products. The genotype of each sample was determined by the size of the amplified fragments, A= 450 bp, B= 840 bp, C= 450 bp & 840 bp and categorised using the ABC scheme.

2.3. MLST genotyping

MLST genotyping was done using method described by Bougnoux et al. (2002). The primers for the seven housekeeping genes (*AAT1a, ACC1, ADP1, MPIb, SYA1, VPS13* and *ZWF1b)* are described by Spampinato and Leonardi (2013). The 50 µl PCR mix contained 0.5 µl of the forward and 0.5 µl of the reverse primers of each of the 7 housekeeping genes, 10 µl of Taq buffer, 10 µl of $MgCl_2$, 2 µl of dNTPs, 0.5 µl of Taq polymerase enzyme, 5 µl of DNA from respective samples and 21.5 µl of sterile distilled water. Each sample was subjected to seven PCR reactions, one for each gene. Agarose gel electrophoresis was performed using 10 µl of the PCR product. The remaining amplified gene content (40 µl) was subjected to DNA sequencing.

2.4. Genotyping and strain identification using the MLST database

The forward and reverse sequences of each of the housekeeping genes from every sample were subjected to further analysis using the Geneious 6 R software (Biomatters, http://www.geneious.com). A consensus sequence conforming to the number of base pairs for each gene was obtained from aligning and trimming the forward and reverse sequences for each gene sequence. The trimmed sequences were compared with the existing allele sequences on the MLST database http://calbicans.mlst.net/sql/singlelocus.asp. Conformity with existing allele sequence produced the allele number and when there is no perfect agreement the MLST database indicated the closest allele and percentage closeness. Allele numbers for the seven genes were then used to generate a diploid sequence type (DST) number unique to that strain. Existing DST numbers were noted. Sequence data were sent to the MLST curator at the Institute Pasteur (Paris) to assign numbers for the alleles and DSTs that were novel.

Using cluster analysis, an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram was generated in Stata® 12 (StataCorp, College Station, Texas) from all the alleles of isolates obtained at all the visits per subject. The method used to interpret the data was based on the similarity coefficient as previously described (Soll, 2000). The algorithm computed a distance between isolated strains from which a number was generated, 0 representing no relationship and 1 indicating identical strains. From 0.9 to 1 indicated microevolution, 0.8–0.9 indicated moderate relationship and less than 0.8 was unrelated. The correlation option was used for the analysis rather than the Euclidian distance as similarities were easier to interpret (Boriollo et al., 2006; Soll, 2000).

To determine the evolutionary relationships of the strains, a tree was drawn using all the strains of *C. albicans* isolated from patients in South Africa present in the MLST database (30 previously isolated from blood

Table 2
C. albicans MLST genotypes in HIV positive women over 6 months.

Sample	Visit	AAT1a	ACC1	ADP1	MPIb	SYA1	VPS13	ZWF1b	DST	Comment
3.1	1	31	3	2	2	2	24	12	2164	Different
3.2	2	35	4	4	4	34	4	4	808	DST at all
3.3	3	31	91	2	2	2	24	12	2177	visits
6.1	1	143	14	8	4	7	10	8	2178	Different
6.2	2	143	92	8	4	7	10	8	2179	DST at all
6.3	3	52	14	8	4	7	10	8	2165	visits
10.1	1	31	2	5	2	2	24	241	2182	Same DST
10.2	2	31	2	5	2	2	24	25	2166	at 2 visits
10.3	3	31	2	5	2	2	24	25	2166	
12.1	1	8	7	8	4	2	10	22	2167	Same DST
12.2	2	8	7	8	4	2	10	22	2167	at 2 visits
12.3	3	70	7	8	4	2	10	22	2168	
27.1	1	8	14	125	4	7	10	8	2183	Same DST
27.2	2	8	14	8	4	7	10	8	124	at 2 visits
27.3	3	8	14	8	4	7	10	8	124	
35.1	1	8	3	8	4	7	13	8	210	Different
35.2	2	8	3	8	4	186	13	8	2186	DST at all
35.3	3	8	3	6	4	7	13	8	2187	visits
41.1	1	8	14	8	4	7	3	8	95	Same DST
41.2	2	8	14	8	4	7	3	22	1909	at 2 visits
41.3	3	8	14	8	4	7	3	22	1909	
42.1	1	70	14	6	4	84	10	8	2169	Same DST
42.2	2	145	14	6	4	76	10	8	2181	at 2 visits
42.3	3	70	14	6	4	84	10	8	2169	
45.1	1	14	14	8	4	50	10	8	2170	Same DST
45.2	2	14	14	8	4	50	10	8	2170	at 2 visits
45.3	3	14	14	30	4	50	10	8	2171	
78.1	1	14	3	6	4	2	10	8	2172	Same DST
78.2	2	70	3	6	4	2	10	8	2173	at 2 visits
78.3	3	14	3	6	4	2	10	8	2172	
82.1	1	35	4	4	4	185	26	4	2185	Different
82.2	2	4	4	4	4	34	26	4	646	DST at all
82.3	3	8	7	8	4	7	26	4	2174	visits
132.1	1	54	93	10	36	184	113	242	2188	Same DST
132.2	2	53	31	10	36	83	113	111	1019	at 2 visits
132.3	3	53	31	10	36	83	113	111	1019	
140.1	1	8	3	6	4	7	10	8	915	Same DST
140.2	2	70	3	6	4	7	10	8	619	at 2 visits
140.3	3	70	3	6	4	7	10	8	619	
166.1	1	8	7	8	4	7	10	8	144	Different
166.2	2	70	7	8	4	7	10	8	661	DST at all
166.3	3	63	7	8	4	7	10	8	2175	visits
173.1	1	70	14	8	4	7	249	8	2189	Different
173.2	2	70	14	8	4	7	10	8	656	DST at all
173.3	3	8	14	8	4	7	10	8	124	visits
185.1	1	8	3	8	4	7	10	8	392	Same DST
185.2	2	8	3	8	4	7	10	8	392	at 2 visits
185.3	3	8	94	8	4	7	10	8	2184	
193.1	1	31	5	5	12	2	21	5	2176	Different
193.2	2	31	95	5	12	2	21	5	2190	DST at all
193.3	3	31	5	5	2	2	21	5	375	visits
10/17 (58.8%) - Same DST at 2 visits, 7/17 (41.2%): Different DST at all visits										

New alleles and diploid sequence types (DSTs) are highlighted in grey,

cultures and the 51 strains of oral cavity isolates of *C. albicans* from this study). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.02014024 was prepared. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were generated (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor, 1969) and are in the units of the number of base

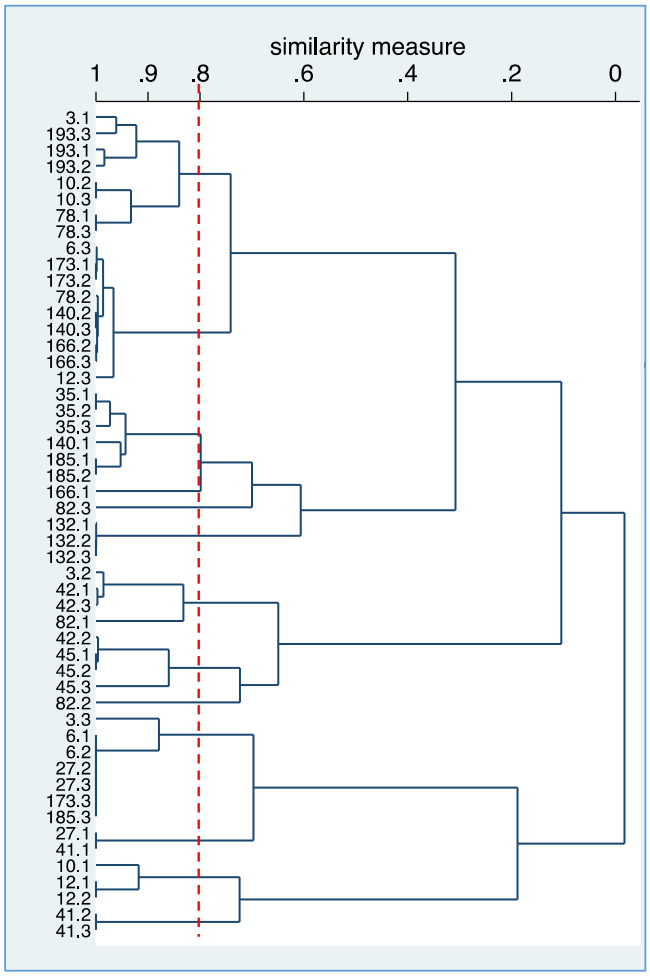

Fig. 1. UPGMA dengrogram to show relatedness of the *C. albicans* isolated from HIV positive women. Where 3.1, 3.2 and 3.3 means patient number 3 and isolates of *C. albicans* at visit 1, visit 2 and visit 3 respectively. This is applicable to all the strains described in this figure.

substitutions per site. The analysis involved 52 nucleotide sequences. Codon positions included were 1st+ 2nd+ 3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 2762 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021). The DST numbers were retained and the sample number from our study was put in parenthesis.

3. Results

3.1. ABC genotyping

Thirty one of the 51 *C. albicans* strains (60.8%) belonged to genotype B. Fourteen stains (27.4%) belonged to genotype A and 6 (11.8%) to genotype C. Sixteen out of the 17 women (94.1%) carried the same ABC genotype at all the three follow up visits (Table 1).

3.2. MLST genotyping

With MLST, strain consistency (indicated by the DST number) was less distinct from visit to visit in the same person. This data was simplified by separating the DST numbers at each visit (Table 2). Ten subjects (58.8%) carried the same strain at 2 visits, and 7 subjects (41.2%) carried different strains at each individual visit (Table 2). Fourteen novel MLST sequences were identified and added to the *C. albicans* MLST database by the curator (Table 2).

Table 3
Similarity matrix and percentage similarities for all *C. albicans* strains isolated over a 6 months.

No.	Patient	Parameters	Comparison of strains			Interpretation
			Visit 1 & 2	Visit 1 & 3	Visit 2 & 3	
1	3	Isolate	3.1 to 3.2	3.1 to 3.3	3.2 to 3.3	Recolonisation
		% Similarity	< 80 (D)	< 80 (D)	< 80 (D)	
2	6	Isolate	6.1 to 6.2	6.1 to 6.3	6.2 to 6.3	Recolonisation
		% Similarity	100 (S)	< 80 (D)	< 80 (D)	
3	10	Isolate	10.1 to 10.2	10.1 to 10.3	10.2 to 10.3	No recolonisation
		% Similarity	81-90 (M)	81-90 (M)	100 (S)	
4	12	Isolate	12.1 to 12.2	12.1 to 12.3	12.2 to 12.3	Recolonisation
		% Similarity	100 (S)	< 80 (D)	< 80 (D)	
5	27	Isolate	27.1 to 27.2	27.1 to 27.3	27.2 to 27.3	Recolonisation
		% Similarity	< 80 (D)	< 80 (D)	100 (S)	
6	35	Isolate	35.1 to 35.2	35.1 to 35.3	35.2 to 35.3	No recolonisation
		% Similarity	100 (S)	91-99 (ME)	91-99 (ME)	
7	41	Isolate	41.1 to 41.2	41.1 to 41.3	41.2 to 41.3	Recolonisation
		% Similarity	< 80 (D)	< 80 (D)	100 (S)	
8	42	Isolate	42.1 to 42.2	42.1 to 42.3	42.2 to 42.3	Recolonisation
		% Similarity	< 80 (D)	91-99 (ME)	< 80 (D)	
9	45	Isolate	45.1 to 35.2	45.1 to 35.3	45.2 to 45.3	No recolonisation
		% Similarity	100 (S)	81-90 (M)	81-90 (M)	
10	78	Isolate	78.1 to 78.2	78.1 to 78.3	78.2 to 78.3	No recolonisation
		% Similarity	81-90 (M)	100 (S)	81-90 (M)	
11	82	Isolate	82.1 to 82.2	82.1 to 82.3	82.2 to 82.3	Recolonisation
		% Similarity	< 80 (D)	< 80 (D)	< 80 (D)	
12	132	Isolate	132.1 to 132.2	132.1 to 132.3	132.2 to 132.3	No recolonisation
		% Similarity	100 (S)	100 (S)	100 (S)	
13	140	Isolate	140.1 to 140.2	140.1 to 140.3	140.2 to 140.3	Recolonisation
		% Similarity	< 80 (D)	< 80 (D)	100 (S)	
14	166	Isolate	166.1 to 166.2	166.1 to 166.3	166.2 to 166.3	Recolonisation
		% Similarity	< 80 (D)	< 80 (D)	100 (S)	
15	173	Isolate	173.1 to 173.2	173.1 to 173.3	173.2 to 173.3	Recolonisation
		% Similarity	100 (S)	< 80 (D)	< 80 (D)	
16	185	Isolate	185.1 to 185.2	185.1 to 185.3	185.2 to 185.3	Recolonisation
		% Similarity	100 (S)	< 80 (D)	< 80 (D)	

(continued on next page)

Table 3 (*continued*)

No.	Patient	Parameters	Comparison of strains			Interpretation
			Visit 1 & 2	Visit 1 & 3	Visit 2 & 3	
17	193	Isolate	193.1 to 193.2	193.1 to 193.3	193.2 to 193.3	No recolonisation
		% Similarity	91-99 (ME)	91-99 (ME)	91-99 (ME)	
Summary: 11 (64.7%) – recolonization, 6 (35.3%) – no recolonisation (microevolution/same strain)						

D: Different strain, S: Same strain, M: Moderately related, ME: Microevolution

The strain relatedness was confirmed with the dendrogram (Fig. 1). On dendrogram 8 distinct clusters were noted with the highest Duda/Hart coefficient of 0.8.

Similarity matrix and percentage similarities for all *C. albicans* strains (Table 3) showed that in 11 subjects (64.7%) recolonisation with different strains occurred in the follow up period while 6 (35.3%) had no recolonisation. These subjects either carried the same strains over the study period or the strains at follow up visits were closely related including occurrence of microevolution.

In Fig. 2, the dendrogram confirms the similarity of our DSTs with previously isolated strains from the blood samples of South African subjects. Furthermore, the relationship of the newly isolated strains to each other is confirmed as the same strains in the previous dendrogram occur in the same clades.

4. Discussion

The majority of our subjects (64.7%) were recolonized with different strains in the follow up period (Table 3). The source of recolonization with different strains have been indicated to be gastrointestinal tract, vagina and skin since few individuals carry more than one predominant strain and replacement of a strain is not always from an.

exogenous source (Odds, 2010). In our study it is difficult to speculate on the source of the recolonising strains since we did not sample yeasts from other body parts. However, recolonisation with exogenous strains has been reported. Tian et al. (2021) found 41% of women to be reinfected with vaginal *C. albicans*. Transmission from the environment, between family members, mother to children and in the hospital setting between patients and hospital staff has been documented (Cliff et al., 2008; Kam & Xu, 2002; Taylor et al., 2003). Consequently, more pathogenic *C. albicans* may be acquired, which could potentially initiate severe mucocutaneous or disseminated candidiasis in immune suppressed subjects like our study population. *C. albicans* is known to survive for 4 months and on surfaces (Kramer et al., 2006; Dire et al., 2023; Gerhardts et al., 2012). In addition, anal and vaginal isolates have been suggested to constitute as a reservoir for possible source for infections (Araujo Paulo de Medeiros et al., 2014; Tan et al., 2021). In addition, transmission of drug resistant and virulent *C. albicans* strains between family members, mother and children have been documented and it can become a potential problem (Müller et al., 1999; Bougnoux et al., 2006; Huët et al., 2021). This highlights the importance of general and personal hygiene, practices of sharing food and fomites, and intimate contacts such as kissing in the transmission.

Microevolution, which is characterized as a minor variation in the genotype of a strain and often occurs due to a single step in mutation. It occurs as an adaptation to new environments by colonizing yeasts and tends to occur more frequently than strain replacement (Sampaio et al., 2005; Moorhouse et al., 2021). *C. albicans* yeasts show a clonal pattern of reproduction but are able to shuffle chromosomes leading to duplicate genes and reassortment of genes (Samaranayake et al., 2003). Therefore, they tend to reveal a high genetic diversity from clonal reproduction and a very high rate of recombination events (d'Enfert et al., 2021). This distinction between microevolution of a colonizing strain and a replacing strain can be detected using genotyping techniques and analysis as shown here in the MLST technique.

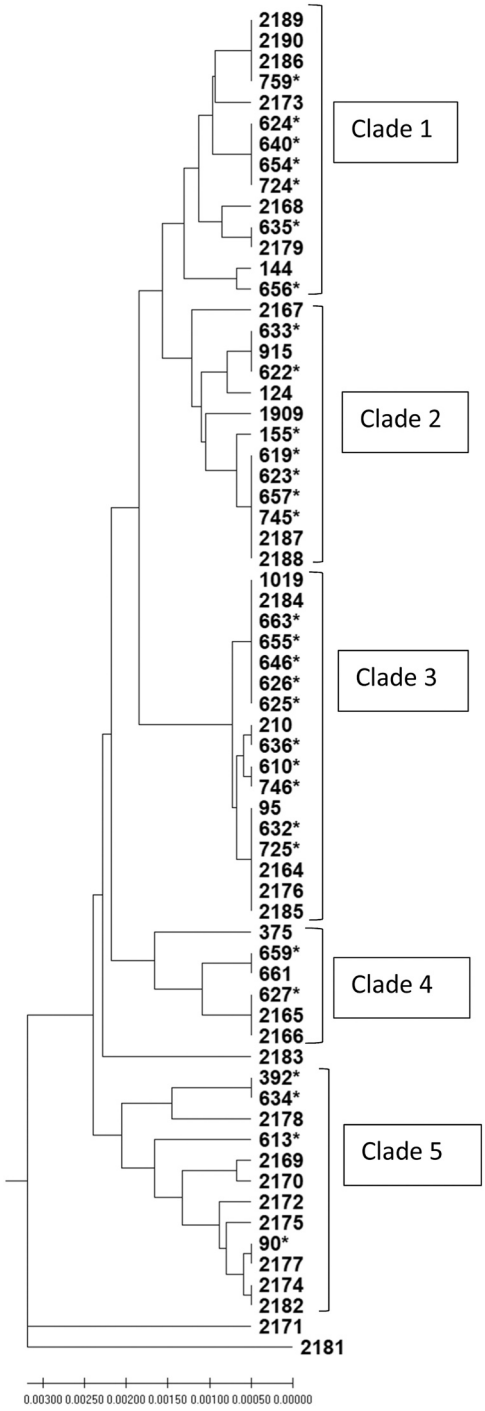

Fig. 2. Evolutionary relationships of *C. albicans* isolated from oral cavities of HIV positive women in this study (New) using old blood culture South African strain as a reference strain.

Although, ABC genotyping enabled the comparison of genotypes from visit to visit in our study population, it proved to be less discriminatory. Therefore, ABC genotyping alone is not recommended when the pleomorphism in the strains differ by more than a single nucleotide (Chaves et al., 2012). This justified MLST genotyping in our study. MLST is the largest global database of strain type information for *C. albicans*. The higher discriminatory ability of MLST genotyping technique (Bougnoux et al., 2002; Muñoz et al., 2019) reflected in the greater

diversity of *C. albicans* strains in Tables 2 and 3.

In our study, high genetic diversity of *C. albicans* genotypes (40 DSTs) has been demonstrated and added 27 previously undocumented DSTs to the MLST database of *C. albicans* (Table 2). Although some of the DSTs in our subjects have been previously documented from South Africa, our samples were the first to be documented on the MLST database from oral samples. This confirms the notion that some strains are prevalent in peculiar geographic locations. Similarly 52 DSTs from 173 patients were reported in China of which 27 DSTs were unique to this region (Tian et al., 2021). In addition, comparison of these strains to previously identified blood isolates showed relatedness and unique nature of South African strains of *C. albicans* (Fig. 2). In a previous study on oral *Candida* genotyping, a particular clade restricted to South Africa was identified (Blignaut et al., 2002). This study was conducted using Ca3 genotyping and therefore it is difficult to confirm if the strains in our subjects were related to the *C. albicans* clade isolated previously in South Africa. The basis for strain typing in Ca3 probing is the variability of the major repeat sequences on the *Candida* genome, while MLST employs single nucleotide polymorphism of selected housekeeping genes. Some workers have however identified a moderate correlation in the clades identified by both methods in spite of the differences in technique (Odds & Jacobsen, 2008). The advantages of MLST genotyping compared with Ca3 probing include lower cost of processing and detection of genetic variations from single nucleotide pleomorphisms in the same subjects followed up over time (which Ca3 cannot detect) and the MLST global database is also considerably larger.

The *C. albicans* samples on the MLST database show a global distribution. This pattern may have risen due to recent acquisition of yeast by human beings, which has compelled adaptation to different environments. Alternatively, it is possible that the yeast has been evolving with humans right from inception (Odds, 2010). Distinct strains have been tied to particular geographic regions, although several strains have been found in multiple locations (Takakura et al., 2008). Some of the previously documented strains present in our subjects have been found elsewhere. DST 95 is the most ubiquitous, found in UK, Switzerland, India, Pakistan, Australia, Morocco, France, and Austria (MLST.net.). DST 124 has been previously isolated in the UK, Israel, France, USA, China, Morocco and Austria (http://pubmlst.org/calbicans/). Determining microvariation and distinguishing a colonizing strain from an exogenous one is technically demanding. Although tedious, MLST is an easy and reliable method with a relatively high discriminatory index. While it is not considered the gold standard, it remains a valuable tool to confirm strain relatedness (Odds & Jacobsen, 2008; Spampinato & Leonardi, 2013).

In two patients (42 and 78) DSTs for the 1st and the 3rd visits were the same, but different on a second visit, and the reason for these results is that only a single randomly collected colony per patients at each visit was subjected to genotyping. This can be considered as a limitation of this study.

This study has shown a unique nature of South African strains of *C. albicans* suggesting independent origin of these strains. This is important in the understanding of molecular epidemiology, transmission and spread of *C. albicans* strains within communities and between countries. These strains may have unique characteristics, therefore warrant further investigations into their virulence, including the adherence ability, biofilm formation, and the production of hydrolytic enzymes and related genes in comparison to the strains elsewhere. In addition, drug resistance to conventional antifungal agents can also be studied.

In conclusion, genotypes of *C. albicans* strains isolated from the oral cavities of HIV positive women over a time period frequently showed unrelated strain, and some evidence of microevolution in the existing strains. This suggests that transmission of *C. albicans* may occur. Mutation or acquisition of virulent and drug resistant strains is a potential problem for immune compromised subjects, which stresses the importance of general hygiene and the prevention of transmission in these patients (Zhang et al., 2014). In addition, this study has shown a unique nature of South African strains of *C. albicans* which can be studied further to understand its pathogenicity and drug resistance pattern.

Ethical approval

Ethical clearance was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg. This study population has been thoroughly described elsewhere with a Certificate no: M10102 (Owotade et al., 2013; Owotade & Patel, 2014).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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