Genetic relatedness among vaginal and anal isolates of *Candida albicans* from women with vulvovaginal candidiasis in north-east Brazil

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Vulvovaginal candidiasis (VVC) is one of the most common causes of vaginitis and affects about 75 % of women of reproductive age. In order to better understand the epidemiology and pathogenesis of this disease, we evaluated genetic relatedness among 62 clinical isolates of *Candida albicans* sequentially obtained from the anus and vagina of patients with sporadic and recurrent VVC. Evaluation of patients' demographic and clinical data, direct examination, and colony forming units (c.f.u.) counts of vaginal and anal samples were also performed. The genotypes of strains were determined with ABC genotyping and Randomly Amplified Polymorphic DNA (RAPD). Genotype A was the most prevalent (93.6 %), followed by genotype C (6.4 %), whereas genotype B was not found. We found the maintenance of the same ABC genotype, regardless of the body site of each patient. Most of the vaginal strains suffered microevolution, whereas most of the anal strains were replaced during the period of study. Vaginal and anal isolates of *C. albicans* obtained simultaneously from the same patient showed the same ABC genotype and high genetic similarity as determined by RAPD. Genotype A seemed to be dominant in both vaginal and anal isolates of patients with VVC. Our results corroborate the hypothesis that there are 'substrains' of the *C. albicans* vaginal clone successfully established, which dominate in an apparently random manner over the course of time. It is suggested that the anal reservoir constitutes a possible source for vaginal infection in most of the cases.

INTRODUCTION

Vulvovaginal candidiasis (VVC) is an infection of the vulva and vagina, caused by different species of the genus *Candida* (Sobel, 1988). Approximately 75 % of women of reproductive age are affected by VVC (Sobel, 1988), whereas about 5 % develop recurrent vulvovaginal candidiasis (RVVC), which is defined by the occurrence of three or more episodes of VVC per year (Fidel & Sobel, 1996). It is widely known that *Candida albicans* is the main aetiological agent of VVC, accounting for 80–90 % of cases, while 10–20 % of them are caused by non-*C. albicans Candida* species, such as *C. glabrata*, *C. tropicalis* and *C. parapsilosis* (Dan *et al.*, 2002; Sobel, 1992). Despite therapeutic advances, VVC has been considered a global public health problem, affecting millions of women of all social strata. It causes great discomfort, impairs sexual relationships and damages work performance (Kent, 1991; Sobel, 1985).

Several studies have investigated the epidemiology of VVC. Nevertheless, the source of infection remains uncertain, with two main hypotheses for recurrent VVC: reinfection from reservoirs of extra genital sites such as the gastrointestinal tract or through sexual transmission (Li *et al.*, 2008; Märdh *et al.*, 2003), or incomplete eradication of the *Candida* strain from the vagina (Sobel, 1985). Therefore, further investigations are mandatory to elucidate the source of infection in women with recurrent VVC.

Holanda *et al.* (2007) observed that patients colonized by *C. albicans* in the anus are about five times more likely to develop VVC. Of note, it is widely believed that endogenous transmission through the gastrointestinal tract is the main source of vaginal yeasts (Fidel & Sobel, 1996).

ABC genotyping was first described by McCullough *et al.* (1999) and is based on the presence or absence of a transposable intron of variable size in the gene that encodes the large ribosomal subunit (gene 25S rRNA). This technique is capable of distinguishing four genotypes of *C. albicans* (genotypes A, B, C and E), besides *Candida dubliniensis* (genotype D; Tamura *et al.*, 2001). Odds *et al.* (2006) stated that ABC genotyping is reliable when strains differ in more than one single nucleotide polymorphism, but it should not be used as a unique method for *C. albicans* typing. RAPD ('Randomly Amplified Polymophic DNA') genotyping utilizes random 'primers' of approximately 10 bp, which bind to various regions along the genome, generating multiple amplification products and detecting several polymorphic sites. It has been one of the most widely used methods for genotyping pathogenic fungi, including *C. albicans* (Ao *et al.*, 2014; Brilhante *et al.*, 2012; da Costa *et al.*, 2012; Muzny *et al.*, 2014; Shokri, 2014; Soll, 2000).

In this study, we evaluated clinical data, and used classical methods for the laboratory based diagnosis of VVC and molecular typing of *C. albicans* strains sequentially obtained from the vagina and anus of patients with VVC, to better understand the pathogenesis and the source of infection of the disease. To the best of our knowledge, this is the first study evaluating genetic relatedness of *C. albicans* strains in this specific clinical scenario in Brazil.

METHODS

Study population and strains. We selected 25 strains of *C. albicans* from the mycological culture collection belonging to the Mycology Laboratory of the Giselda Trigueiro Hospital, obtained from 12 patients between May 2003 and May 2005 (first period of study), while 37 strains of *C. albicans* were obtained from 10 women who attended Januário Cicco Maternity School and Integrated Clinical Analysis Laboratory, from the Federal University of Rio Grande do Norte, during November 2011 to August 2012 (second period of study, Table 1). Only patients who agreed to take part in a surveillance confidential study, in accordance with the Local Research Ethics committee from the University Hospital Onofre Lopes, were enrolled in this study. Of note, the same patient (patient 05) was included in both periods of study (Table 1). The patients were aged between 20 and 47 years and presented with clinical symptoms of VVC (vaginal discharge, itching, burning, dysuria, oedema, erythema and/or dyspareunia), while no symptoms on the anus or perianal region were observed. Seven patients had sporadic VVC, but did not respond to antifungal therapy, which allowed sequential strains to be obtained (weekly) from the same episode of VVC, while 14 women had the recurrent condition, which allowed sequential strains to be obtained (monthly) from different episodes of VVC. Antifungal treatment started after clinical and laboratorial diagnosis of VVC, at the first time point of sample collections (Table 1). Patients with clinical evidence of any malignancy and/or AIDS were excluded from the study.

Sampling, culturing procedures and identification of yeasts. Vaginal and anal secretions obtained from patients in the second period of study were collected with a sterile swab, subsequently placed in saline solution (0.9 % w/v). Samples were subjected to direct examination and inoculated onto the surface of Sabouraud Dextrose Agar (SDA; Difco) supplemented with 50 mg ml^{-1} of chloramphenicol

(Ariston) and 25 mg ml^{-1} of rose Bengal (Vetec) for c.f.u. counts, and also onto the surface of CHROMagar Candida (Difco) to check for purity and colony colour. Petri dishes were incubated at 30 °C, for 48–96 h. The yeasts isolated were identified according to classical methods (Yarrow, 1998) and phenotypic identification was further confirmed with ABC genotyping (McCullough *et al.*, 1999).

DNA extraction, PCR and RAPD analysis. *C. albicans* strains actively growing overnight in YPD broth (yeast extract 10 g l^{-1}; glucose 20 g l^{-1}; peptone 20 g l^{-1}) at 30 °C were used for DNA extraction using PrepMan® Ultra Protocol according to the manufacturer's instructions (Applied Biosystems). A pair of primers spanning the site of the transposable intron in the 25S rDNA was used for ABC genotyping: CA-INT-L (5′-ATAAGGGAAGTCGGCAAAA-TAGATCCGTAA-3′) and CA-INT-R (5′-CCTTGGCTGTGGTTT-CGCTAGATAGTAGAT-3′), according to McCullough *et al.* (1999).

The following reference strains were used: *C. albicans* ATCC 90028 (genotype A), SC5314 (genotype A), ATCC 90029 (genotype B), CY1123 (genotype C), and *C. dubliniensis* CBS 7987.

The single primer B14 (5′-GATCAAGTCC-3′) was used for RAPD analysis. Briefly, 1.0 µl of DNA (40 ng µl^{-1}), 2.5 µl of 10 × PCR buffer (100 mM tris-HCl, pH 8.3, 500 mM KCl, 3.5 mM MgCl$_2$), 5 µl of dNTPmix (100 mM each dNTP), 1.0 µl of primer (50 pmol µl^{-1}), 0.13 µl of tween 20 and 1.0 unit of *Taq* DNA polymerase were added to a final volume of 25 µl. The samples were amplified in a Thermocycler (Amplitherm, TX 96) using the following cycling parameters: one initial cycle of 94 °C for 5 min followed by 45 cycles of 30 s at 94 °C, 1 min at 36 °C, 2 min at 72 °C and a final cycle of 10 min at 72 °C.

PCR products were size-separated by agarose gel electrophoresis (1.2 % agarose) for 2 h at 95 V when ABC genotyping was employed, and for an initial step of 30 min at 100 V followed by a period of 4.5 h at 55 V when RAPD genotyping was used. The gel was stained in a 0.5 µg ml^{-1} ethidium bromide buffer solution (1 × tris-acetate-EDTA). *C. albicans* SC5314 and ATCC 90028 were used as control strains for each PCR.

Computer-assisted RAPD data analysis. Gel images were analysed with the GelCompar II software (Applied Maths). The similarities between the profiles were calculated using the Dice coefficient, to generate the matrices of similarity coefficients to dendrogram constructions. For profile clustering, the unweighted pair-group method with arithmetic averages (UPGMA) with a tolerance of 2 % was used.

Classification of genetic relatedness was based on Soll (2000). Strains were considered 'Identical' when the DNA fingerprints of isolates were indistinguishable and 'highly related' when the DNA fingerprints were highly similar but nonidentical (about 90 % similarity). Isolates were considered 'moderately related' when they presented a similarity coefficient of about 80 %, according to a similarity coefficient threshold above the average similarity coefficient for a set of presumed unrelated isolates, and 'unrelated' when isolates presented a similarity coefficient near or below this threshold (⩽75 %).

Statistical analysis. The Mann–Whitney *t*-test using the statistical program GraphPad Prism (Graph Pad Software) was used to compare c.f.u. counts between vaginal and anal secretion samples. A *P*-value of <0.05 was considered significant.

RESULTS

A total of 62 clinical isolates of *C. albicans* were included in the present study. Thirty-six isolates were obtained from vaginal secretion while 26 were isolated from the anus of

Table 1. Strains of *Candida albicans* obtained from patients with VVC included in this study

Patient	Clinical condition	Isolate number	Anatomical site	ABC typing	Antifungal treatment*	Period of collection
01	Recurrent	01 1CV	Vagina	A	Data unavailable	May 2003 to May 2005 (first period
		01 1CA	Anus	A	Data unavailable	of study)†
02	Recurrent	02 3CV	Vagina	A	Data unavailable	
		02 4CV	Vagina	A	Data unavailable	
		02 1CA	Anus	A	Data unavailable	
03	Recurrent	03 2CV	Vagina	A	Data unavailable	
		03 3CV	Vagina	A	Data unavailable	
		03 6CV	Vagina	A	Data unavailable	
04	Recurrent	04 3CV	Vagina	A	Data unavailable	
		04 3CA	Anus	A	Data unavailable	
05	Recurrent	05 1CV.1	Vagina	A	Data unavailable	
06	Recurrent	06 1CV	Vagina	A	Data unavailable	
		06 1CA	Anus	A	Data unavailable	
07	Recurrent	07 2CV	Vagina	A	Data unavailable	
		07 2CA	Anus	A	Data unavailable	
08	Recurrent	08 1CV	Vagina	A	Data unavailable	
		08 1CA	Anus	A	Data unavailable	
09	Recurrent	09 1CV	Vagina	A	Data unavailable	
		09 1CA	Anus	A	Data unavailable	
10	Recurrent	10 2CV	Vagina	A	Data unavailable	
		10 3CA	Anus	A	Data unavailable	
11	Recurrent	11 1CV	Vagina	A	Data unavailable	
		11 1CA	Anus	A	Data unavailable	
12	Recurrent	12 1CV	Vagina	A	Data unavailable	
		12 4CV	Vagina	A	Data unavailable	
05	Recurrent	05 1CV.2 (LMMM 45)	Vagina	A	Fluconazole§, ¶	November 2011 to August 2012
						(second period of study)
13	Sporadic	13 1CV (LMMM 52)	Vagina	A	None	
		13 1CA (LMMM 53)	Anus	A	None	
14	Sporadic	14 1CV (LMMM 56)	Vagina	A	None	
		14 1CA (LMMM 57)	Anus	A	None	
		14 2CV (LMMM 62)	Vagina	A	Clotrimazole§, #	
		14 2CA (LMMM 63)	Anus	A	Clotimazole§, #	
		14 3CV (LMMM 66)	Vagina	A	Miconazole‡, #	
		14 3CA (LMMM 68)	Anus	A	Miconazole‡, #	
15	Sporadic	15 1CV (LMMM 72)	Vagina	A	None	
		15 2CV (LMMM 90)	Vagina	A	Nystatin‖, **	
		15 2CA (LMMM 91)	Anus	A	Nystatin‖, **	
		15 3CV (LMMM 99)	Vagina	A	None	
		15 3CA (LMMM 100)	Anus	A	None	
16	Sporadic	16 1CV (LMMM 74)	Vagina	C	None	

Table 1. cont.

Patient	Clinical condition	Isolate number	Anatomical site	ABC typing	Antifungal treatment*	Period of collection
		16 1CA (LMMM 75)	Anus	C	None	
		16 2CV (LMMM 92)	Vagina	C	Nystatin‖, **	
		16 2CA (LMMM 93)	Anus	C	Nystatin‖, **	
17	Sporadic	17 1CV (LMMM 76)	Vagina	A	None	
		17 1CA (LMMM 77)	Anus	A	None	
18	Sporadic	18 1CV (LMMM 88)	Vagina	A	Nystatin‖, **	
		18 1CA (LMMM 89)	Anus	A	Nystatin‖, **	
		18 2CV (LMMM 94)	Vagina	A	Nystatin‖, **	
		18 2CA (LMMM 95)	Anus	A	Nystatin‖, **	
		18 3CV (LMMM 96)	Vagina	A	Nystatin‡, **	
		18 3CA (LMMM 97)	Anus	A	Nystatin‡, **	
		18 4CV (LMMM 98)	Vagina	A	Nystatin‡, **	
19	Sporadic	19 1CV (LMMM 102)	Vagina	A	Myconazole§, #	
		19 1CA (LMMM 103)	Anus	A	Myconazole§, #	
		19 4CV (LMMM 193)	Vagina	A	None	
		19 4CA (LMMM 194)	Anus	A	None	
20	Recurrent	20 1CV (LMMM 115)	Vagina	A	Fluconazole‖, ¶	
		20 1CA (LMMM 117)	Anus	A	Fluconazole‖, ¶	
		20 2CV (LMMM 200)	Vagina	A	Fluconazole‡, ¶	
		20 2CA (LMMM 201)	Anus	A	Fluconazole‡, ¶	
21	Recurrent	21 1CV (LMMM 162)	Vagina	A	None	
		21 1CA (LMMM 163)	Anus	A	None	

*Data concerning the use of antifungal drugs up to a month previously to the time of collection.

†Antifungal treatment was instituted after the first collection in the first period of study, but data on the antifungal drug are not available.

‡Currently under antifungal therapy.

§Administration of antifungal drugs for less than 10 days.

‖Administration of antifungal drugs for less than a month.

¶Oral administration of 150 mg tablet, single dose.

#Topical administration of vaginal cream during seven days.

**Topical administration of vaginal cream during 14 days.

1C, First collection; 2C, second collection; 3C, third collection; 4C, fourth collection; 6C, sixth collection.

the patients (Table 1). For most occasions (24), vaginal and anal clinical strains were obtained simultaneously from the same patient at the same time point, while in nine and six occasions isolates were sequentially obtained (weekly or monthly) from either the vagina or the anus of the patients, respectively (Table 1). Clinical manifestations and predisposing factors are listed in Table 2.

In direct examination, budding cells were the most prevalent fungal structures found in both vaginal and anal secretions, present in 18 vaginal samples (18/19; 94.7 %) and nine anal samples (9/16; 56.3 %). Pseudohyphae and true hyphae were observed in most of the vaginal samples (14/19; 73.7 %) and leukocytes were found in all vaginal samples.

Vaginal secretion samples showed significantly higher c.f.u. counts than the anal secretion samples ($P<0.0001$, Fig. 1). c.f.u. counts from vaginal samples sequentially obtained from the same patient showed no correlation with antifungal treatment and reduction of symptoms presented by most of the patients (Fig. 2). Nevertheless, on one specific occasion the reduction in c.f.u. counts in sequential vaginal samples
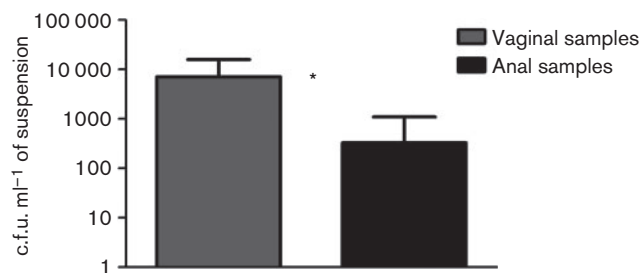

Fig. 1. c.f.u. counts of *Candida albicans* from vaginal (7139 ± 8763) and anal (331 ± 762) secretions obtained from patients with VVC, determined after 96 h incubation at 30 °C on SDA. Each bar represents the mean ± SD of the results obtained for all the isolates of each group. *$P<0.0001$ (Mann–Whitney *t*-test comparing the vaginal samples with anal samples regarding the number of c.f.u.).

Table 2. Clinical manifestations and predisposing factors presented by patients included in this study

Clinical manifestation	N*/total (%)
Vaginal discharge	26/27 (96.3 %)
Itching	19/27 (70.4 %)
Erythema	13/27 (48.1 %)
Dyspareunia	11/27 (40.7 %)
Burning†	6/17 (35.3 %)
Oedema	7/27 (25.9 %)
Dysuria	6/27 (22.2 %)
Predisposing factor	**N*/total (%)**
Active sex life	27/27 (100 %)
Pregnancy	16/27 (59.3 %)
Nylon underwear	14/27 (51.9 %)
Tight clothes	11/27 (40.7 %)
Allergy	7/27 (25.9 %)
Contraceptive	5/27 (18.5 %)
Tampon	5/27 (18.5 %)
Diabetes	4/27 (14.8 %)
Antibiotic therapy (last 30 days)	1/27 (3.7 %)
Intrauterine device	1/27 (3.7 %)
Others‡	0/27 (0 %)

*Number of occasions where the clinical manifestation or predisposing factor was present when positive vaginal cultures for *Candida albicans* were obtained (27 occasions in total). Data were unavailable for nine occasions in the first period of study.
†Data obtained only for the second period of study in 17 occasions in total.
‡Others: corticosteroids, immunosuppressants, asthma, vaginal trauma and hormonal replacement.

obtained from patient 14 was associated with good prognosis, because this patient was cured for VVC, while the increase in c.f.u. counts of the vaginal samples from patient 18 correlated with poor prognosis, since there was a return of all symptoms of VVC in the last culture of this patient (Fig. 2).

Most of the isolates of *C. albicans* included in the study belonged to genotype A (58/62; 93.6 %), while only four strains (4/62; 6.4 %), obtained from the same patient (patient 16), belonged to genotype C. Genotypes B, E and D (*C. dubliniensis*) were not found (Table 1, Fig. 3). We were able to find six different clusters with approximately 80 % similarity between the isolates when RAPD genotyping was used (Fig. 4). We could not detect any cluster enriched for either sporadic or recurrent VVC since most of the clusters were composed of isolates obtained from patients with the two different VVC conditions (Fig. 4).

Vaginal and anal strains obtained simultaneously from the same patient showed the same ABC genotype (Table 1) and high genetic similarity (greater than or equal to 90 % similarity) in most of the cases (15/24; 62.5 %) when RAPD was used (Table 3). Nevertheless, there were some occasions (4/24; 16.7 %) where the anal and vaginal isolates were considered unrelated (less than 75 % similarity; Table 3), for instance, strains 01 1CV and 01 1CA, and strains 15 2CV and 15 2CA (Fig. 4).

Maintenance of the same ABC genotype was observed among vaginal and anal sequential strains (Table 1). Nevertheless, when RAPD was employed a trend to microevolution (similarity greater than 75 % and less than 100 %) of the sequential vaginal strains isolated from the same patient was detected, being observed in five (5/9; 55.6 %) patients (patients 02, 03 and 12 in the first period of study and patients 14 and 20 in the second period of study). However, a complete vaginal strain replacement (similarity lower than 75 %) was observed in four (4/9;
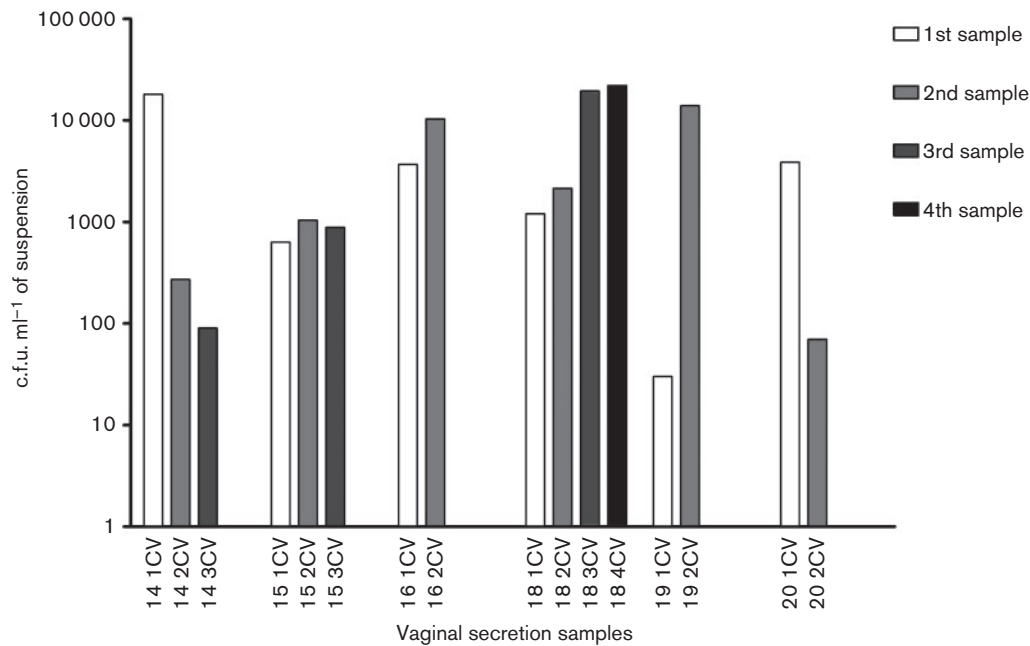
Fig. 2. c.f.u. counts of *Candida albicans* from vaginal secretion samples sequentially obtained from patients with VVC, determined after 96 h incubation at 30 °C on SDA.

44.4 %) patients (15, 16, 18 and 19) in the second period of study (Table 3; Fig. 4).

A trend to replacement of the anal strain in the course of time was detected when RAPD technique was used, as it can be observed for anal strains obtained from patients 15, 16, 18 and 19 in the second period of study (Fig. 4), corresponding to four out of six (66.7 %) of the occasions (Table 3).
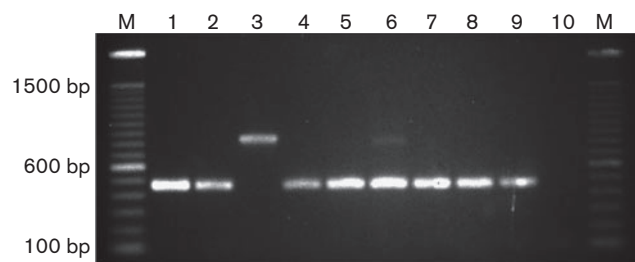
Fig. 3. Representative gel electrophoresis illustrating DNA banding patterns of *Candida albicans* genotypes A (product of 450 bp), B (product of 840 bp), and C (products of 450 bp and 840 bp) obtained through ABC genotyping. Lanes 1 to 10: 1, reference strain ATCC 90028 (genotype A); 2, reference strain SC5314 (genotype A); 3, reference strain ATCC 90029 (genotype B); 4, strain 15 1CV (genotype A); 5, strain 15 2CV (genotype A); 6, reference strain CY1123 (genotype C); 7, strain 15 2CA (genotype A); 8, strain 15 3CV (genotype A); 9, strain 15 3CA (genotype A); 10, negative control; M, molecular size marker; bp, base pairs.

DISCUSSION

It is noteworthy that the proliferation of yeast and bud-to-hypha transition are important in establishing VVC, as demonstrated by the results of direct examination and c.f.u. counts. In fact, the presence of filamentous structures has been associated with symptoms of VVC (Ferrer, 2000). Our results relative to c.f.u. counts demonstrate a lack of association between the concentration of *C. albicans* in the vagina and the clinical manifestations presented by patients with VVC, as observed by other authors (Lopes Consolaro *et al.*, 2004; Giraldo *et al.*, 2000), although the increase or decrease in c.f.u. counts was related to poor or good prognosis, respectively.

Genotype A seems to be dominant over the other ABC genotypes in both vaginal and anal isolates of patients with VVC. Our results corroborate with the theory that the genotype A of *C. albicans* is the most prevalent worldwide, which was also observed in several other studies evaluating clinical isolates of *C. albicans* from various anatomical sites and different geographical regions by ABC genotyping (Emmanuel *et al.*, 2012; Jacobsen *et al.*, 2008; Millar *et al.*, 2002; Odds *et al.*, 2006, 2007).

We found by RAPD genotyping several distinct genotypes causing VVC, although most of the isolates were clustered into only two groups (cluster 4 and cluster 5), suggesting the dominance of particular genotypes of *C. albicans* causing VVC and colonizing the anal mucosa of patients with VVC, corroborating with the results from studies conducted in China (Fan *et al.*, 2008; Ge *et al.*, 2012; Li & Bai, 2007; Li *et al.*, 2008).

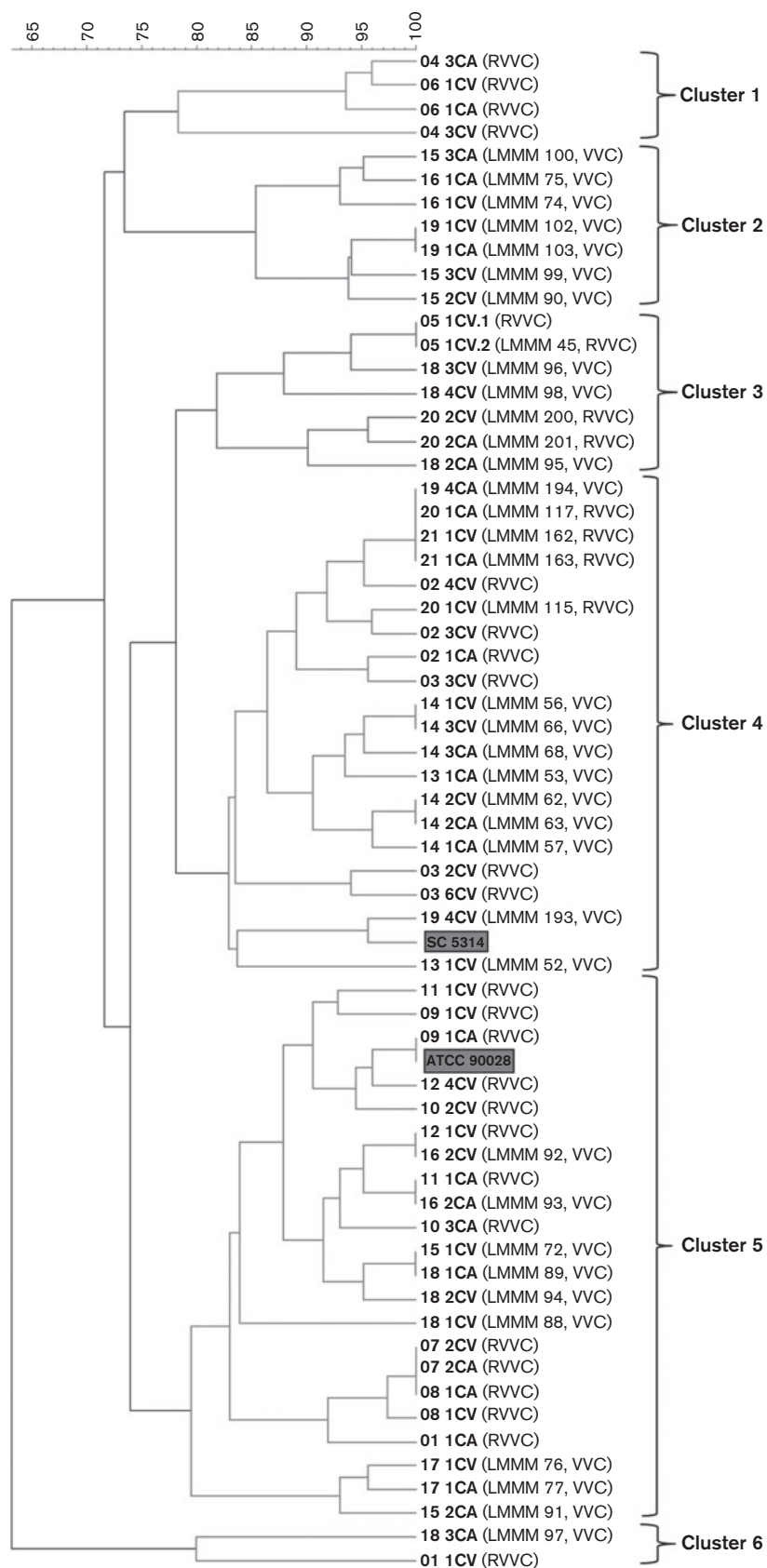Dice (Tol2.0%–2.0%) (H>0.0% S>0.0%) [0.0%–100.0%]
RAPD B14

Fig. 4. Clustering of *Candida albicans* strains based on the dendrogram generated by GelComparII program using the UPGMA method concerning the banding patterns generated by amplification using the primer B14. The reference strains ATCC 90028 and SC5314 are highlighted. Strains identified by isolate number and clinical condition.

Table 3. Distribution of pair of strains (vaginal and anal strain) and sequential isolates of *Candida albicans* according to the degree of genetic relatedness and genetic alteration during the course of time, respectively.

Level of genetic relatedness* (RAPD genotyping)	Genetic similarity (%)	Number of pair of strains† (vaginal and anal strain) N/total (%)
Unrelated	⩽75 %	4/24 (16.7 %)
Moderately related	≈ 80 %	5/24 (20.8 %)
Highly related	≈ 90 %	12/24 (50 %)
Identical	100 %	3/24 (12.5 %)
Level of genetic alteration (RAPD genotyping)	**Vaginal sequences‡ N/total (%)**	**Anal sequences‡ N/total (%)**
Replacement	4/9 (44.4 %)	4/6 (66.7 %)
Microevolution	5/9 (55.6 %)	2/6 (33.3 %)
Maintenance	0/9 (0 %)	0/6 (0 %)

*Classification of genetic relatedness according to Soll (2000).

†Pair of strains: vaginal and anal clinical strains obtained simultaneously from the same patient at the same time point. These included 24 pairs of strains obtained from 16 patients.

‡Sequences: vaginal and anal strains sequentially obtained (weekly or monthly) from either the vagina or the anus of the same patient. These included nine vaginal sequences obtained from nine patients and six anal sequences obtained from six patients.

Other studies also have shown high genetic similarity among isolates of *C. albicans* obtained simultaneously from the vagina and anus in women with VVC (Sampaio *et al.*, 2003; Shi *et al.*, 2007). It is suggested that the vaginal pathogenic strains probably originated from the anal reservoir and may be the source of infection of VVC at least for most occasions. Nevertheless, we found genetically unrelated vaginal and anal strains obtained from the same patient for a few cases, suggesting the probable existence of different strains causing vaginal infection and colonizing the anus. Therefore, we cannot completely rule out the possibility of reinfection from sexual transmission or even incomplete eradication of vaginal infecting strains as the cause of recurrence of infection.

Several studies have evaluated the genetic relationship between vaginal isolates of *Candida* spp. sequentially obtained from patients with RVVC, verifying three different conditions as the most frequent scenario: strain maintenance (Sampaio *et al.*, 2003, 2005), microevolution (Chong *et al.*, 2007; Lockhart *et al.*, 1996) or replacement in the course of time (Amouri *et al.*, 2012).

In our study, the most common scenario was microevolution of the strains of *C. albicans* in the vaginal environment, cooperating with the hypothesis that there are 'substrains' of the clone established, which dominate in an apparently random manner during the establishment of infection (Lockhart *et al.*, 1996).

To the best of our knowledge, no studies have evaluated the genetic relationship of anal isolates sequentially obtained of patients with VVC. In our study, we observed that these isolates have a tendency to be replaced, contrary to what was found with regard to vaginal isolates, but consistent with the higher genetic heterogeneity found in colonization sites as compared to sites of infection (Ge *et al.*, 2012; Jacobsen *et al.*, 2008).

Our results suggest no association between the genotype presented by the strain of *C. albicans* and the clinical condition of the patient (sporadic or recurrent VVC), confirming the results reported by Lian *et al.* (2004).

Finally, we must consider a limitation of our study with regard to the genotyping techniques employed. It is known that RAPD may present low reproducibility and ABC genotyping has limited discriminatory power. Despite the fact that novel genotyping techniques are available for *C. albicans* with higher discriminatory power and reproducibility, a comparison between RAPD and multilocus sequence typing (MLST) to type *C. albicans* showed a direct concordance, according to Robles *et al.* (2004). In a set of 29 *C. albicans* isolates evaluated by RAPD, MLST identified three clusters of genetically related isolates, with 82.7 % concordance with RAPD analysis. When MLST was applied to a subset of 22 isolates of unrelated origins, it identified 21 independent diploid sequence types (DSTs), resulting in a discriminatory power of 99.6 %. These DSTs were 96.9 % concordant with the genotypes identified by RAPD analysis (Robles *et al.*, 2004).

In conclusion, this study contributed to the understanding of the pathogenesis of vaginal candidiasis. Further studies with a higher number of patients and the employment of more discriminatory genotyping techniques are mandatory to confirm these findings.

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