Multilocus enzyme electrophoresis typing of *Candida albicans* populations isolated from healthy children according to socioeconomic background

Tipagem de populacões de Candida albicans isoladas de crianças saudáveis que apresentam um fundo socioeconômico por eletroforese de enzima multiloco

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Abstract

The aim of this research was to evaluate the genetic diversity within and between *C. albicans* populations isolated from the oral cavity of healthy Brazilian children classified into five socioeconomic categories (A to E). Multilocus Enzyme Electrophoresis (MLEE) analysis was the method used to assess genetic diversity. High genetic diversity was observed in all populations that showed predominance of some *C. albicans* subtypes (Electrophoretic Types – ETs). However, no correlation was observed between a specific ET and a specific population of children. Clustering analysis showed one or more highly related ET clusters, suggesting the existence of indirect and direct propagation routes of *C. albicans* among healthy children. Microevolutionary changes were observed in some *C. albicans* populations isolated from children with the same or very similar socioeconomic condition. Furthermore, low transition of *C. albicans* subtypes can be occurring among certain populations of children coming from high and medium/high, or high and medium/low, or medium/high and medium/low socioeconomic categories, which can also be explained by their own socioeconomic and cultural characteristics.

Key Words: *Candida albicans*. MLEE. Genetic diversity. Healthy children. Socioeconomic category.

Resumo

O objetivo desta pesquisa foi avaliar o grau de diversidade genética dentro e entre populações de *C. albicans* isoladas da cavidade bucal de crianças saudáveis brasileiras classificadas em cinco categorias socioeconômicas (A até E), através da análise de Eletroforese de Enzimas Multilocos (MLEE). Alta diversidade genética foi observada em todas as populações, as quais mostraram predominância de alguns subtipos de *C. albicans* (Tipos Eletroforéticos – ETs). Contudo, nenhuma correlação foi observada entre ET-específico e população-específica de crianças. A existência de um ou mais grupos de ET altamente relacionados foi mostrada pela análise de agrupamento, o que sugere a existência de rotas de propagação direta e indireta de *C. albicans* entre crianças saudáveis. Alterações microevolucionárias foram observadas em algumas populações de *C. albicans* isoladas de crianças que tiveram a mesma, ou muito próxima, condição socioeconômica. Além disso, baixa transição de subtipos de *C. albicans* podem estar ocorrendo entre certas populações de crianças provenientes de alta e média/alta, ou alta e média/baixa, ou média/alta e média/baixa, categorias socioeconômicas, o que pode ser esclarecido pelas suas próprias características socioeconômica e cultural.

Palavras-chave: *Candida albicans.* MLEE. Diversidade genética. Crianças saudáveis. Categoria socioeconômica.

Introduction

Candida albicans and related species are found ubiquitously and commensally in the microbiota of human cavities (rectal, oral, vaginal, urethral, nasal, and aural) and skin[1]. The reasons of their existence in the microbiota of healthy people remain unknown. However, nutritional factors, interactions with bacterial microbiota, and the presence of salivary antibodies were suggested to influence the incidence of those yeasts[2]. In addition, these species are considered opportunistic pathogens capable of causing infections, varying from harmless mucocutaneous disorders to the individual up to invasive diseases involving almost all organs. The frequency of infections caused by *Candida* has been increasing worldwide due to a multiplicity of predisposing factors (AIDS, diabetes, leukemia, cancer...)[3,4], which facilitates the conversion of the commensal form to the parasitic existence[5,6]. The increase of these infections has been associated with immunological deficiencies according to the observations of various cases of oropharyngeal candidiasis in patients with AIDS[7]. The progression of the colonization for infection in mucous membranes was referred as a process that depends on the host defense mechanism and on the ability of *Candida* spp. to overcome such mechanism[8].

There has been strong interest in acquiring better understanding of the pathogenesis, epidemiology, genetics and outcome of infections caused by *C. albicans.* This has led to the development of extensive research, employing fingerprinting methods such as Multilocus Enzyme Electrophoresis (MLEE)[9-16], Random Amplified Polymorphic DNA (RAPD)[15,17], Restriction Endonucleases Analysis (REA)[18,19], Southern Blot hybridization with the Ca3 probe [15,20-23], and Electrophoretic Karyotyping (EK)[24,25]. Strain delineation by MLEE has permitted evaluating the genetic structure and diversity of populations[26,27], and has provided high discriminatory power and reproducibility[12,15,26-29]. Considered neutral markers (invariable when they suffer environment selective pressures),

metabolic isoenzymes present great potentiality in the taxonomic, systematic, genetic, evolution and epidemiologic characterization of *C. albicans* and other yeasts of medical importance[9-16,30-40].

The aim of this research was to evaluate by MLEE and clustering analysis, the genetic diversity in *C. albicans* populations isolated from the oral cavity of healthy Brazilian children classified into five socioeconomic categories (A, B, C, D, and E). Concisely, the results permitted evaluating (*i*) the genetic diversity degrees among isolates in each population, (*ii*) the existence of subtypes and highly related isolate clusters, (*iii*) the distribution and prevalence of these subtypes and highly related isolates clusters in each population, (i*v*) non-existing correlation between subtypes or isolate clusters and a population of healthy children (different socioeconomic categories), and (*v*) microevolution within and between isolate populations.

Material and Methods

Population. The study involved 75 *C. albicans* samples isolated from the oral cavity of 75 clinically healthy children (randomly isolated), with ages varying between six and nine years, of both genders, classified into 5 socioeconomic categories (A = 19, B = 17, C = 15, D = 12, and E = 12) according to the criteria adopted by the Brazilian Association of Advertisers and by the Brazilian Institute of Market Research (ABA/ABIPEME), from the municipal district of Piracicaba, State of São Paulo, Brazil[41]. Isolates were previously identified[41] in our laboratory (tube germ formation, chlamydospore test, growth in chromogenic medium CHROMagar Candida®, and carbohydrate assimilation and fermentation test), and the prevalence of *C. albicans* (approximately 47% of the total population studied – approximately 2% of non-*C. albicans*) did not differ substantially between groups A (central area), B (central area and/or outlying area), C (central area and/or outlying area), D (outlying area), and E (outlying area)[41].

Cellular extract preparation. Yeast cultures were grown in flasks containing 50mL of YEPD medium (yeast extract 1% wt/vol, peptone 2% wt/vol, and D-glucose 2% wt/vol) at 37ºC for 18h, under constant agitation at 150rpm (Shaker Incubator mod. NT 712, Nova Técnica Instrumentos e Equipamentos de Laboratório Ltda.)[42,43]. After growth, cells were centrifuged at 3,000 × *g* for 5 minutes and washed twice in a 0.9% wt/vol NaCl solution, submitting each wash to the same centrifuge force[44,45]. Pellets (~500mL) were transferred to 2mL microtubes (Biospec Products, Inc.) containing cold distilled water (approximately 8ºC) and glass beads (1:1:1). These mixtures remained in ice (4ºC) for 5 minutes and, afterwards they were agitated 4 times in a BeadBeater® machine (Biospec Products, Inc.) at 4,200rpm for 30 seconds, with one-minute intervals. Cell fragments were centrifuged at 5,000 × *g*, 4ºC for 5 minutes. The upper aqueous phases resultants were applied in Whatman n3 (wicks) filter papers, 12x5mm in size, and maintained at -70 °C until the moment of the application[16,46].

Electrophoresis and specific enzyme staining. Enzymes were separated in starch gel (Penetrose 30® –Refinações de Milho Brasil Ltda) at 13% wt/vol, with the dimension of 200x120x10mm. Wicks were then immediately soaked in 5 µL (0.02% wt/vol) of bromophenol-blue solution and, afterwards, they were perpendicularly applied on a gel longitudinal cut (20mm). Electrophoresis was performed in a horizontal and continuous system, under a 130-volt tension at 4ºC overnight (bromophenol-blue migration equivalent to 80mm). To assure result reproducibility, the *C. albicans*$^{CBS-562}$ type-strain (Centralbureau voor Schimmelcultures, Delft, The Netherlands) was systematically placed in the ends of each gel. After the electrophoretic run, the gel was put on an acrylic base, and it was sliced (1.5mm layers) with the aid of rulers and a n15 nylon thread. The layers were carefully put inside white porcelain containers and submitted to a staining process by methods previously described for 11 systems (15 enzyme loci)[15,47,48]. The enzymatic activities analyzed were: alcohol de-

hydrogenase, sorbitol dehydrogenase, manitol-1-phosphate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, glucose dehydrogenase, glucose-6-phosphate dehydrogenase, aspartate dehydrogenase, catalase, peroxidase, and leucine aminopeptidase (Table 1). Enzymatic expressions of malate dehydrogenase, isocitrate dehydrogenase, and sorbitol dehydrogenase showed two and three genetically interpretative loci (*Mdh-1, Mdh-2*, and *Mdh-3*; *Idh-1* and *Idh-2*; *Sdh-1* and *Sdh-2*).

Genetic interpretation of MLEE patterns. MLEE patterns were interpreted following a commonly accepted rule, which allows the deduction of the allelic composition of a diploid organism. The electromorphs (bands) of each enzyme were numbered and/or alphabetically sorted in descending disposition regarding the anodal enzymatic mobility, and were compared with the alleles of the corresponding structural genic locus. *C. albicans* populations were characterized by the allelic combinations of 15 enzyme loci, so that different allelic combinations of polymorphic loci designated electrophoretic types (ETs). Thus, the percentile index of polymorphic loci (frequency of the most common allele < 0.99), the average number of alleles per locus, the average number of alleles in each polymorphic locus, and the number of alleles between heterozygotes and homozygotes, were also established[27,49]. The lack of enzymatic activity was interpreted as two null alleles of the corresponding genic locus[12,14,32,47,48,50,51].

Clustering analysis. The genetic diversity of ea ch *C. albicans* population was determined by the Nei' coefficient of genetic distance, $d_{ij} = -In\left[\frac{\sum_k |x_{ki}x_{kj}|}{\sqrt{\sum_k x_{ki}^2 x_{kj}^2}}\right]$, which accepts the use of data from allelic and genic frequencies[52]. Thus, genetic distance matrices (trellis diagrams) were prepared and treated by the SAHN grouping method (Sequential, Agglomerative, Hierarchic, Non-overlapping Clustering Methods) UPGMA algorithm (Unweighted Pair-Group Method Using an Arithmetic Average), in order to generate trees with two-dimensional classifications, denominated dendrograms[53]. The Pearson product-moment correlation coefficient

$$r_{jk} = \frac{\sum_{i=1}^{n}(X_{ij} - \overline{X}_j)(X_{ik} - \overline{X}_k)}{\sqrt{\sum_{i=1}^{n}(X_{ij} - \overline{X}_j)^2 \sum_{i=1}^{n}(X_{ik} - \overline{X}_k)^2}}$$

was used as a measure of the agreement between the genetic distance values implied by the UPGMA dendrograms and those of the original genetic distance matrices (d_{ij})[53]. Such agreements were interpreted as follows: $0.9 \leq r$ – very good fit; $0.8 \leq r < 0.9$ – good fit; $0.7 \leq r < 0.8$ – poor fit; $r < 0.7$ very poor fit. These analyses were done with the aid of the NTSYS pc version 1.70 software. The *C. albicans*$^{CBS-562}$ type-strain (Centralbureau voor Schimmelcultures, Delft, The Netherlands) was included in this experiment in order to establish the cophenetic correlation among isolates, and to determine method reproducibility[54].

Results

Genetic interpretation of MLEE patterns. The enzyme profiles of the *C. albicans* samples on different gels were reproducible after three repetitions of each electrophoretic run. The genetic interpretation of MLEE patterns showed intrinsic genetic characteristics for each *C. albicans* population:

Population of socioeconomic class A (19 isolates): 14 (93.3%) out of 15 enzymatic loci were polymorphic to two, three or four alleles (2 alleles: Adh, Cat, Lap, Mdh-1, Mdh-2 and Po; 3 alleles: Asd, G6pdh, Idh-1, Idh-2, M1p, Mdh-3 and Sdh-2; 4 alleles: Gdh). Only 1 (6.7%) enzymatic locus was monomorphic (Sdh-1). The average number of alleles per locus was equal to 2.53, while the average number of alleles per polymorphic locus was equal to 2.69. The combination of the existing alleles in 15 enzymatic loci showed 17 (89.4%) ETs. Heterozygotes revealed two and three enzymatic bands (2 bands: Adh, Asd, G6pdh, Gdh, Idh-1, Idh-2, Lap, M1p, Mdh-2,

Tabela 1 – Sistemas e soluções utilizados para análise de MLEE a partir de enzimas metabólicas de *C. albicans*.
Table 1 – *Systems and solutions utilized for MLEE analysis from metabolic enzymes of* C. albicans.

Enzyme			Compound for Staining				
EC number	Name	Symbol	Substrate	Buffer	Salt	Coenzyme	Dye Catalyser
1.1.1.1.	Alcohol dehydrogenase	ADH	Ethanol (3 mL) Isopropanol (2 mL)	200 mM Tris-HCl pH 8.0 (50 mL) [1]		NAD 1% (2mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.14.	Sorbitol dehydrogenase	SDH	Sorbitol (250 mg)	Tris-HCl 50 mM pH 8.0 (50 mL) [2]		NAD 1% (2mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.17.	Mannitol-1-phosphate dehydrogenase	M1P	Mannitol-1-phosphate (5 mg)	Tris-HCl 100 mM pH 8.5 (50 mL) [3]		NAD 1% (2mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.37.	Malate dehydrogenase	MDH	2M Malic acid (6 mL) [4]	Tris-HCl 200 mM pH 8.0 (40 mL) [1]		NAD 1% (2mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.42.	Isocitrate dehydrogenase	IDH	1M Isocitric acid (2 mL) [5]	Tris-HCl 200 mM pH 8.0 (40 mL) [1]	100 mM $MgCl_2$ (1 mL) [6]	NADP 1% (1mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.47.	Glucose dehydrogenase	GDH	D-glucose (500 mg)	Tris-HCl 200 mM pH 8.0 (50 mL) [1]		NAD 1% (2mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.1.1.49.	Glucose-6-phosphate dehydrogenase	G6PDH	Glicose-6-phosphate disodium salt (100 mg)	Tris-HCl 200 mM pH 8.0 (50 mL) [1]	100 mM $MgCl_2$ (1 mL) [6]	NADP 1% (1mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.4.3.x.	Aspartate dehydrogenase	ASD	Aspartic acid (50 mg)	Sodium phosphate pH 7.0 (50 mL) [7]		NAD 1% (2mL)	PMS 1% (500 µL) MTT 1.25% (1 mL)
1.11.1.6.	Catalase [8]	CAT					
1.11.1.7.	Peroxidase	PO	H_2O_2 3% (1 mL)	100mM Sodium acetate pH 4.5 (50 mL) [9]			o-dianisidine 2HCl (16mg)
3.4.11.1.	Leucine aminopeptidase	LAP	L-leucine b-naphthylamide HCl (30 mg)	100mM Potassium phosphate pH 5.5 (50 mL) [10]	100 mM $MgCl_2$ (1 mL) [6]		Black K (30 mg)

Tampão do eletrodo: Tris-citrato pH 8,0 [83,2g de $C_4H_{11}NO_3$ (Tris), 33,09g de $C_6H_8O_7 . H_2O$ (Ácido cítrico), 1L de H_2O]; Tampão do gel: Tampão do eletrodo diluído 1:29; [1] 24,2g de $C_4H_{11}NO_3$ (Tris), 1L de H_2O (pH ajustado com HCl); [2] 6,05g de $C_4H_{11}NO_3$ (Tris), 1L de H_2O (pH ajustado com HCl); [3] 12,1g de $C_4H_{11}NO_3$ (Tris), 1L de H_2O (pH ajustado com HCl); [4] 26,8g de $C_4H_6O_5$ (DL-ácido málico) e 16g de NaOH em 0,1L de H_2O (precaução: reação potencialmente explosiva); [5] 29,41g de $C_6H_5O_7Na_3 . 2H_2O$ (DL-ácido isocítrico) em 0,1L de H_2O; [6] 2,03g de $MgCl_2 . 6HCl$ (Cloreto de magnésio) em 0,1L de H_2O; [7] Misturar partes iguais de 27,6g de $NaH_2PO_4 . H_2O$ (Fosfato de sódio monobásico) em 1L de H_2O e 53,6g de $Na_2HPO_4 . 7H_2O$ (Fosfato de sódio dibásico heptahidratado) em 1L de H_2O, então diluir a mistura 1:25 com H_2O; [8] Incubar a fatia do gel por 30 minutos a 0ºC em 50mL de tampão de 0,1M fosfato de sódio pH 7, então decantar a solução, e imergir o gel em 50mL de solução de iodeto de potássio 1,5% (KI) por 2 minutos. Por conseguinte, enxaguar a fatia do gel com água, e imergir o gel em 50mL de solução de peróxido de hidrogênio (H_2O_2) 0,03%. Misturar cuidadosamente e remover a solução corante quando zonas brancas surgirem sobre o fundo azul-escuro; [9] 13,61g de $C_2H_3O_2Na . 3H_2O$ (Acetato de sódio), 1L de H_2O; [10] 13,61g de KH_2PO_4 (Fosfato de potássio), 1L de H_2O.*Electrode buffer: Tris-citrate pH 8.0 [83.2g of $C_4H_{11}NO_3$ (Tris), 33.09g of $C_6H_8O_7 . H_2O$ (Citric acid), 1L of H_2O]; Gel buffer: Electrode buffer diluted 1:29; [1] 24.2g of $C_4H_{11}NO_3$ (Tris), 1L of H_2O (pH adjusted with HCl); [2] 6.05g of $C_4H_{11}NO_3$ (Tris), 1L of H_2O (pH adjusted with HCl); [3] 12.1g of $C_4H_{11}NO_3$ (Tris), 1L of H_2O (pH adjusted with HCl); [4] 26.8g of $C_4H_6O_5$ (DL-malic acid) and 16g of NaOH in 0.1L of H_2O (caution: potentially explosive reaction); [5] 29.41g of $C_6H_5O_7Na_3 . 2H_2O$ (DL-isocitric acid) in 0.1L of H_2O; [6] 2.03g of $MgCl_2 . 6HCl$ (Magnesium chloride) in 0.1L of H_2O; [7] Mix equal parts of 27.6g of $NaH_2PO_4 . H_2O$ (Sodium phosphate monobasic monohydrate) in 1L of H_2O and 53.6g of $Na_2HPO_4 . 7H_2O$ (Sodium phosphate dibasic heptahydrate) in 1L of H_2O, then dilute the mixture 1:25 with H2O; [8] Incubate gel slice for 30 minutes at 0ºC in 50mL of 0.1M sodium phosphate pH 7.0 buffer, then pour off solution, and immerse it in 50mL of 1.5% potassium iodide solution (KI) for 2 minutes. Then rinse gel slice with water, and immerse it in 50mL of 0.03% hydrogen peroxide (H_2O_2) solution. Mix gently and remove stain solution when white zones appear on dark-blue background; [9] 13.61g of $C_2H_3O_2Na . 3H_2O$ (Sodium acetate), 1L of H_2O; [10] 13.61g of KH_2PO_4 (Potassium phosphate), 1L of H_2O.*

Mdh-3, Po and Sdh-2; 3 bands: Mdh-2). Among homozygotes, one allele was observed in the Adh, Gdh, Idh-2, Lap, Po, Sdh-1 and Sdh-2 loci, two alleles in the Asd, Cat, Idh-1, M1p, Mdh-1 and Mdh-3 loci, and three alleles in the G6pdh locus (Table 2).

Population of socioeconomic class B (17 isolates): 6 (40%) out of 15 enzymatic loci were polymorphic to two alleles (Adh, G6pdh, Lap, Mdh-1, Mdh-2 and Po). Nine (60%) enzymatic loci were monomorphic (Asd, Cat, Gdh, Idh-1, Idh-2, M1p, Mdh-3, Sdh-1 and Sdh-2). The average number of alleles per locus was equal to 1.40, while the average number of alleles per polymorphic locus was equal to 2. The combination of the existing alleles in 15 enzymatic loci showed 11 (64.7%) ETs. Heterozygotes revealed two and three enzymatic bands (2 bands: Adh, G6pdh, Lap, Mdh-1, Mdh-2 and Po; 3 bands: Mdh-2). Among homozygotes, one allele was observed in the Adh, Asd, Cat, Gdh, Idh-1, Idh-2, Lap, M1p, Mdh-1, Mdh-3, Po, Sdh-1 e Sdh-2 loci, and two alleles in the G6pdh locus (Table 2).

Population of socioeconomic class C (15 isolates): 5 (33.3%) out of 15 enzymatic loci were polymorphic to two or three alleles (2 alleles: Mdh-1, Mdh-2, Po and Sdh-2; 3 alleles: Adh). Ten (66.7%) enzymatic loci were monomorphic (Asd, Cat, G6pdh, Gdh, Idh-1, Idh-2, Lap, M1p, Mdh-3 and Sdh-1). The average number of alleles per locus was equal to 1.40, while the average number of alleles per polymorphic locus was equal to 2.20. The combination of the existing alleles in 15 enzymatic loci showed 11 (73.3%) ETs. Heterozygotes revealed two and three enzymatic bands (2 bands: Adh, Mdh-1, Mdh-2, Po and Sdh-2; 3 bands: Mdh-2). Among homozygotes, one allele was observed in the Adh, Asd, Cat, G6pdh, Gdh, Idh-1, Idh-2, Lap, M1p, Mdh-1, Mdh-2, Mdh-3, Po and Sdh-1 loci, and two alleles in the Sdh- 2 locus (Table 2).

Population of socioeconomic class D (12 isolates): 9 (60%) out of 15 enzymatic loci were polymorphic to two alleles (Adh, Asd, G6pdh, Gdh, Idh-1, M1p, Mdh-2, Po and Sdh-2). Six (40%) enzymatic loci were monomorphic (Cat, Idh-2, Lap, Mdh-1, Mdh-3 and Sdh-1). The average number of alleles per locus was equal to 1.60, while the average number of alleles per polymorphic locus was equal to 2. The combination of the existing alleles in 15 enzymatic loci showed 6 (50%) ETs. Heterozygotes revealed two and three enzymatic bands (2 bands: Adh, Asd, Gdh, Idh-1, M1p, Mdh-2, Po and Sdh-2; 3 bands: Mdh-2). Among homozygotes, one allele was observed in the Adh, Cat, Gdh, Idh-1, Idh-2, Lap, Mdh-1, Mdh-3, Po and Sdh-1 loci, and two alleles in the G6pdh, M1p and Sdh-2 loci (Table 2).

Population of socioeconomic class E (12 isolates): 10 (66.7%) out of 15 enzymatic loci were polymorphic to two or three alleles (2 alleles: Asd, G6pdh, Gdh, Idh-1, Lap, M1p, Mdh-2, Po and Sdh-2; 3 alleles: Adh). Five (33.3%) enzymatic loci were monomorphic (Cat, Idh-2, Mdh-1, Mdh-3 and Sdh-1). The average number of alleles per locus was equal to 1.73, while the average number of alleles per polymorphic locus was equal to 2.11. The combination of the existing alleles in 15 enzymatic loci showed 12 (100%) ETs. Heterozygotes revealed two and three enzymatic bands (2 bands: Adh, Asd, Gdh, Idh-1, M1p, Mdh-2, Po and Sdh-2; 3 bands: Mdh-2). Among homozygotes, one allele was observed in the Asd, Cat, Gdh, Idh-1, Idh-2, M1p, Mdh-1, Mdh-3, Sdh-1 and Sdh-2 loci, and two alleles in the Adh, G6pdh, Lap and Po loci (Table 2).

Such results indicated that 31 healthy children (A = 12; B = 4; C = 7; D = 2; E = 6) were carriers of different *C. albicans* ETs in the oral cavity. However, identical ETs were found in children coming from socioeconomic categories as follows: a) only A (ET1); b) only B (ET10); c) A and B (ET31); d) A, B and C (ET33); e) A, D and E (ET23 and ET24); f) B and C (ET9 and ET37); g) B, C and E (ET32); h) B and E (ET34); and, i) D and E (ET4 and ET28). Identical ETs were not identified in children of socioeconomic classes B and D, C and D, or only E (Table 3, Fig. 1).

Clustering analysis. The genetic diversity among isolates in their respective populations of healthy children was evaluated by UPGMA dendrograms (Fig. 2). Such results showed coexistence of highly related or in-

Tabela 2 – Perfis alélicos em 43 ETs enzimáticos de *C. albicans* isolada de 75 crianças saudáveis provenientes de cinco categorias socioeconômicas.

Table 2 - *Allelic profiles in 43 enzymatic ETs of* C. albicans *isolated from 75 healthy children coming from five socioeconomic categories.*

	No. of	Alleles of 15 enzymatic loci*													
ET	Isolates	*Adh*	*Asd*	*Cat*	*G6pdh*	*Gdh*	*Idh-1*	*Idh-2*	*Lap*	*M1p*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Po*	*Sdh-1*	*Sdh-2*
	TS	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*ab*	*ab*	*cc*	*ab*	*aa*	*bb*
A socioeconomic class																
1	2	*ab*	*ab*	*aa*	*ab*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
6	1	*ab*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
16	1	*bb*	*ab*	*aa*	*aa*	*bb*	*ac*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
17	1	*bb*	*ab*	*aa*	*aa*	*bb*	-	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
18	1	*bb*	*ab*	*aa*	*bb*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*ab*	*aa*	*bb*
20	1	*bb*	*ab*	*aa*	*cc*	*ab*	*ac*	*aa*	*aa*	*ab*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
21	1	*bb*	*ab*	*aa*	*cc*	*ab*	*ac*	*aa*	*aa*	*ab*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
22	1	*bb*	*ab*	*aa*	*cc*	*ab*	*ac*	*aa*	*ab*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
23	1	*bb*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
24	2	*bb*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
26	1	*bb*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bc*	*aa*	*ab*	*bb*	*aa*	*aa*	*bb*
27	1	*bb*	*ab*	*aa*	*cc*	*bb*	*ac*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
29	1	*bb*	*ab*	*aa*	*cc*	*cd*	*bb*	*bc*	*aa*	*cc*	*cc*	-	*ab*	*ab*	*aa*	*bb*
30	1	*bb*	*bb*	*aa*	*bb*	*bb*	*aa*	*aa*	*aa*	*bc*	*aa*	*ab*	*bb*	*ab*	*aa*	*bb*
31	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
33	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*aa*	*aa*	*bb*
41	1	*bb*	*cc*	*bb*	*bb*	*ab*	*ac*	*aa*	*aa*	*ab*	*aa*	*ab*	*cc*	*ab*	-	*cd*
B socioeconomic class																
7	1	*ab*	*bb*	*aa*	*bb*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
9	1	*ab*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
10	4	*ab*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
11	1	*ab*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*aa*	*aa*	*bb*
14	1	*ab*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*ab*	*bb*	*ab*	*ab*	-	*aa*	*aa*	*bb*
31	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
32	4	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
33	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*aa*	*aa*	*bb*
34	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*ab*	*aa*	*bb*
35	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	-	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
37	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	-	*aa*	*bb*	*aa*	*ab*	-	*aa*	*aa*	*bb*
C socioeconomic class																
9	1	*ab*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
13	1	*ab*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*ab*	*aa*	*bb*
15	1	*ab*	*bb*	*aa*	*cc*	*bb*	*aa*	-	*aa*	*bb*	*ab*	*bb*	-	*ab*	*aa*	*bb*
32	5	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
33	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*aa*	*aa*	*bb*
36	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	-	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
37	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	-	*aa*	*bb*	*aa*	*ab*	-	*aa*	*aa*	*bb*
38	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	-	*aa*	*bb*	*aa*	*ab*	-	*ab*	*aa*	*aa*
39	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	-	*aa*	*bb*	*aa*	*ab*	-	*ab*	*aa*	*bb*
40	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	-	*aa*	*bb*	*aa*	*bb*	-	*ab*	*aa*	*bb*
42	1	*bc*	*bb*	*aa*	*cc*	*bb*	*aa*	-	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*

Tabela 2 – Perfis alélicos em 43 ETs enzimáticos de *C. albicans* isolada de 75 crianças saudáveis provenientes de cinco categorias socioeconômicas.

Table 2 - *Allelic profiles in 43 enzymatic ETs of* C. albicans *isolated from 75 healthy children coming from five socioeconomic categories.*

ET	No. of Isolates	Alleles of 15 enzymatic loci*														
		Adh	*Asd*	*Cat*	*G6pdh*	*Gdh*	*Idh-1*	*Idh-2*	*Lap*	*M1p*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Po*	*Sdh-1*	*Sdh-2*
	TS	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*ab*	*ab*	*cc*	*ab*	*aa*	*bb*
D socioeconomic class																
3	1	*ab*	*ab*	*aa*	*cc*	*ab*	*ac*	*aa*	*aa*	*aa*	*aa*	*ab*	*cc*	*aa*	*aa*	*aa*
4	1	*ab*	*ab*	*aa*	*cc*	*ab*	*ac*	*aa*	*aa*	*ab*	*aa*	*ab*	*cc*	*aa*	*aa*	*ab*
19	1	*bb*	*ab*	*aa*	*bb*	*bb*	*ac*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
23	4	*bb*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
24	3	*bb*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
28	2	*bb*	*ab*	*aa*	*cc*	*bb*	*ac*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
E socioeconomic class																
2	1	*ab*	*ab*	*aa*	*bb*	*ab*	*ac*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
4	1	*ab*	*ab*	*aa*	*cc*	*ab*	*ac*	*aa*	*aa*	*ab*	*aa*	*ab*	*cc*	*aa*	*aa*	*ab*
5	1	*ab*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*ab*
8	1	*ab*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*ab*
12	1	*ab*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*ab*	*aa*	*ab*
23	1	*bb*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*aa*	*aa*	*bb*
24	1	*bb*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
25	1	*bb*	*ab*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*ab*	*aa*	*bb*
28	1	*bb*	*ab*	*aa*	*cc*	*bb*	*ac*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
32	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	*cc*	*ab*	*aa*	*bb*
34	1	*bb*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*aa*	*bb*	*aa*	*ab*	-	*ab*	*aa*	*bb*
43	1	*cc*	*bb*	*aa*	*cc*	*bb*	*aa*	*aa*	*cc*	*bb*	*aa*	*ab*	*cc*	*bb*	*aa*	*bb*

* Heterozigotos estão presentes como *ab*, *ac*, *bc* e *cd*. (-) alelo nulo. TS corresponde a linhagem-tipo de *C. albicans*$^{CBS-562}$. ** Heterozygotes are present as ab, ac, bc and cd. (-) null allele. TS corresponds to C. albicans*$^{CBS-562}$ *type-strain.*

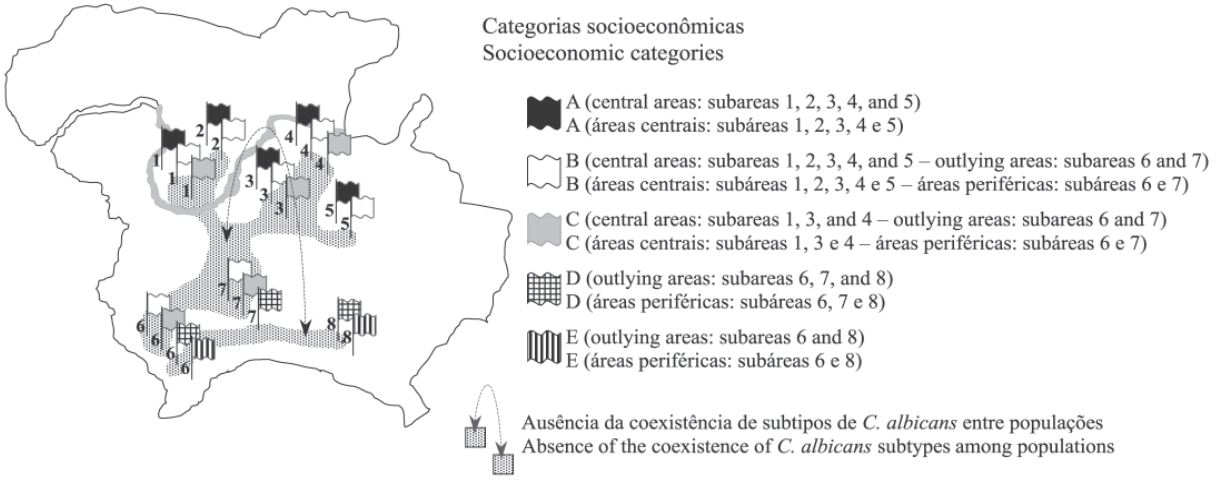

Figura 1 – Subtipos de *C. albicans* (ETs) coexistentes na maioria das populações de crianças saudáveis.

Figure 1 – C. albicans *subtypes (ETs) coexisting in most populations of healthy children.*

Tabela 3 - Distribuição de 43 ETs enzimáticos de *C. albicans* em 75 crianças saudáveis provenientes de cinco categorias socioeconômicas.

Table 3 - *Distribution of 43 enzymatic ETs of C. albicans in 75 healthy children coming from five socioeconomic categories.*

ET	Socioeconomic classes				
	A	B	C	D	E
1	**2**	-	-	-	-
2	-	-	-	-	**1**
3	-	-	-	**1**	-
4	-	-	-	**1**	**1**
5	-	-	-	-	**1**
6	**1**	-	-	-	-
7	-	**1**	-	-	-
8	-	-	-	-	**1**
9	-	**1**	**1**	-	-
10	-	**4**	-	-	-
11	-	**1**	-	-	-
12	-	-	-	-	**1**
13	-	-	**1**	-	-
14	-	**1**	-	-	-
15	-	-	**1**	-	-
16	**1**	-	-	-	-
17	**1**	-	-	-	-
18	**1**	-	-	-	-
19	-	-	-	**1**	-
20	**1**	-	-	-	-
21	**1**	-	-	-	-
22	**1**	-	-	-	-
23	**1**	-	-	**4**	**1**
24	**2**	-	-	**3**	**1**
25	-	-	-	-	**1**
26	**1**	-	-	-	-
27	**1**	-	-	-	-
28	-	-	-	**2**	**1**
29	**1**	-	-	-	-
30	**1**	-	-	-	-
31	**1**	**1**	-	-	-
32	-	**4**	**5**	-	**1**
33	**1**	**1**	**1**	-	-
34	-	**1**	-	-	**1**
35	-	**1**	-	-	-
36	-	-	**1**	-	-
37	-	**1**	**1**	-	-
38	-	-	**1**	-	-
39	-	-	**1**	-	-
40	-	-	**1**	-	-
41	**1**	-	-	-	-
42	-	-	**1**	-	-
43	-	-	-	-	**1**
	∑ 19	∑ 17	∑ 15	∑ 12	∑ 12

n corresponde ao número de ETs de *C. albicans* entre *n* crianças saudáveis provenientes de várias classes socioeconômicas e – corresponde a ausência de ET.

n correspond to the number of *C. albicans* ETs among *n* healthy children coming from several socioeconomic classes and – corresponds to ET absence.

distinguishable *C. albicans* subtypes ($0.012 > d_{ij} \geq 0$) among some healthy children coming from the same socioeconomic category. However, variations of highly related or indistinguishable ($0.012 > d_{ij} \geq 0$), and moderately related or non-related ($d_{ij} \geq 0.012$) isolate numbers were observed in each population of children (Table 4). Thus, the larger percentile index of polymorphism ($d_{ij} \geq 0.012$) occurred among isolates from healthy children coming from socioeconomic categories A (47.3% of isolates), followed by E (33.3% of isolates), B (17.6% of isolates), C (13.3% of isolates), and D (8.3% of isolates), whose indexes of genetic distance were of $0.151 \geq d_{ij} \geq 0$, $0.148 \geq d_{ij} > 0$, $0.123 \geq d_{ij} > 0$, $0.127 \geq d_{ij} > 0$, and $0.039 \geq d_{ij} > 0$, respectively.

The genetic diversity analysis among populations of isolates showed an ancestral convergence in populations B and C, or D and E. However, a low genetic divergence was detected in populations A and BC, A and DE, or BC and DE which, on average, corresponded to >1 and <2.3 allelic substitutions for each 100 loci, from a common ancestral population (Fig. 3).

Discussão

In our research, quantitative and qualitative variations of polymorphic loci, of the average number of alleles per locus, and of the average number of alleles per polymorphic locus were observed in all *C. albicans* populations coming from healthy children. These variations have been observed in several genetic diversity studies of *C. albicans* populations isolated from immunocompromised and immunocompetent patients[11,12,14,15,32,38,39,50]. Like previous results of MLEE studies[11,12,38,39], the heterozygote patterns obtained in the present analysis were also consistent with the diploid nature of *C. albicans*[55]. Pujol *et al.* (1993) reported that different allelic frequencies in different populations could be associated with geographical isolation, the same when each separate population remains in panmixia[38].

The combination of the existing alleles in 15 enzymatic loci showed a quantitative varia-

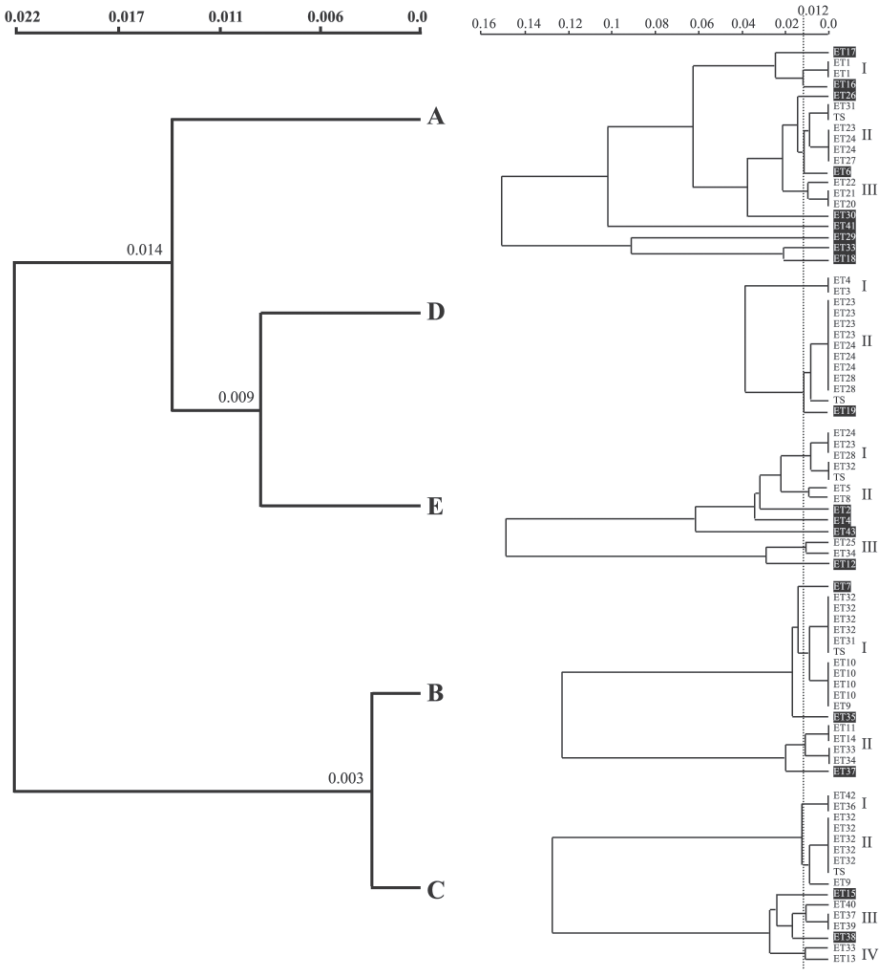

Figure 2 – Diversidade genética dentro e entre populações de *C. albicans* isoladas da cavidade bucal de crianças saudáveis provenientes de cinco categorias socioeconômicas. Dendrogramas UPGMA (0,92980 ≤ r_{jk} ≤ 0,94560 – muito bom ajuste) gerados a partir das matrizes de distância genética d_{ij} (Nei, 1972).

Figure 2 – *Genetic diversity within and between C. albicans populations isolated from the oral cavity of healthy children coming from five socioeconomic categories. UPGMA dendrograms (0.92980 ≤ r_{jk} ≤ 0.94560 – very good fit) generated from matrices of genetic distance d_{ij} (Nei, 1972).*

Tabela 4 – Relação do número de isolados altamente relacionados ou indistingüíveis (0.012 > d_{ij} ≥ 0) e moderadamente relacionados ou não relacionados (d_{ij} ≥ 0.012), obtidos pela análise de agrupamento de populações de *C. albicans*.

Table 4 – *List of the number of highly related or indistinguishable (0.012 > d_{ij} ≥ 0) and moderately related or non related (d_{ij} ≥ 0.012) isolates, obtained by clustering analysis of C. albicans populations.*

Socioconomic clategories	Isolates 0.012 > d_{ij} ≥ 0		number of clusters (0.012 > d_{ij} ≥ 0)	Isolates d_{ij} ≥ 0.012		Total
	n	%		*n*	%	
A	10	52.6	3	9	47.4	19
B	14	82.4	2	3	17.6	17
C	13	86.7	4	2	13.3	15
D	11	91.7	2	1	8.3	12
E	8	66.7	3	4	33.3	12

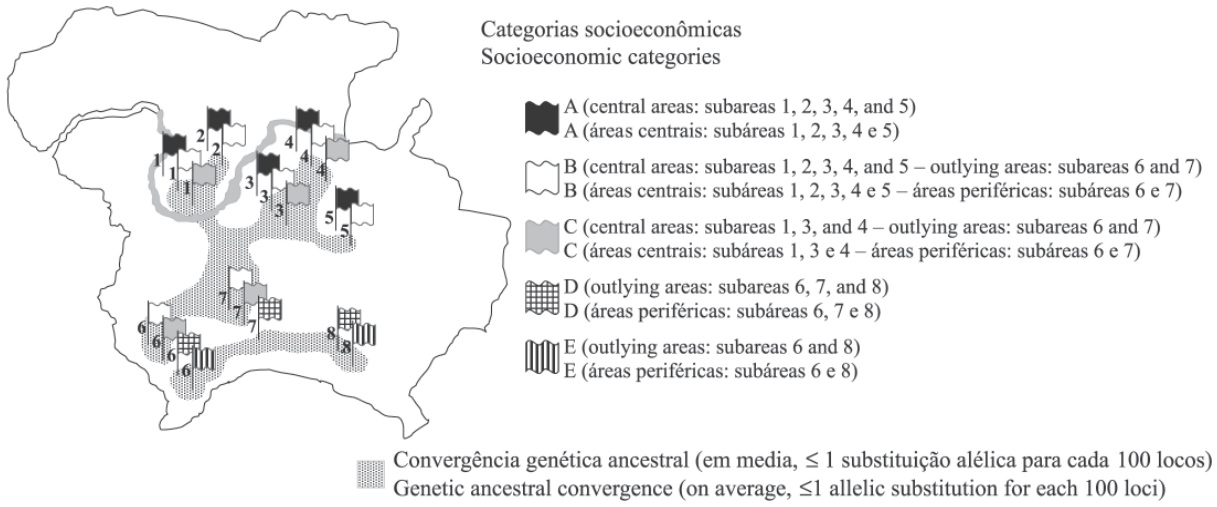

Figure 3 – Convergência ou divergência ancestral (>1 e <2,3 substituição alélica para cada 100 locos) entre isolados de *C. albicans* provenientes de populações de crianças saudáveis que apresentam uma base socioeconômica.
Figure 3 – *Ancestral convergence or divergence (>1 and <2.3 allelic substitutions for each 100 loci) among C. albicans isolates coming from healthy children populations with a common socioeconomic background (Fig. 3).*

tion of subtypes (ETs) in the healthy children populations suggesting the existence of high genetic diversity of *C. albicans* (A = 17 ETs$^{89,5\%}$, B = 11 ETs$^{64,7\%}$, C = 11 ETs$^{73,3\%}$, D = 6 ETs$^{50\%}$, and E = 12 ETs$^{100\%}$). The predominance and coexistence of some ETs (ET1, ET4, ET9, ET10, ET23, ET24, ET28, ET31, ET32, ET33, ET34 and ET37) was observed within and between some children populations. These results also suggest the existence of strain groups selected and better adapted than others in the oral cavities of those healthy children. Soll *et al.* (1991) also demonstrated the existence of *Candida* spp. strains selected and better adapted in certain human niches[56]. Although certain ETs were identified exclusively in certain children populations, no correlation was observed between a specific ET and a specific population of children. Some researchers have demonstrated the prevalence of *C. albicans* (60% to 95%) and *Candida* spp. in approximately 50% of the populations of healthy individuals[2,41,57] regardless of socioeconomic factors[41].

The isoenzymatic typing of *C. albicans* oral isolates from clinically healthy children (Piracicaba, Brazil) has revealed a way of multiclonal colonization for those yeasts[14]. Mehta *et al.* (1999) have analyzed the distribution of *C. albicans* genotypes among healthy family members of a same city (United States) by electrophoretic karyotyping, RAPD and REA with *Hinf*I and *Eco*RI. Their results demonstrated the existence of a genotypic intrafamiliar identity (each member of a family as a carrier of the same genotype). However, different genotypes were also observed inter and intrafamiliarly[24]. Pujol *et al.* (1993) identified 41 *C. albicans* subtypes (74.5% of isolates) in HIV-seropositive patients from a limited geographical area (Montpellier, France) by MLEE and population genetics[9]. Those researchers suggested that the high genetic diversity (11 of 21 enzymatic loci being polymorphics) could be correlated with the existence of some clonal strains that present widespread geographical distribution, as it is the case of some bacteria[58,59] and protozoa[60,61,62]. Important biological and medical consequences were pointed out with clonal reproduction, once the correlation between the genetic composition and medical characteristics could facilitate effective method selection for the control of the pathological expression of *C. albicans* in immunocompromised individuals[39].

In contrast with the high genetic diversity of *C. albicans* observed in healthy children populations, a low genetic diversity has

been detected in immunocompromised patients. The epidemiologic analysis of *C. albicans* isolated from seven patients (Oslo, Norway) submitted to bone marrow transplant showed the existence of 8 ETs (13.1% of isolates) and a low genetic diversity among those yeasts (4 of 10 enzymatic loci being polymorphics)[12]. In some patients, the colonization with one or more ETs in different anatomical sites remained during medical follow-up. However, no correlation was observed between those ETs and the sensitivity to some antifungal (amphotericin B – AMB – and flucytosine) or anatomical sites (oral cavity, groin, and feces)[12]. Boerlin *et al.* (1995) identified 3 atypical *C. albicans* ETs (23% of isolates) colonizing the oral cavity of HIV-seropositive asymptomatic patients (Lausanne, Switzerland). This lower genetic diversity (1 of 16 enzymatic loci being polymorphic) among the isolates was also observed without correlation with clinical parameters, and confirmed by Southern blot hybridization with probe Ca3 analysis. Such results were suggestive of probable colonization by atypical *C. albicans* subtypes from different origins and without a single limited source of contamination[32]. *C. albicans* populations isolated from HIV-seropositive patients (Lausanne, Switzerland) with and without oropharingeal candidiasis symptoms, from patients with invasive candidiasis, and from healthy individuals could not be distinguished by MLEE analysis, given that low genetic diversity was found (10 of 18 enzymatic loci being polymorphic) among isolates. In addition, 52 ETs (27.5% of isolates) were identified without correlation with clinical aspects and reduced *in vitro* sensitivity to fluconazole (FCZ)[11].

The simultaneous occurrence of genetically different *C. albicans* strains in HIV-seropositive patients (Montpellier, France) suffering of oropharyngeal candidiasis was also demonstrated by MLEE analysis. Low genetic diversity (10 of 21 enzymatic loci being polymorphics) among the isolates and 20 ETs (12.5% of isolates) were identified in a population of patients. However, there was predominance of a single *C. albicans* genetic type in the oral cavity of patients. This fact could result from the interspecies competition, which could be altered by the selective pressure of antifungal treatments[63]. Using MLEE, Nébavi *et al.* (1998) also demonstrated that most of HIV-seropositive patients (Abdjan, Ivory Coast) suffering from oropharyngeal candidiasis were colonized by identical or variant ETs of *C. albicans* during antifungal therapy (AMB, KTZ, NYS). These researchers identified 27 ETs (40.3% of isolates) and a low genetic diversity (10 of 21 enzymatic loci being polymorphics) among isolates[51]. MLEE analyses were also performed in *C. albicans* isolates from patients (Montpellier, France) suffering of recurrent oropharyngeal candidiasis that successively developed clinical resistance to the fluconazole (FCZ) and itraconazole (ITZ). These analyses revealed that the infection of the patients occurred for one or more ETs during antifungal therapy, which could be (*i*) selected from a mixed population or (*ii*) acquired from an exogenous source. Besides, 14 ETs (14.3% of isolates) and low genetic diversity (12 polymorphic enzymatic loci) were identified in a population of *C. albicans* isolates, without correlation to antifungal sensitivity tests[50].

The genetic diversity of isolates in their respective populations was evaluated through UPGMA dendrograms. The coexistence of highly related or indistinguishable *C. albicans* ($0.012 > d_{ij} \geq 0$) was observed in some healthy children of the same socioeconomic category, probably emerging from a common ancestral strain[55,63]. These results suggest (*i*) the existence of one or more highly related *C. albicans* oral isolate clusters and usually predominant in healthy children populations with a common socioeconomic background, and (*ii*) the existence of direct and indirect propagation routes of *C. albicans* in populations of healthy children, which could be determined by complementary studies as, for instance, the isolation of *C. albicans* from a shared environment (education and sport schools, and their respective professionals...). Schmid *et al.* (1999) have showed high genetic similarity among not geographically related *C. albicans* clusters by

Southern blot hybridization with a Ca3 probe. Their results suggested that there was a former small radial propagation of strains among geographically adjacent regions[64]. The frequent and common mechanisms involved in the genetic diversity of *Candida* species could explain this genetic similarity. These mechanisms comprise chromosomal rearrangements, chromosomal alterations and genic expression control[50,65,66]. Besides, repetitive sequences in tandem and subtelomeric and telomeric sequences can be involved in organization and chromosomal rearrangements[67,68]. Using MLEE and grouping analyses, other researchers have also showed the existence of highly related *C. albicans* clusters isolated from healthy and immunocompromised patients without correlations with the clinical aspects, antifungal sensitivity or geographical regions[11,15,50,51]. Lupetti *et al.* (1995) used electrophoretic karyotyping and identified two similar *C. albicans* clusters displaying prevalence in healthy individuals and HIV-seropositive patients (Pisa, Italy)[25]. Their observations were suggestive that commensal strains can be probable agents of subsequent oral candidiasis in immunocompromised patients, as also suggested by other researchers[69,70], although strain substitution can also happen[25].

The genetic diversity analysis among populations showed ancestral convergence in *C. albicans* populations isolated from healthy children of the B and C (mean/high), or D and E (mean/low) socioeconomic categories. Ancestral divergence was observed among *C. albicans* populations isolated from children of socioeconomic categories A (high) and BC (medium/high), A (high) and DE (medium/low), or BC (medium/high) and DE (medium/low) that, on average, corresponded between >1 and <2.3 allelic substitutions for each 100 loci, from a common ancestral population. These results suggest that microevolutionary changes can occur in some *C. albicans* populations isolated from healthy children that present the same socioeconomic status. However, microevolution investigation in *C. albicans* population commensals, comparing other host parameters (nutritional and hygienic habits, hormonal changes, age…) could be explored. Other epidemiologic and microevolutionary studies of *C. albicans* have been performed using Southern blot hybridization with DNA probe Ca3[20-23,64,71-73]. Some of these studies have also demonstrated the existence of regional specificity and genetically similar and highly predominant subgroups of *C. albicans* in various types of infections from various patients living in different geographic areas. Such results were indicative of the existence of a ubiquitous group displaying the predominant etiological agent of candidiasis, which could arise from its high prevalence as a commensal. In addition, strong epidemiologic and microevolutionary agreements were demonstrated by Ca3 fingerprinting, MLEE, and RAPD analyses during the characterization of *C. albicans* isolated from several anatomical sites of immunocompetent and immunocompromised patients[15].

Using MLEE analysis, the results obtained in the current research showed high genetic diversity of *C. albicans* oral isolates and predominance and coexistence of some subtypes (ETs) in Brazilian populations of clinically healthy children classified into five socioeconomic categories (A, B, C, D, and E). However, no correlation was observed between a specific ET and a specific population of children. The existence of one or more highly related ET clusters was showed by clustering analysis, suggesting the existence of indirect and direct propagation routes of *C. albicans*, which could demand certain complementary studies as, for instance, the isolation of *C. albicans* from shared environments. The genetic diversity analyses among populations showed (*i*) ancestral convergence in the *C. albicans* populations isolated from healthy children of socioeconomic categories B and C (medium/high), or D and E (medium/low), and (*ii*) ancestral divergence among *C. albicans* populations isolated from children of socioeconomic categories A (high) and BC (medium/high), A (high) and DE (medium/low), or BC (medium/high) and DE (medium/low). These results suggest that microevolutionary changes can occur in some *C. albicans*

populations isolated from healthy children that present a common socioeconomic status. Furthermore, a low transition of *C. albicans* subtypes can be occurring among certain populations of children (low transition between A and BC, A and DE, or BC and DE), which can also be explained by their own socioeconomic and cultural characteristics. However, microevolution investigations of *C. albicans* commensal populations, comparing other host parameters, could be explored. Finally, MLEE analysis could be used for current and retrospective analyses of *C. albicans* isolated from healthy and immunocompromised individuals, in order to detect the existence of a predominant group in candidiasis. Such procedures could lead to the development of strategies for prevention of transmissibility of these yeasts groups in healthy or immunocompromised children, regardless of their socioeconomic and cultural conditions.

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