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Clusters of patients with candidaemia due to genotypes of *Candida albicans* and *Candida parapsilosis*: differences in frequency between hospitals

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Abstract

The presence of clusters (identical genotypes infecting different patients) suggests patient-to-patient transmission or a common source for strains. We report the results of a genotyping study based on microsatellite markers of *Candida albicans* (n = 179) and *Candida parapsilosis* (n = 76) causing candidaemia, to assess and compare the percentage of patients grouped in clusters during the study period (January 2010 to December 2012). The study was performed in two large tertiary hospitals in Madrid, Spain. We detected 145 *C. albicans* genotypes (21 in clusters) and 63 *C. parapsilosis* genotypes (seven in clusters). Clusters involved two to seven patients each. Most of the clusters in the two centres involved two patients for both species, but the number of patients included in each cluster differed between hospitals. Considering both species, the percentage of patients per cluster ranged from 19% to 38% ($p < 0.05$) in Hospital A and B respectively. Up to 2.9% of genotypes were present in both hospitals. Clusters of *C. albicans* and *C. parapsilosis* genotypes causing candidaemia differed between hospitals, suggesting differences in strain transmission. Occasionally, the same genotypes were found in patients admitted to different hospitals located in the same city.

Keywords: *Candida albicans*, *Candida parapsilosis*, clusters, genotyping, Madrid, microsatellites, Spain
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Introduction

Candida spp. are the main cause of invasive fungal infections, with *Candida albicans* and *Candida parapsilosis* being the most common entities in most geographical areas [1]. Exogenous strains of *C. albicans* and *C. parapsilosis* can infect patients admitted to hospital and cause outbreaks of candidaemia [2]. Highly discriminatory genotyping procedures are useful when investigating the genetic relationships between isolates [2]. The presence of clusters (identical genotypes infecting different patients) suggests patient-to-patient transmission or a common source for strains. We previously observed that up to 11% of *C. albicans* genotypes and 18% of *C. parapsilosis* genotypes

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causing candidaemia in patients admitted to Gregorio Marañón Hospital in Madrid (Spain) were clusters [3,4]. These data suggest highly effective transmission of strains in hospitals. However, it is unknown whether this percentage is similar to that found in other large hospitals.

We report the results of a genotyping study based on microsatellite markers of *C. albicans* and *C. parapsilosis* causing candidaemia in patients admitted to two large tertiary hospitals in Madrid, Spain that aimed to assess and compare the percentage of patients grouped in clusters in the centres over a specific period. We also included isolates collected at a third hospital in Rome, Italy, to study the presence of genotypes clustering in a non-geographically related institution.

Materials and methods

Hospital description and collection of isolates

Gregorio Marañon (Hospital A) serves a population of approximately 715 000 inhabitants, and Ramón y Cajal (Hospital B) serves a population of approximately 600 000 inhabitants. Both hospitals care for all types of patients at risk of acquiring candidaemia, including patients with haematological malignancies, solid organ transplant recipients, patients with central venous catheters, patients admitted to medical and surgical intensive care units, and neonates (only Hospital A). The distance between the hospitals is 11 km.

During the study period (January 2010 to December 2012), a total of 230 and 123 episodes of candidaemia were recorded in patients admitted to Hospital A and Hospital B, respectively. One isolate per patient was studied (incident isolate). The number of *C. albicans* and *C. parapsilosis* isolates included in each centre is shown in Table 1.

In both hospitals, blood samples were obtained for culture using standard procedures and incubated in the automated Bactec 9240 (from 2010) and Bactec™-FX system (2011–2012) (Becton-Dickinson, Cockeysville, MD, USA).

Blood cultures with presumptive visualization of yeasts in the Gram stain were subcultured on CHROMagar (CHROMagar, Paris, France) in Hospital A, and on CandiSelect agar (BioRad, Marnes La Coquette, France) in Hospital B. Plates were incubated for 24–48 h at 35–37°C. Presumptive identification was confirmed by internal transcribed spacer sequencing (Hospital A) [5] and by matrix-assisted laser desorption/ionization time of flight mass spectrometry on a Microflex LT (Daltonik, Bremen, Germany) (Hospital B) [6].

Genotyping procedure

Genotyping was based on microsatellite markers. The microsatellite markers used for *C. albicans* were CDC3, EF3 and HIS3 [7,8] and CAI, CAIII and CAVI [9]. The microsatellite markers used for *C. parapsilosis* were CP1, CP4a, CP6, and B, as previously described [10,11]. Capillary electrophoresis was carried out in a 3130xl analyser, and data were analysed using GeneMapper 4.0 software (Applied Biosystems-Life Technologies Corporation, Foster City, CA, USA).

Genetic diversity analysis

The allelic composition was studied for each locus, as *C. albicans* and *C. parapsilosis* are diploid and can be homozygous or heterozygous for each marker. The parameters of genetic diversity studied for each locus were calculated as previously reported [3,4]. The number of alleles per locus observed heterozygosity (Ho) (direct count calculated as the number of heterozygous genotypes over the total number of genotypes analysed for each locus); expected heterozygosity (He) (where $He = 1 - \Sigma pi^2$, where *pi* is the frequency of the *i*th allele) [12]; Wright's fixation index ($F = 1 - (Ho/He)$), which shows the relationships between Ho and He and detects an excess or deficiency of heterozygotes [13]; and, finally, the probability of identity for

TABLE 1. Number of *Candida albicans* and *Candida parapsilosis* isolates studied; the number of genotypes and the percentage of clusters and patients in cluster are also shown

	Candida albicans			*Candida parapsilosis*		
	Hospital A	**Hospital B**	**Overall**	**Hospital A**	**Hospital B**	**Overall**
Number of isolates	116	63	179	46	30	76
Mean number of alleles	21.7	16.0	22	17.8	12.0	20.25
Observed heterozygosity[a]	0.664	0.612	0.644	0.525	0.408	0.48
Expected heterozygosity[a]	0.804	0.768	0.797	0.842	0.768	0.825
Wright's index[b]	0.155	0.191	0.192	0.366	0.458	0.418
Probability of identity[c]	7.98×10^{-9}	7.19×10^{-8}	1.30×10^{-8}	1.19×10^{-6}	2.16×10^{-5}	2.31×10^{-6}
Number of genotypes	101	49	145	42	22	63
No. of clusters (%)	9 (9%)	10 (20.4%)	21 (14.4%)	3 (7.1%)	4 (18.1%)	7 (11.1%)
No. of patients in cluster (%)	**24 (20.7)%**	**24 (38.1%)**	53 (29.6%)	**7 (14.9%)**	**12 (40%)**	20 (26.3%)

Diversity parameters calculated are shown by each species and per hospital. Mean number of alleles, and the observed and expected heterozygosity are shown as mean per locus. Numbers in bold indicate significant differences.
[a]Observed and expected heterozygosities ranged from 0 (no heterozygosity) to 1 (highest heterozygosity).
[b]Wright's index indicates a deficiency of heterozygosity (positive values) or excess heterozygosity (negative values).
[c]Total probability of identity indicates the probability of finding two identical genotypes after randomly selecting two isolates.

unrelated individuals ($PI = 1 - \Sigma pi^4 + \Sigma\Sigma(2pipj)^2$; where *pi* and *pj* are the frequencies of the *i*th and *j*th alleles, respectively), which measures the probability that two randomly drawn diploid genotypes will be identical, assuming observed allele frequencies and random assortment [14].

Genotyping results were converted to binary data by scoring the presence or absence of each allele. Data were treated as categorical, and the genetic relationship between all the genotypes found was studied by constructing a minimum spanning tree (BioNumerics version 6.6; Applied Maths, Sint-Martens-Latem, Belgium). Isolates were considered to have identical genotypes when they showed the same alleles for all loci; identical genotypes were confirmed. A cluster was defined as the same genotype infecting two or more patients. We compared the number of clusters and the percentage of patients in the clusters found in Hospital A and Hospital B using the chi-square test. We also included isolates causing candidaemia (*C. albicans*, *n* = 89; *C. parapsilosis*, *n* = 46) in patients admitted to Policlinico Universitario Agostino Gemelli, Rome, Italy during the study period to study the presence of isolates clustering in a non-geographically related hospital.

Results

Analysis of genetic diversity

We studied the *C. albicans* and *C. parapsilosis* isolates from the two hospitals to analyse genetic diversity parameters (Fig. 1a,b).

The diversity of the *C. albicans* isolates was as follows: number of alleles, 132 (mean 22 alleles per locus); observed and expected heterozygosity, 0.64 and 0.79; and probability of identity, 1.3×10^{-8}. The diversity of the *C. parapsilosis* isolates was as follows: number of alleles, 81 (mean 20.25 alleles per locus); observed and expected heterozygosity, 0.48 and 0.83; and probability of identity, 2.31×10^{-6}. The parameters for each hospital are shown in Table 1.

Of the 145 *C. albicans* genotypes found, 21 were in clusters (Fig. 1a). We also found 63 *C. parapsilosis* genotypes, seven of which were in clusters (Fig. 1b). Clusters involved two to seven patients each (Tables 2 and 3).

Analysis of clusters

The number of genotypes found, the number of clusters, and the percentage of patients in clusters are shown in Table 1.

Nine and ten *C. albicans* clusters were found in Hospital A and Hospital B, respectively; however, the percentage of patients in each cluster was higher in Hospital B (38.1%) than in Hospital A (20.7%) ($p < 0.05$) (Table 1). In the case of *C. parapsilosis*, three and four clusters were found in Hospital A and Hospital B, respectively; similarly, the proportion of patients in each cluster was higher in Hospital B (40%) than in Hospital A (15%) ($p < 0.05$) (Table 1). Considering both species, 19.1% and 38% of patients admitted to Hospital A and Hospital B, respectively, were in clusters ($p < 0.05$). Tables 2 and 3 and Fig. 1 summarize data for the patients involved in the clusters. Patients in the cluster infected by *C. albicans* were not generally admitted to the same unit, with the exception of three clusters in the neonatology ward of Hospital A (CA-34, CA-227 and CA-371) and four clusters found in the oncology, internal medicine, haematology and intensive care departments of Hospital B (CA-100, CA-230, CA-290 and CA-303) (Table 2 and Fig. 1a). In contrast, no clusters of patients admitted to the same wards and infected by *C. parapsilosis* were found in Hospital A, but in hospital B, two clusters were found in patients admitted to the general intensive care unit (CP-31 and CP-70) (Table 3 and Fig. 1b).

Some strains clustered in patients admitted to Hospitals A and B (*C. albicans* (genotypes CA-48, CA-59, CA-63, CA-165 and CA-310); *C. parapsilosis* (genotype CP-117)) (Tables 2 and 3). Up to 8.2% were infected by the genotypes found in Hospitals A and B, and up to 2.9% of genotypes were present in both hospitals. We also studied the presence of genotypes clustering in patients admitted to Hospital A, Hospital B and Policlinico Universitario Agostino Gemelli in Rome. Six genotypes were found in the hospital in Rome and in Hospital A (CP23 and CP-111), in Hospital B (CA-71, CA-100, CA-159), or in all three hospitals (CA-165).

Discussion

Our study shows that the number of patients grouped in clusters differed between the two hospitals; the highest number of patients per cluster was found in hospital B. This percentage was higher for both *C. albicans* and *C. parapsilosis* and suggests different rates of strain transmission in each hospital.

The study of the genetic diversity of *Candida* isolates causing candidaemia provides information on the distribution of genotypes within the hospital and can reveal the presence of clusters. Microsatellite markers have high discriminatory power and reproducibility and are useful for studying the genetic diversity of *Candida* spp. [2].

We previously observed a high number of *C. albicans*-infected patients in clusters admitted to Hospital A [4]. In the present study, we describe the genetic diversity of *C. albicans* and *C. parapsilosis* isolates from patients with candidaemia admitted to two tertiary hospitals in Madrid as well as genotypes clustering in both hospitals and a hospital in Rome. Genetic diversity between *C. albicans* and *C. parapsilosis* differed, probably owing to the different number of isolates studied. The overall

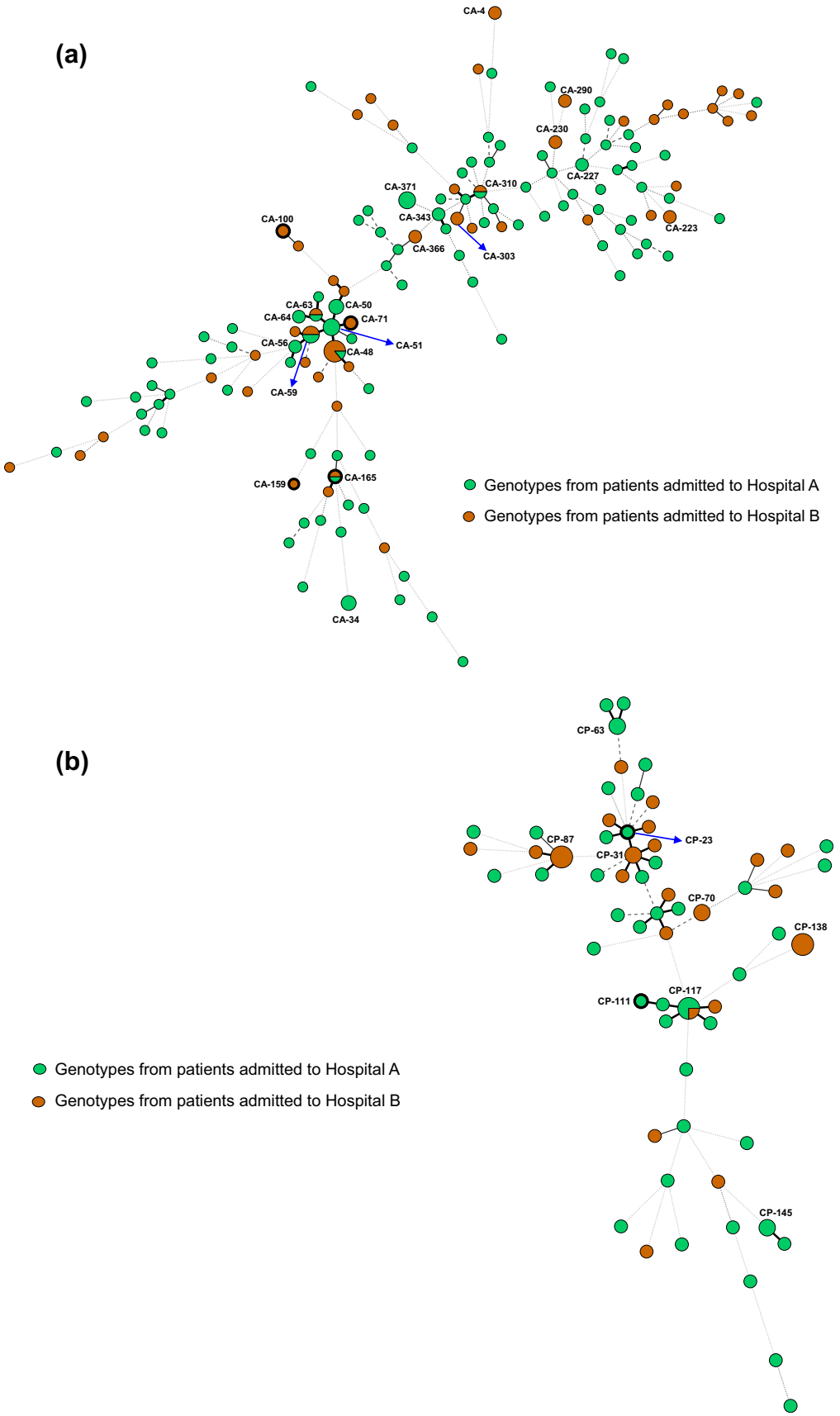

TABLE 2. Clusters of *Candida albicans* found in Hospitals A and B

Genotype code	Allelic composition (bp)						No. of patients involved	Ward of admission at the time of blood sample collection	Hospital	Date of blood culture collection (month/day/year)
	CDC3	EF3	HIS3	CAI	CAIII	CAVI				
CA-34	120–124	143–148	149–191	203–216	92–92	234–273	3	Neonatology	A	8/2/10 12/10/10 12/16/10
CA-50	116–128	146–146	154–154	206–228	95–95	239–239	3	Angiology Neonatology	A	7/17/10 11/23/10 12/16/10
CA-51	116–128	135–146	154–154	206–228	95–95	239–239	4	Paediatrics Oncology Geriatric General Surgery	A	4/30/10 7/15/10 12/18/10 8/23/11
CA-56	116–116	135–146	154–154	206–206	95–95	239–239	2	Reanimation Neonatology		3/1/10 1/6/12
CA-64	116–128	135–149	154–154	206–228	95–95	239–239	2	Infectious diseases Oncology	A	7/13/10 1/2/12
CA-227	116–116	125–134	162–162	234–293	95–109	246–246	2	Neonatology	A	3/2/10 4/15/10
CA-343	116–124	130–130	162–195	216–228	95–98	246–276	3	Angiology Internal Medicine	A	7/7/10 4/20/11
CA-371	116–124	130–130	166–195	216–228	95–98	251–276	4	Neonatology	A	9/21/10 9/24/10 10/5/10 10/24/10
CA-4	124–124	130–138	199–221	228–231	104–104	280–299	2	Haematology Intensive care unit (ICU)	B	4/6/11 6/9/11
CA-71	116–128	135–146	154–154	228–228	95–95	239–239	2	Urology ICU	B	3/9/11 9/15/10
CA-303	116–124	130–130	162–187	216–231	95–98	246–269	2	ICU	B	1/7/11 12/28/11
CA-290	116–124	125–134	162–221	304–307	95–115	246–246	2	Haematology	B	9/3/12 9/5/12
CA-230	116–124	125–134	162–162	234–298	95–95	246–246	2	Internal Medicine	B	9/12/12 9/10/12
CA-223	116–124	134–134	162–203	249–287	109–109	246–284	2	General Surgery Oncology	B	7/9/12 7/15/12
CA-366	116–124	130–130	183–195	216–228	95–98	265–276	2	Urology ICU	B	12/26/11 11/23/10
CA-100	116–124	128–143	154–154	185–185	92–95	239–262	2	Oncology	B	11/10/10 11/11/10
CA-59	116–116	135–146	154–154	206–228	95–95	239–239	4	Urology Neurology Paediatrics ICU	A B 	2/2/10 11/29/12 3/7/12 11/20/12
CA-310	116–124	130–130	162–187	228–228	95–98	246–269	2	General Surgery Oncology	A B	2/25/10 11/10/10
CA-63	116–128	135–135	154–154	206–228	95–95	239–239	2	ICU	A B	1/25/11 9/9/10
CA-165	112–116	129–135	149–162	231–255	92–95	234–246	2	Internal Medicine ICU	A B	5/12/10 6/8/10
CA-48	116–128	135–146	154–154	206–206	95–95	239–239	7	Neonatology Internal Medicine Digestive Urology General Surgery	A B 	8/5/12 9/3/12 9/10/12 5/31/12 6/5/12 11/9/10 11/29/10

genetic diversity found in both species did not differ much from that found in each centre.

We found that the number of patients per cluster differed significantly depending on the centre. The differences in the percentage of patients per cluster between Hospital A and Hospital B also suggest different rates of strain transmission; although we did not investigate the reasons for this observation, it could be a consequence of differences in catheter care, or the type of patients admitted to each hospital.

We found that up to 2.9% of genotypes were present in the two hospitals in Madrid; some genotypes were also found in the hospital in Rome. Genotypes clustering in different hospitals

FIG. 1. Minimum spanning tree indicating the *Candida albicans* genotypes (a) and *Candida parapsilosis* genotypes (b). The figure shows the different genotypes found (circles). The number of patients in each cluster is indicated by the size of the circles; the hospital of admission is indicated by the colour of the circle (green circles, Hospital A; brown circles, Hospital B). Connecting lines between circles show the similarity between profiles: a bold solid line indicates differences in only one marker, a solid line indicates differences in two markers, long dashes indicate differences in three markers, and short dashes indicate differences in four or more markers. Genotypes in clusters are labelled CA (i.e. *C. albicans*) or CP (i.e. *C. parapsilosis*) followed by the number of the cluster. Circles in bold indicate genotypes also found in Hospital Policlinico Universitario Agostino Gemelli, Rome, Italy.

TABLE 3. Clusters of *Candida parapsilosis* found in Hospitals A and B

Genotype code	Microsatellite fragments (bp)				No. of patients involved	Ward of admission at the time of blood sample collection	Hospital	Date of blood culture collection (month/day/year)
	CP1	CP4a	CP6	B				
CP-145	242–242	256–256	254–267	145–145	2	Emergency	A	5/7/10
						Oncology		8/21/10
CP-63	245–245	291–24	267–269	113–129	2	Neonatology	A	8/30/10
						Oncology		8/6/11
CP-31	224–245	300–300	317–317	129–129	2	Intensive care unit (ICU)	B	12/12/11
								11/14/11
CP-70	245–245	303–303	286–286	127–129	2	ICU	B	7/31/11
								8/1/11
CP-87	245–245	325–342	317–317	127–127	4	Oncology	B	7/19/11
								7/17/11
						Otolaryngology		7/14/11
						ICU		9/28/11
CP-138	208–242	240–240	312–321	133–133	4	ICU	B	2/24/10
						Internal Medicine		7/8/11
								3/2/11
						Infectious diseases		12/21/11
CP-117	239–245	240–240	265–265	133–133	4	Angiology	A	10/19/10
						General surgery		4/30/11
						ICU	A	11/30/11
							B	9/12/10

The genotype code, the allelic composition, the number of patients involved, the wards of admission of the patients at the time of diagnosis, and date of the blood culture collection are shown for each cluster.

have been described in hospitals located in the same country [15–17] or even in different countries [18]. However, these studies are limited by the use of less discriminative typing procedures, analysis of mainly *C. albicans* isolates, and the study of isolates recovered from the intensive care unit. The high percentage of genotypes found in more than one centre is difficult to explain, and different hypotheses could be proposed. First, certain genotypes may be predominant in the population or in the environment, as previously reported with *Staphylococcus aureus,* or so-called bacterial high-risk strains could adapt well to the hospital environment [19]. Second, the possible absence of a sexual cycle means that some clones could expand in the environment. Finally, microsatellite markers are limited owing to homoplasy (alleles with identical size but different sequences) or sexual recombination that can lead to strains with the same genotype but a different epidemiological source [15,20]. Next-generation sequencing analysis may help to identify various genotypes with the same microsatellite profile.

Our study is subject to a series of limitations. First, it was not designed to prove nosocomial transmission, because we did not include environmental isolates or isolates from the hands of the healthcare workers to trace an epidemiological link. Finally, the presence of genotypes clustering different hospitals should be proved through highly discriminatory approaches such as whole genome sequencing.

In conclusion, *C. albicans* and *C. parapsilosis* clusters causing candidaemia differed between hospitals, suggesting differences in strain transmission. Furthermore, some genotypes can be found in patients admitted to hospitals located in different cities. Genotyping should be performed to evaluate the impact of campaigns to decrease patient-to-patient *Candida* transmission in the hospital.

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Transparency declaration

This study does not present any conflicts of interest for its authors.

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