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Nosocomial candiduria in women undergoing urinary catheterization. Clonal relationship between strains isolated from vaginal tract and urine

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We determined the incidence of nosocomial candiduria associated with indwelling urinary catheters in 42 women with and without *Candida* spp. vaginal colonization being treated in the intensive care unit (ICU). We established a relationship between strains initially isolated from the vaginal tract and those subsequently recovered from urine samples through the use of random amplified polymorphic DNA (RAPD). The overall incidence of nosocomial candiduria in these patients was 21.4%. Vaginal colonization by *Candida* spp. was detected in 11 patients (26.2%) of whom 6 (54.5%) developed candiduria. In comparison, only 3 (9.7%) cases of candiduria were found in women who were not colonized by the yeast (RR: 4.4, 95% CI 1.61–86.8, $P = 0.005$). The dendrogram obtained by RAPD using 14 primers showed that the strains isolated from vagina and urine samples in five women had high similarity values (SAB >0.9) forming independent clusters. Our study suggests that women vaginally colonized by *Candida* spp. in an ICU setting have a high risk of acquiring nosocomial candiduria and that strains isolated from both sites in a single patient may be genetically related.

Keywords *Candida* spp., candiduria, molecular epidemiology

Introduction

Nosocomial candidiasis has increased significantly in recent years and is currently an important cause of morbidity and mortality in patients admitted to intensive care units (ICU). The urinary tract is the most common site of infection and vesical catheterization, associated with more than 90% of these infections, is the main risk factor [1–3]. Other factors contributing to this infection are prolonged stays in the ICU, female gender, prolonged vesical catheterization and antibiotic use [4–6].

The Center for Disease Control and Prevention has not established a clear definition for nosocomial urinary tract infection (UTI) caused by *Candida* spp. or other yeasts [7,8]. Some have defined it as the isolation of $\geq 10^5$ colony forming units (CFU)/ml of *Candida* spp. in urine collected at least 72 h after the patient has been admitted to the hospital who had a previously negative urine culture [5]. We previously reported that catheterized ICU patients with urine yeast counts greater than 2×10^4 CFU/ml required antifungal treatment, while cultures of patients with lower counts became negative spontaneously [4]. Independent of the CFU/ml count, *Candida* UTI is mostly asymptomatic [2].

In Chile, according to the Ministry of Health (MINSAL), 22% of nosocomial UTI in ICU patients between 1999 and 2000 were caused by *Candida* spp., making them the most important etiological agents of UTI [9]. *Candida albicans* was the third most common etiologic recovered from UTI in these patients after *Escherichia coli* and *Klebsiella pneumoniae* [10,11].

Compared to men, women are at twice the risk of developing nosocomial candiduria most probably due

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to anatomical differences of their genital organs [4]. Furthermore, this higher rate of candiduria may also be the result of vaginal colonization found in 5 to 30% of healthy and sexually active women [12]. Until now, the objective of the majority of studies was to establish an endogenous origin in invasive candidasis by investigating the relationship between commensal and infective strains of the etiologic agent [13–15]. However, there is no evidence to establish a clonal relationship between *Candida* spp. isolated from nosocomial candiduria and adjacent colonized sites in a single patient.

In recent years, molecular tools have helped to establish parental and phylogenetic relationships among microorganisms and have had a significant impact on the identification of etiologic agents causing nosocomial infections [16–20]. Random amplified polymorphic DNA (RAPD) has emerged as one of the most commonly applied tools in molecular epidemiology of *Candida* spp. which has aided in determining the source of infection [17,21–23].

The objective of this study was to use molecular fingerprinting by RAPD to determine the relationship between nosocomial candiduria and vaginal yeasts colonization in women admitted to ICU with indwelling urinary catheter.

Materials and methods

Over a period of 16 months a cohort study was carried out of women who had undergone vesical catheterization while hospitalized in the ICU. Entry criteria included the absence of renal diseases, lack of antifungal treatments, and having negative cultures inoculated with urine samples collected within 24 h of vesical catheterization. At the same time, a single sample of the vaginal tract was obtained with a cotton swab from each patient. Urine samples were collected systematically every 72 h until the patient was discharged from the ICU. Candiduria was defined as the presence of *Candida* yeast in urine sample at a count $\geq 2 \times 10^4$ CFU/ml.

Urine and vaginal samples were inoculated onto Petri dishes containing Sabouraud dextrose-agar with chloramphenicol, incubated at 37°C for five days and observed daily for growth. Isolates were identified by the germ tube test, micromorphology on cornmeal-Tween 80-agar and carbohydrate assimilation patterns [24]. Isolates were transferred to dehydrated gelatin discs until further genetic tests were conducted [25].

Genomic DNA extraction was done using the Wizard® Genomic DNA Purification Kit (Promega). The final DNA concentration of each strain was adjusted to 10 pg/μL with TE buffer and stored at −20°C. RAPD reactions were done using 100 pg of DNA, 1X Taq pol buffer, 100 μM of each nucleotide, 2 mM $MgCl_2$, 10 pmoles of primer and 1U of Taq DNA polymerase (Gibco BRL) in a final volume of 25 μl per reaction. The RAPD program was done as follows: initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 2 min and extension at 72°C for 2 min, and a final extension step at 72°C for 5 min. A series of 14 primers (OP AA, OP BA, OP AC, OP AE) 10 nucleotides long, and arbitrary sequence were used, each one with ≥60% of G+C (Operon Technologies, Inc.). The final amplification products were separated by gel electrophoresis in 1% agarose gels with ethidium bromide staining using 100 bp Ladder as DNA standard (Gibco BRL).

Bands observed with two isolates of the same species were scored as present or absent and their similarity was mathematically determined according to the Dice coefficient with the following formula: $S_{AB} = 2E/(2E + a + b)$ [26]. For S_{AB} calculation RAPDistance software was used. $S_{AB} = 1$ suggests that both species share the same genetic profile and S_{AB} values 0.01 to 0.99 indicate different degrees of genetic relationship among isolates [27]. A dendrogram was constructed using the results obtained with the 14 primers and the data was analyzed by the UPGMA algorithm [28]. Furthermore, average similarity among isolates recovered from a single patient (patient S_{AB}) and average similarity among strains obtained from all patients studied and controls (control S_{AB}) were determined. The controls were three isolates of *Candida albicans* and four of *Candida glabrata* recovered from seven different ICU patients with nososcomial candiduria who had vesical catheterization unrelated to the cases and whose urine counts of the yeasts were $\geq 2 \times 10^4$ CFU/ml.

The computer program STATISTICA (StatSoft, Inc., 1993) was used to carry out a *t* student test for independent samples with different variances to determine if differences obtained between the average S_{AB} of patients and controls were statistically significant. EPI info 6.0 (CDC) software was used to calculate the relative risk, confidence interval and statistical significance of nosocomial candiduria in women with and without vaginal colonization.

Results

Forty-two women with indwelling urinary catheters who were admitted to the ICU were enrolled during the 16 month study period. Of these, nine asymptomatic patients developed nosocomial candiduria (21.4%) with counts ranging from 4.6×10^4 to 1×10^5 CFU/ml. Vaginal colonization was detected in 11of the 42

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Table 1 Results of urine cultures according to *Candida* spp. in the vaginal tract of ICU patients with indwelling urinary catheter

Results of urine culture for yeasts	Vaginal sample with *Candida* spp.		Vaginal sample without *Candida* spp.		Total	
	n	%	n	%	n	%
Positive	6	54.5	3	9.7	9	21.4
Negative	5	45.5	28	90.3	33	78.6
Total	11	100.0	31	100.0	42	100.0

RR, 4.4; CI, 95% =1.61–86.8; P =0.005.

enrolled individuals (26.2%), of whom 6 developed candiduria (54.5%) as compared to 3/31 women without evidence of *Candida* spp. colonization (9.7%). This difference resulted in a relative risk (RR) of 4.4, confidence interval (CI) [95%] = 1.61 to 86.8, and P = 0.005 (Table 1). In 5 of the 6 patients, the same *Candida* spp. was recovered from both urine and vaginal specimens. Seven isolates of *C. albicans* were obtained from 3 patients (3 from vagina and 4 from urine) and 5 of *C. glabrata* from the other two (2 from vagina and 3 from urine).

Fig. 1 shows the profiles of the *C. albicans* (A) and *C. glabrata* (B) strains isolated from the vaginal tract and urine of the 5 patients, as well as control strains. This primer (OP BA 13) generated highly related profiles for both *Candida* species isolated from the same patient. The one exception was strain 105.1 of *C. glabrata* isolated from urine (Fig. 1 B, lane 5) that showed a different pattern from the other strains recovered from the same patient. Different profiles were found with isolates recovered from different patients and those of the control strains.

Table 2 shows the sequence and results obtained with each of the 14 primers relative to the total number of bands and the similarity coefficients (S_{AB}). All primers allowed for the detection of polymorphisms among *C. glabrata* strains, while 13 primers detected polymorphisms in *C. albicans* isolates. Primer OP BA 15 is considered species-specific since it generated the same profile for all *C. albicans* strains giving a $S_{AB} = 1$. Primer OP BA 13 generated the greatest number of bands for *C. albicans* isolates (Fig. 1 A) and similar results were noted with OP BA 01 for *C. glabrata*. For *C. albicans,* five primers (OP BA 03, 09, 10, 13 and OP AA 14) generated unique profiles for strains isolated from a single patient, which permitted their discrimination from each other and controls isolates. For *C. glabrata* unique profiles were obtained with primers OP BA 03, 06, 13 and 18. Therefore, primers OP BA 03 and 13 generated intra-species profiles for both *Candida* species. For *C. albicans* S_{AB} analysis with the 14 primers generated a mean S_{AB} value of 0.93 ± 0.07 for isolates from a single patient and a value of 0.75 ± 0.15 for unrelated strains (P =0.0008). Likewise, average S_{AB} for *C. glabrata* isolates from a single patient was 0.90 ± 0.13 and 0.72 ± 0.21 for unrelated strains (P =0.014).

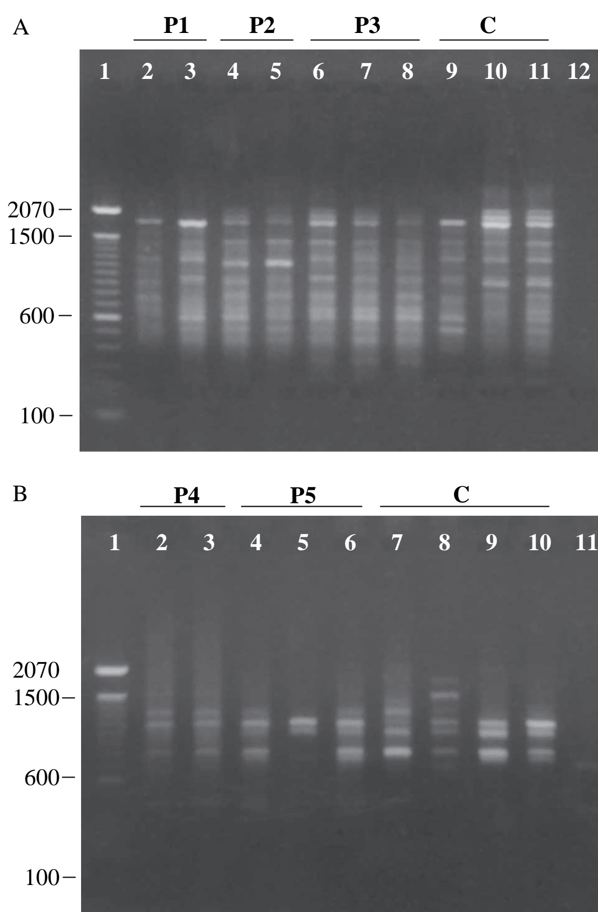

Fig. 1 RAPD electrophoretic profiles obtained with primer OP BA 13. (A) *Candida albicans* strains: Lane 1; 100 bp ladder, Lane 2, 4 and 6; vaginal tract isolates, Lane 3, 5, 7 and 8; urine isolates, Lane 9–11; control strains (C) and Lane 12; negative control. P1, P2, P3; strains isolated per patient. (B) *C. glabrata* strains: Lane 1; 100bp ladder, Lane 2 and 4; vaginal tract isolates, Lane 3, 5 and 6; urine isolates, Lane 7–10; control strains (C) and Lane 11; negative control. P4 and P5; strains isolated per patient.

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Table 2 Primers used in RAPD analysis for *Candida albicans* and *Candida glabrata*, according to the number of bands amplified and similarity coefficient between strains per patients and controls

Primer	Sequence 5′–3′	C. albicans			C. glabrata		
		Total no. of bands	Patients S_{AB}	Controls S_{AB}	Total no. of bands	Patients S_{AB}	Controls S_{AB}
OP BA 01	TTCCCCACCC	6	0.94	0.73	14	0.50	0.19
OP BA 02	TGCTCGGCTC	4	0.97	0.65	7	0.95	0.47
OP BA 03 *	GTGCGAGAAC	13	0.78	0.68	11	1.00	0.67
OP BA 05	TGCGTTCCAC	3	0.88	0.83	6	0.92	0.74
OP BA 06 +	GGACGACCGT	9	0.95	0.83	11	0.90	0.83
OP BA 09 |	GGAACTCCAC	7	1.00	0.71	4	1.00	0.89
OP BA 10 |	GGACGTTGAG	11	0.85	0.63	3	0.88	0.92
OP BA 13 *	AGGGCGAATG	16	1.00	0.68	8	1.00	0.74
OP BA 15 °	GAAGACCTGG	8	1.00	1.00	4	0.96	0.98
OP BA 16	CCACGCATCA	5	1.00	0.97	5	0.94	0.91
OP BA 18 +	CTCGGATGTC	5	0.86	0.93	6	1.00	0.79
OP AA 14 |	AACGGGCCAA	15	0.84	0.54	5	0.94	0.51
OP AC 17	CCTGGAGCTT	4	1.00	0.53	6	0.84	0.67
OP AE 12	CCGAGCAATC	9	0.94	0.82	5	0.78	0.80
Average S_{AB}		115	0.93 ± 0.07	0.75 ± 0.15	95	0.90 ± 0.13	0.72 ± 0.21

Average S_{AB} between strains per patient and unrelated strains ($P = 0.0008$ for *C. albicans* and $P = 0.014$ for *C. glabrata*).
Intra-species primer activity in; *C. albicans* (|), *C. glabrata* (+) and both species (*). Primer with species-specific activity in *C. albicans* (°).

The dendrogram shows the presence of two large clusters clearly distinguishing both *Candida* species. In each cluster, subgroups are observed, i.e., vaginal tract and urine isolates from a single patient formed a unique cluster with similarity coefficients greater than 0.9 (90%). Strains comparisons among patients and patients and controls showed lower similarity values than the established threshold and were excluded from the cluster. The exception was *C. glabrata* strain 105.1o isolated from urine with a similarity coefficient lower than 0.9 indicating that this isolate has a distant clonal relationship with the other strains isolated from the same patient (Fig. 2).

Discussion

The incidence of nosocomial candiduria in 42 women with vesical catheterization who had been admitted to the ICU was 21.4%, which is similar to that previously reported in Brazil [4]. Surveillance studies have shown that *Candida* urinary tract infections (UTI) in ICU patients is considerably more frequent than previously reported and that *Candida* spp. are today one of the main agents of nosocomial UTI in these units [2,4,6,9–11]. *Candida* spp. vaginal colonization increases the risk of nosocomial candiduria at a RR level of 4.4. These results are in agreements with previous observations from our group that demonstrated that over 60% of women with nosocomial candiduria had the same *Candida* species in vaginal and urine samples [4].

In recent years, a great number of molecular studies have assisted our understanding of the epidemiology and source of nosocomial *Candida* fungemia. However, there have not been similar detailed studies on the source and transmission of this yeast in candiduria. Several methods have been successfully used in nosocomial candidiasis, such as RFLP, electrophoretic karyotyping, hybridization and RAPD. To determine similarity by RAPD at least five primers must be separately analyzed. The information must then be combined to determine the similarity thresholds (S_{AB}), where a value close to 0.9 or 90% is used to discriminate highly related strains [13,22,29].

The global analysis in this study of the strains per patient demonstrated a high similarity value among the isolates ($S_{AB} \geq 0.90$) which formed independent clusters. This value is higher than the value obtained from strains of the same species isolated from different patients and those used as the controls. This suggests that the strains isolated from the vagina and subsequently from urine of the same patient are genetically related and would correspond to the same strain despite not sharing an identical amplification profile. This observation may be explained by studies that have indicated that commensal or infective isolates present in a patient for a long time may undergo microevolution events generating subgenotypes [15,23,30]. Lockhart *et al.* showed that subgenotypes of *Candida* spp. coexist in a single site being able to dominate under determined host conditions, events called ‘substrain shuffling’ [31].

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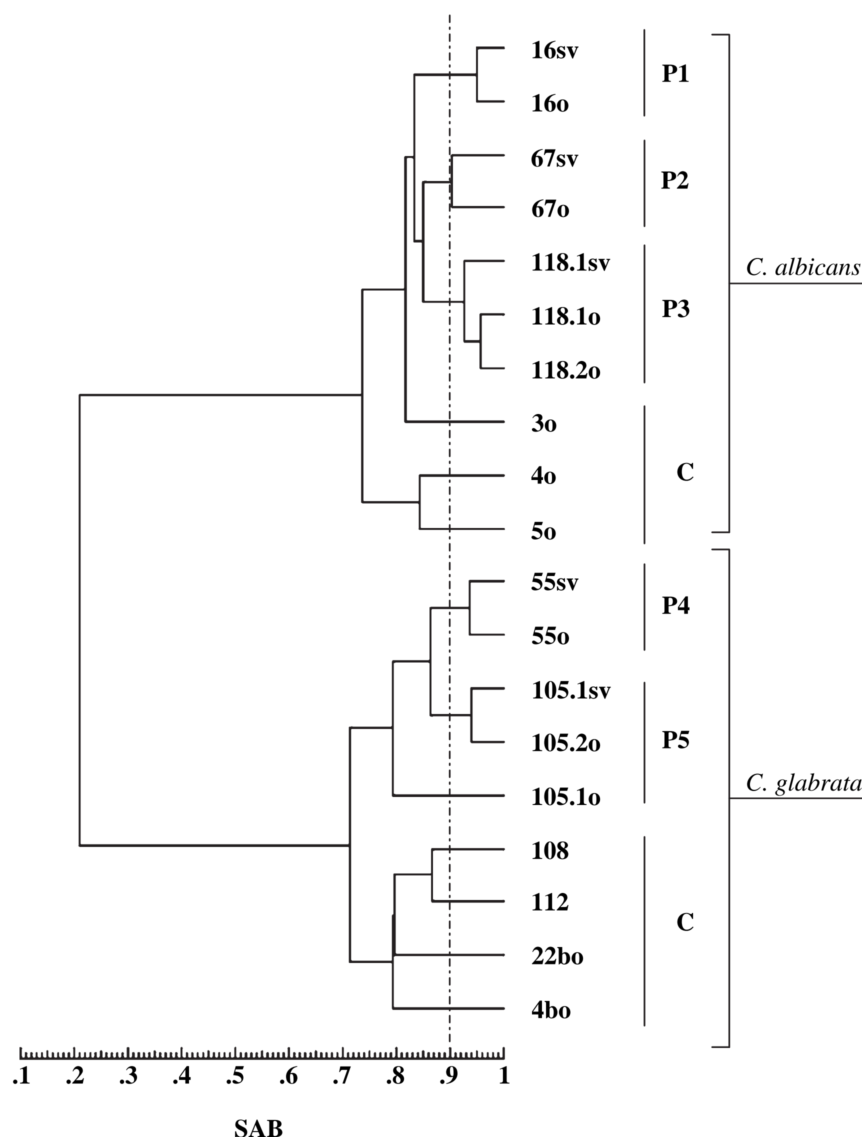

Fig. 2 Dendrogram of isolates from vaginal samples (sv) and urine (o) of patients (P1 to P5) and control strains (C), generated from the S_{AB} values with the 14 primers. P1, P2 and P3: *C. albicans* strains isolated per patient. P4 and P5: *C. glabrata* strains isolated per patient. C: control strains according to species. The established threshold of 0.90 is represented by a segmented line.

Marco *et al*. studied nosocomial candidemia by DNA fingerprinting with the complex probe Ca3 establishing that strains isolated from different anatomical sites before and after candidemia may be grouped into the same clusters in the majority of patients. In addition they noted that health care workers carrying *Candida* spp. strains from colonized patients may be contributing to cross-contamination [13]. The similarity of the *Candida* spp. strains that colonized and were present in urine in our study suggest an endogenous origin rather than the hospital environment as the cause of nosocomial UTI.

The low similarity coefficient obtained from one strain of *C. glabrata* (105.1 o) in one of the patients may be explained by the simultaneous colonization by more than one strain and/or by different yeast species, not related to host conditions [29].

Using RAPD to independently fingerprint strains with the 14 primers, we were easily able to obtain identifiable amplification products and profiles among the *C. albicans* and *C. glabrata* strains. Furthermore, we identified seven primers that discriminated intra-species, two of which generated unique visual patterns for strains of both species isolated from each patient. These would therefore be good candidates for further nosocomial outbreak studies and for establishing clonality between strains of the same species of *Candida*. Previously, Robert *et al*. established a relationship among strains isolated from different sites using one primer to genetically type the *C. albicans* isolates

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recovered from patients admitted to a burn unit [23]. Species-specific profiles obtained in other studies could have a role in the identification of the main species of *Candida* [32–34] as was observed in this study with primer OPBA 15.

In conclusion, our study suggests that women vaginally colonized by *Candida* spp have a high risk of acquiring nosocomial candiduria and that strains isolated from both sites in a single patient are genetically related.

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