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Original Article

Investigation of the relationship between virulence factors and genotype of *Candida* spp. isolated from blood cultures

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Abstract

Introduction: The aim of study was to investigate the virulence factors of phospholipase, proteinase, esterase production and biofilm formation in *Candida* species isolated from patients with candidemia, and to assess their relationship with *Candida* genotypes derived after repetitive sequence-based polymerase chain reaction (rep-PCR) fingerprinting.
Methodology: Fifty-two strains were identified to species level according to conventional methods and sequencing. The DiversiLab system was used for the genotyping. Enzyme activities and biofilm formation were evaluated using microbiological methods.
Results: The 52 strains were identified as follows: 29 *C. parapsilosis*, 19 *C. albicans*, 2 *C. glabrata*, and 2 *C. tropicalis*. Phospholipase and proteinase activities were observed to have statistically significant differences between *C. albicans* and non-*albicans Candida* (NAC) strains ($p < 0.05$), with *C. albicans* strains showing higher virulence. Rep-PCR revealed eight major genotypes (A-H).The 19 *C. albicans* and the 33 non-*albicans Candida* isolates yielded seven (A-G) and four (A, B, C, H) genotypes, respectively. *C. albicans* strains were not shown to have a predominant genotype and showed higher phospholipase and proteinase activitiy than did NAC, regardless of genotype. Genotype H (52%) was the predominant genotype for the NAC including 27 *C. parapsilosis* strains, but the majority of strains showed low virulence.
Conclusions: NAC species were the most common causative agent for candidemia. Genotyping showed low transmission of *C. albicans* strains, but transmission of *C. parapsilosis* was high. In candidemia, several *Candida* virulence factors may be responsible at the same time. However, different genotypes of *Candida strains* showed different virulence activity.

Key words: *Candida* spp.; clonal relationship; genotyping; virulence factors.

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Introduction

In the development of candidemia, virulence factors as well as the weakening of the defense mechanisms of the host play an important role [1-4]. Most studies on virulence factors that contribute to pathogenesis use *C. albicans*. Although *C. albicans* is most often associated with invasive fungal infection, non-*albicans Candida* species have also emerged as clinically important pathogens [5-11]. In recent years, the number of studies related to virulence factors of non-*albicans Candida* has increased. The main virulence factors of *Candida* spp. that have been studied are production of phospholipase, proteinase, esterase and biofilm formation.

Genotyping of *Candida* strains allows investigation of nosocomial candidiasis to identify outbreak-related strains, distinguish epidemic from endemic or sporadic strains, and determine the origins of infection. There are many methods used for epidemiological typing, such as multi-locus enzyme electrophoresis, electrophoretic karyotyping, random amplified polymorphic DNA, microsatellite length polmorphism, pulsed-field gel electrophoresis (PFGE), and multi-locus sequence typing. However, these methods are not practical for the clinical laboratory because they are time consuming and labor intensive. Repetitive sequence-based polymerase chain reaction (rep-PCR) is based on the amplification of repetitive DNA fragments dispersed in bacterial genomes. Primers used for rep-PCR complement these repetitive sequences and allow for specific binding, providing reproducible, unique rep-PCR DNA fingerprint patterns. The DiversiLab system (bioMérieux, Marcy l'Etoile, France) (rep-PCR) can be used to obtain species-level identification and for the genotyping study of *Candida* spp. in epidemiological studies. The rep-PCR method has several advantages due to its speed, high reproducibility, and ease of use. The discriminatory power of rep-PCR is good [12].

In studies conducted in recent years, it has been reported that the virulence factors of distinct genotypes of *Candida* spp. may be different [1,13-16].

The aim of this study was to investigate the virulence factors of production of phospholipase, proteinase, esterase and biofilm formation in *Candida* species isolated from the blood cultures of patients with candidemia in an adult intensive care unit (ICU), and to assess their relationship with *Candida* genotypes.

Methodology

Yeast isolates

Strains were obtained from June 2011 to July 2012 in the clinical microbiology laboratories of the Kayseri Education and Research Hospital, Turkey (1,000 beds). A total of 52 non-repetitive strains isolated from the positive blood cultures of adult ICU patients were included in the study. Strains were isolated in the clinical microbiology laboratory of the Kayseri Education and Research Hospital, Turkey. The identification of the isolates, virulence assays and genotyping by rep-PCR were performed in the clinical microbiology laboratory of Erciyes University School of Medicine. All isolates were obtained from different patients. These isolates were identified according to a germ tube test, morphology on corn-meal agar with Tween-80, and a carbohydrate assimilation test using a commercially available API AUX C 20 kit (bioMerieux). Sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA was used as the reference method in the analysis. *C. albicans* ATCC 90028 was used as the reference strain.

Sequencing analysis

Definitive species identification was determined by sequencing the ITS1-5.8S-ITS2 region as described by White *et al*. [17]. The ITS region was amplified using the ITS1 forward primer 5′-TCCGTAGGTGAACCTGCGG-3′ and the ITS4 reverse primer 5′-TCCTCCGCTTATTGATATGC - 3′. Sequence data were generated using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Foster City, USA) and sequenced using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Sequences were entered into nucleotide BLASTN tool search provided by GenBank for species identification.

Rep-PCR

DNA from each isolate was extracted using the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Carlsbad, USA) following the manufacturer's instructions. All DNA samples were amplified using the DiversiLab *Candida* kit for DNA fingerprinting (bioMérieux) following the manufacturer's instructions. Two microliters of DNA were added to the rep-PCR master mix in a total volume of 25 µL per reaction. The thermal cycler parameters were as follows: initial denaturation of 94°C for 2 minutes, followed by 35 cycles of denaturation at 92°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 70°C for 90 seconds, with a final extension at 70°C for 3 minutes.

The rep-PCR based fingerprint patterns of all isolates were obtained in gel-like images using DiversiLab version 2.1.66 web-based interpretation software. For the analysis, the Pearson correlation coefficient was used in the similarity calculation, and unweighted pair group method with arithmetic mean (UPGMA) was used to automatically compare the rep-PCR profiles. Strains with a similarity of 90% or more were categorized as the main clones, and those in the main clone category with a similarity of 95% or more were categorized as subclones. Isolates with a similarity of 90% or less were considered to be different clones.

Yeast suspensions for enzymatic activities

Yeast suspensions were prepared from the isolates included in the study to evaluate phospholipase, proteinase, esterase activities and biofilm formation. A loopful of the stock culture was streaked onto Sabouraud dextrose agar (SDA, Oxoid, Basingstoke, UK) with chloramphenicol and incubated at 37°C for 24 to 48 hours. Yeast cells were harvested and suspended in sterile phosphate buffered solution (PBS) at an optical density (OD) of 0.5 McFarland. The final suspension was adjusted to contain $2.5\text{-}5\times10^6$ yeast cells/mL [18-20].

Phospholipase activity

To determine the enzymatic activity of phospholipase, the egg yolk agar method of Price *et al*. [18], modified by Samaranayak e*et al*. [22], was employed. Ten microliters of the previously prepared suspension of isolates was inoculated onto the test medium The plates were incubated at 37°C for 48 hours and 72 hours. Phospholipase activity (Pz) was calculated by dividing the diameter of the colony by the diameter of the colony plus precipitation zone. The Pz was scored as follows: Pz = 1, negative phospholipase activity; Pz = 0.64–0.99, positive

phospholipase activity; Pz ≤ 0.63, very strong phospholipase activity [4].

Proteinase activity

To determine proteinase activity, bovine-serum albumin agar defined by Staib [20]was used. The final pH was adjusted to 4.5. Ten microliters of the previously prepared suspension from each isolate were inoculated onto the test medium. The plates were incubated at 37°C for 48 hours and 72 hours. The enzymatic activity was determined by the formation of a halo around the yeast colony, and measured in terms of the ratio of the diameter of the colony to the total diameter of the colony plus the zone of precipitation (Pz), according to the method described by Price *et al*. [18]. According to this system, Pz = 1.0 indicated that the test strain was negative for proteinase, while a value of Pz ≤ 0.63 signified that the test strain was releasing large amounts of proteinases (strongly positive). Values of Pz between 0.64 and 0.99 indicated positive proteinase activity.

Esterase activity

The esterase activity of the isolates was assessed in Tween-80 agar according to Slifkin [23]. Ten microliters of the previously prepared suspension from each isolate was carefully inoculated on the Tween-80 opacity test medium and incubated at 37°C for 48 hours and 72 hours. This activity was considered to be positive in the presence of a halo viewed with transmitted light around the inoculation site [23]. Test isolates were tested three times.

Biofilm formation

To induce biofilm formation, sterile 96-well microplates were used based on the method of Toledo-Arana *et al*. [21]. Using a loop, one colony of each isolate was placed into tubes containing 2 mL of brain-heart infusion broth (BHIB) medium with glucose (0.25%). The tubes were incubated at 37°C for 24 hours. Subsequently, the suspensions were diluted at a ratio of 1:20 using freshly prepared BHIB. From this final solution, 200 µL was placed into each well of the microplates. After a 24-hour incubation at 37°C, the microplates were rinsed thrice with PBS and then inverted to blot, and 200 µL of 1% crystal violet was added to each well, followed by incubation at room temperature for 15 minutes. After incubation, the microplate was again rinsed thrice with PBS. Next, 200 µL of ethanol (acetone mixture 80:20 w/v) was added to each well. The plates were read at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Biotek EL x 808, Biotek, Winooski, USA) and the OD was recorded for each well. Three wells were used for each strain, and the arithmetical mean of three readings was used in analysis. *Enterococcus faecalis* ATCC 29212 was used as the positive control strain. Sterile BHIB without microorganism was employed as the negative control. The cutoff value was determined as the average OD of the negative control well plus 2 standard deviations (SDs). Samples with an OD higher than the cut off value were considered positive.

Statistical analysis

All statistical analyses were performed using SPSS version 15.0. Normal distributions of the variables were analyzed by visual (histogram and o-probability graphics) and analytic methods (Shapiro-Wilk test). Wilcoxon's test, the Mann-Whitney U test, and Fisher's exact test were used, as the data were ordinal. $P < 0.05$ was considered significant. For the statistical analysis of virulence factors between *C. albicans* and non-*albicans Candida*, the Chi-square test was used.

Table 1. Frequency of virulence factor positivity of the isolated *Candida* strains according to species

	Number of positive strains/total number of strains			
	Biofilm	**Phospholipase**	**Esterase**	**Proteinase**
C. parapsilosis	9/29	2/29*	7/29	9/29‡
C. albicans	5/19	18/19†	5/19	14/19§
C. tropicalis	1/2	0/2	2/2	2/2**
C. glabrata	0/2	0/2	0/2	0/2
Total	**15/52**	**20/52**	**14/52**	**25/52**

*Phospholipase activity was shown to be very strongly positive in 2 of the strains. †Phospholipase activity was shown to be very strongly positive in 16 of the strains and positive in 2 strains. ‡Proteinase activity was shown to be very strongly positive in 5 of the strains and positive in 4 strains. §Proteinase activity was shown very strongly positive in 11 of the strains and positive in 3 strains. **Proteinase activity was shown to be very strongly positive in two strains.

Table 2. Frequency of virulence factors between *C. albicans* and non-*albicans Candida* strains (NAC)

Virulence factors		Strains		Total n = 52 (%)
		***C. albicans* n = 19 (%)**	**NAC n = 33 (%)**	
Biofilm	-	14 (73.7)	23 (65.7)	37 (71.1)
	+	5 (26.3)	10 (30.3)	15 (28.8)
Esterase	-	14 (73.7)	24 (72.7)	38 (73)
	+	5 (26.3)	9 (27.2)	14 (28.8)
Proteinase	-	5 (26.3)	22 (66.6)	27 (51.9)
	+	14 (73.7)	11(33.3)	25 (48)
Phospholipase	-	1 (11.1)	31 (93.7)	32 (61.5)
	+	18 (94.7)	2 (6)	20 (38.4)

- Strains with no virulence factor activity. +Strains with positive or strongly positive virulence factor activity.

Table 3. Number of isolated *Candida* strains with positive (+) or negative (-) virulence factor activity according to the major genotypes

Genotypes	Strain	Biofilm		Esterase		Proteinase		Phospholipase	
		+	-	+	-	+	-	+	-
Genotype A (n = 2)	*C. albicans* (n = 1)	-	1	1	-	-	1	1	-
	NAC (n = 1)	1	-	1	-	1	-	-	1
Genotype B (n = 6)	*C. albicans* (n = 5)	1	4	2	3	3	2	5	-
	NAC (n = 1)	-	1	-	1	-	1	-	1
Genotype C (n = 2)	*C. albicans* (n = 1)	-	1	-	1	1	-	1	-
	NAC (n = 1)	-	1	-	1	-	1	-	1
Genotype D (n = 2)	*C. albicans* (n = 2)	2	-	-	2	2	-	2	-
	NAC (n = 0)	-	-	-	-	-	-	-	-
Genotype E (n = 2)	*C. albicans* (n = 2)	-	2	-	2	2	-	2	-
	NAC (n = 0)	-	-	-	-	-	-	-	-
Genotype F (n = 4)	*C. albicans* (n = 4)	2	2	2	2	3	1	4	-
	NAC (n = 0)	-	-	-	-	-	-	-	-
Genotype G (n = 3)	*C. albicans* (n = 3)	-	3	-	3	3	-	3	-
	NAC (n = 0)	-	-	-	-	-	-	-	-
Genotype H (n = 27)	*C. albicans* (n = 0)	-	-	-	-	-	-	-	-
	NAC (n = 27)	8	19	6	21	7	20	1	26
Sporadic (n = 4)	*C. albicans* (n = 1)	-	1	-	1	-	1	-	1
	NAC (n = 3)	1	2	2	1	3	-	1	2

NAC: Non-*albicans Candida* strains

Results

Of the 52 isolates, 19 (36.5%) were identified as *C. albicans*, while 29 (55.7%) were *C. parapsilosis*, 2 (3.8%) were *C. tropicalis*, and 2 (3.8%) were identified as *C. glabrata. C. parapsilosis* have become more frequently occurring etiological agents for invasive fungal infections in the ICU.

The frequencies of the virulence factor activities of the different *Candida* species are shown in Table 1. When groups were divided according to *C. albicans* and non-*albicans Candida*, the phospholipase, proteinase, esterase activities and biofilm formation were observed in 18 (94.7%), 14 (73.7%), 5 (26.3%), and 5 (26.3%) *C. albicans* and in 2 (6%), 11 (33.3%), 9 (27.2%), and 10 (30.3%) non-*albicans Candida* isolates, respectively (Table 2). The phospholipase and proteinase activities of *C. albicans* isolates were found to be higher than those of non-*albicans Candida* isolates. These differences were statistically significant ($p < 0.05$). The 2 *C. glabrata* isolates were negative for all these virulence factors. Phospholipase activity was shown to be strongly positive in 16 of the *C. albicans* strains. For *C. parapsilosis*, it was shown to be very strongly positive in two strains. Proteinase activity of *C. albicans* was shown to be positive in 3 and very strongly positive in 11 strains. For *C. parapsilosis* strains, it was shown to be positive in 4 and very strongly positive in 5. Proteinase activity was shown to be very strongly positive in 2 *C. tropicalis* strains (Table 1). Although biofilm formation and esterase activities of non-*albicans Candida* strains were higher than those of *C. albicans* strains, these differences were not statistically significant ($p > 0.05$).

Rep-PCR analysis showed the presence of a total of 8 major genotypes: A (3.8%), B (11.5%) (2 subtypes), C (3.8%), D (3.8%), E (3.8%), F (7.7%) (2 subtypes), G (5.8%) (2 subtypes), and H (52%) (2 subtypes). Four of the strains were identified as sporadic (7.7%). Genotype H (52%) was the predominant genotype in this hospital during the study period. Of the 19 *C. albicans* strains, 5 belonged to genotype B, 4 to F, 3 to G, 2 to D, 2 to E, 1 to A, 1 to C, and 1 to a sporadic genotype. Of the 33 non-*albicans Candida* strains, 27 belonged to genotype H, 1 each belonged to genotypes A, B, and C, and 3 were sporadic. None of the 33 non-*albicans Candida* strains belonged to genotypes D, E, F, or G. The genotype H group comprised only *C. parapsilosis* strains for which species it was the predominant genotype (27 of the 29 isolates).

C. albicans strains with high phospholipase and proteinase activities belonged to genotypes B, D, E, F, and G. For *C. albicans*, most strains had high phospholipase and proteinase activity regardless of genotype, and for genotype H, the majority of strains had low virulence factors (Table 3).

Discussion

Candidemia is an increasingly common challenge in hospitalized patients. The general risk factors for *Candida* infections include immunocompromised status, diabetes mellitus, indwelling devices, and intravenous drug use [24]. This infection is most commonly observed in patients in ICUs. *C. albicans* is the predominant cause of candidemia [5]. However, non-*albicans Candida* species such as *C. parapsilosis*, *C. glabrata*, *C. krusei*, and *C. tropicalis* have been seen more frequently as the etiologic agent of candidemia in recent years [6-11]. Our results agree with these findings.

In previous studies, it has been reported that the enzymatic activity of *Candida* spp. may vary depending on the species of isolates. The virulence of *Candida* species is not attributed to a single factor, but to a combination of several factors such as phospholipase, proteinase, esterase activity and biofilm formation [1,4,25].

Phospholipase catalyzes the hydrolysis of phospholipids, which are major components of all cell membranes, and leads to cell lysis. Thus, phospholipase has been considered to be a major virulence factor. Mohan das and Ballal [4] reported that *C. albicans* species isolated from blood produced more phospholipase than did non-*albicans Candida* spp. In three studies conducted in Turkey, phospholipase activity of *C. albicans* strains isolated from blood cultures was found to be positive in 60.3%–100% of strains, while no such activity was detected in non-*albicans Candida* spp. [1,3,14]. In our study, phospholipase activity was observed in 94.7% of *C. albicans* strains. In contrast, only 2 (6%) of the 29 *C. parapsilosis* strains expressed phospholipase activity. *C. tropicalis* and *C. glabrata* strains did not have any phospholipase activity.

Proteinase is responsible for adhesion and tissue damage. It has been reported that more than 90% of *Candida* isolates produce proteinase. Gökce *et al*. [3] found proteinase production at a level of 89.7% in *C. albicans* strains, whereas only 25.8% of non-*albicans Candida* strains were had proteinase activity. Inci *et al*. [1] reported that proteinase activity was detected in 95% of *C. albicans* versus 24% of non-*albicans Candida* strains under aerobic conditions. Similar to the above studies, Mohandas and Ballal [2] reported

that the proteinase-producing capacity of non-*albicans* *Candida* strains was less than that of *C. albicans* (50.45% *versus* 67.34% of strains). In our study, proteinase activity was detected in 14 of the 19 *C. albicans versus* 11 (9 *C. parapsilosis* and 2 *C. tropicalis*) of the 33 non-*albicans Candida* strains, and in none of the *C. glabrata* strains. These differences between *C. albicans* and non-*albicans Candida* strains were statistically significant($p < 0.05$).

The role of esterase enzyme activity in the pathogenesis of candidemia has not been well characterized. In previous studies, it has been reported that both *C. albicans* and non-*albicans Candida* strains show esterase activity [14,26]. However, Akyol and Cerikcioglu [15] reported that esterase activity was not significant in *C. parapsilosis* isolates. Inci *et al*. [1] reported that esterase activity was detected in only 1 of 20 *C. albicans* strains they studied. In the present study, esterase activity was detected in 5 of the 19 *C. albicans versus* 9 (7 *C. parapsilosis* and 2 *C. tropicalis*) of the 33 non-*albicans Candida* strains and in none of the 2 *C. glabrata* strains. These differences were not statistically significant.

One of the most extensively investigated virulence factors of *Candida* spp. is biofilm formation. Infections related to biofilm are difficult to treat, because antimicrobial resistance is very high in biofilm-producing *Candida* strains [27,28]. Previous studies have shown that the biofilm formation rate was higher in non-*albicans Candida* species than in *C. albicans* [3,14,29,30]. Furthermore, this activity was found to be higher in *C. parapsilosis* strains isolated from blood and catheters than in *C. parapsilosis* strains isolated from other body sites [31,32]. In our study, biofilm formation was detected in 10 out of 33 non-*albicans Candida* spp. (9 *C. parapsilosis* and 1 *C. tropicalis*) *versus* 5 of 19 *C. albicans*, and again, in none the *C. glabrata* strains. These differences between *C. albicans* and non-*albicans Candida* strains were not statistically significant.

Molecular typing can provide significant information on nosocomial transmission and helps differentiate endogenous from exogenous infections [33]. In our study, genotyping of strains in terms of rep-PCR based fingerprinting showed the presence of a total of eight major genotypes. Four of the strains were identified as sporadic. Molecular typing of the 19 *C. albicans* isolates yielded seven genotypes. No predominant genotype was found for *C. albicans*. Moreover, the isolation of these strains occurred at time points far apart from one another. Thus, it seems that the origin of candidemia in these patients could be endogenous. Previous studies have reported that *C. albicans* is usually responsible for endogenous infections [16,34]. *C. parapsilosis* strains can cause nosocomial infection in the absence of patient colonization [35,36] and are often isolated from the hands of hospital staff. *C. parapsilosis* therefore causes usually exogenous infections and is the most common cause of catheter-related candidemia [15,37-39]. In our study, molecular typing of the 33 non-*albicans Candida* isolates yielded four genotypes, and three were sporadic. Of the 29 *C. parapsilosis* strains, 27 were shown to belong to the dominant genotype H. The isolation dates of these strains were in proximity. As all patients infected with *C. parapsilosis* had a central venous catheter and stayed in the same adult ICU, it appears that the origin of candidemia in these patients was exogenous and thus a nosocomial infection.

Akyol and Cerikcioglu [15] investigated virulence factors and genotyping of *C. parapsilosis* strains isolated from blood and urine using the random amplified polymorphic DNA PCR (RAPD-PCR) method. One of three different genotypes predominated (genotype II, 90.6%). All of the blood isolates belonged to RAPD genotype II. For this genotype, most strains showed higher proteinase activity and biofilm formation, but none of strains showed phospholipase and esterase activity. The phospholipase, proteinase, esterase, and biofilm activities of this genotype, however, were not found to have statistically significant different activity levels [15]. Gültekin *et al*. [14] investigated the virulence factors (phospholipase, esterase activity and biofilm formation) in 46 *Candida* species isolated from the blood cultures of patients with invasive fungal infections and evaluated the clonal relationships among isolates with RAPD-PCR. In that study, all of the *C. albicans* strains had phospholipase and esterase activity, but they were not biofilm producing. In contrast, all of the *C. parapsilosis* strains were negative for phospholipase and esterase activity, but positive for biofilm formation. The results of RAPD genotyping showed that *C. albicans* strains demonstrated five to eight different patterns, whereas 77.7% of *C. parapsilosis* strains were found to have a single pattern. Thus, they concluded that candidemia caused by *C. parapsilosis* was associated with exogenous spread. In our study, rep-PCR was used for genotyping. The DiversiLab system for DNA fingerprinting is a powerful tool for tracing the source and spread of microbial infection, contamination, or epidemics. Due *et al*. [40] reported that there was an

excellent correlation between restriction fragment length polymorphism (RFLP), multilocus sequence typing (MLST), and the DiversiLab system for molecular epidemiology. In our study, the rep-PCR analysis showed the presence of seven major genotypes for *C. albicans* and one major genotype for *C. parapsilosis* strains. The different genotypes of *Candida* strains showed different virulence activity. Most of the *C. albicans* strains had high phospholipase and proteinase activity, regardles of genotype, and very few had esterase activity and biofilm formation. For the non-*albicans Candida* strains the predominant genotype was genotype H, which did not appear to be correlated with any of the virulence factors studied.

A limitation of our study was the small number of isolates and different species.

Conclusions

The enzymatic activity of *Candida* strains may vary depending on the species of isolates. *C. albicans* strains showed higher phospholipase and proteinase activitiy than did non-*albicans Candida* strains. *C. parapsilosis* have become more frequently etiological agents for candidemia in our ICU, but most of these strains had low virulence. The genotyping of *Candida* strains is important in order to determine the origin of infection. The non-*albicans Candida* spp. were found to be clustered in genotype H, while *C. albicans* strains were of different genotypes. Genotyping showed very low transmission of *C. albicans* strains in patients with candidemia, whereas *C. parapsilosis* strain transmission was high. Because of the small number of *Candida* strains in genotypes, we did not find any correlation between virulence factors and genotypes. Rep-PCR can be used for the determination of the clonal relationships among *Candida* strains and sources of infection.

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