Frequency and distribution of group I intron genotypes of *Candida albicans* colonising critically ill patients

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ABSTRACT

A study is performed to examine the distribution and frequency of 25S rRNA intron genotypes of *Candida albicans* isolated from different anatomical sites of patients in an intensive care unit (ICU) setting. Germ-tube positive *Candida* isolates (n=65) from 65 patients are included and isolates are characterised by 25S intron genotyping, whereby all can be subdivided into four genotypes (A-D). Results demonstrated that there were no significant differences between the frequency and genotype distribution of the *Candida* isolates and the anatomical site of colonisation. Furthermore, analysis of the transposable intron region in the 25S rRNA gene demonstrated equal distribution, regardless of age and anatomical site of isolation (groin, throat, etc.). Therefore, there does not appear to be any selective pressure associated with any anatomical site, resulting in an ecological shift in the frequency of genotypes present. This suggests that *C. albicans* intron genotypes equally colonise those sites of the body examined in this study. Although such an ecological finding as this is interesting, it perpetuates the continued need to find a genotypic typing scheme that helps to identify the source (nosocomial or endogenous) and mode of entry of *C. albicans* into patients in the ICU setting, resulting in *C. albicans* bloodstream infection.

KEY WORDS: Candida albicans.
Candida dubliniensis.
Candidiasis. Genetic techniques.
Polymerase chain reaction.
RNA, ribosomal, 25S.

Introduction

Candida albicans is a common opportunistic pathogen that can be responsible for both superficial infections and invasive disease in susceptible hosts.[1] Many healthy humans are colonised with this organism but only a very small minority develop invasive candidiasis. Therefore, it is of interest to examine whether such invasiveness is due to intrinsic genetic regulation within the organism, host deficiencies, or a combination of both.

Soll[2] suggests that modulation of gene expression could play a crucial role in the transition of this organism from coloniser to pathogen. Therefore, the ability to determine the likelihood of a given colonising *C. albicans* strain becoming invasive would assist clinicians in making a risk-based decision regarding pre-emptive antifungal therapy in susceptible patients.

Recently, several new genotyping methods have been described for *C. albicans*, including multilocus genotyping[3] and short-sequence repeat polymorphism typing.[1] Most recently, Tamura *et al*.[4] described the presence of a special genotype (genotype E) of *C. albicans* isolated from sputum and urine, based on a transposable intron region in the 25S rRNA gene. To date, however, there have been no reports evaluating the relationship between the frequency of intron I genotypes based on polymorphisms within this transposable region and site of isolation from critically ill patients.

The aim of this study is to examine the frequency of genotypes based on molecular rearrangements at the 25S rRNA locus and anatomical site of isolation in a collection of *C. albicans* isolates recovered from critically ill patients.

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Materials and methods

Source of yeast isolates

Germ-tube positive *Candida* isolates (n=65) from 65 critically ill patients were included in the study. Patients included 40 males (age range: <12 months to 80 years) and 25 females (age range: <12 months to 89 years). Specimens taken from colonisation sites were cultured on Columbia blood agar (Oxoid CM0129; Oxoid, Basingstoke, England) supplemented with 5% (v/v) defibrinated horse blood at 37°C for 24 h.

Phenotypic characterisation and antifungal susceptibility testing

All isolates with presumptive yeast colony morphology were plated on to CHROMagar (CHROMagar Microbiology, Paris) at 37°C for 48 h and isolates with typical *C. albicans* and

Table 1. Distribution and frequency of group I intron genotypes of *C. albicans* and *C. dubliniensis* obtained from 65 patients in an intensive care unit by gender, age and specimen source.

		Number (%) of strains of the following genotype			
	Total	A	B	C	D
Sex					
Male	40	29 (72.5%)	6 (15%)	2 (5%)	3 (7.5%)
Female	25	17 (68%)	6 (24%)	2 (8%)	0
Subtotal	65	46 (70.8%)	12 (18.4%)	4 (6.2%)	3 (4.6%)
Age (years)					
0-10	4	3	1		
11-20	3	2	1		
21-30	3	3			
31-40	3	2	1		
41-50	14	10	2	1	1
51-60	9	5	3	1	
61-70	18	15	2	1	
71-80	10	6	2	1	1
81-90	1	1			
Subtotal	65	46	12	4	3
Source					
Throat	23	16	4	1	2
Urine	4	3	1		
Rectum	10	7	2	1	
Sputum	16	10	3	2	1
Endotracheal tube	3	1	2		
Groin	3	3			
Other*	6	6			
Subtotal	65	46	12	4	3

*peritoneal dialysis fluid, central line tip, nasal, pleural fluid and secretions.

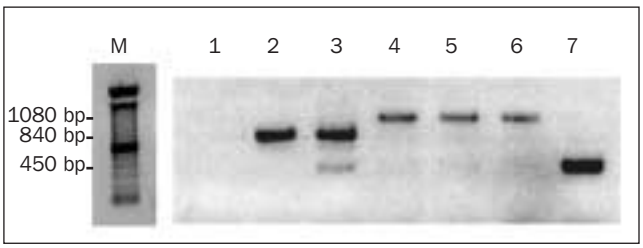

Fig. 1. Banding profiles of genotypes A-D of clinical isolates from various anatomical sites from patients in ICU. M: 100 bp molecular weight marker (Biorad Inc., USA); lane 1: PCR-negative control (molecular grade water; Biowhittaker Inc., USA); lane 2: genotype B; lane 3: genotype C; lanes 4-6: genotype D; lane 7: genotype A.

C. dubliniensis morphological appearance were examined for the production of germ tubes, as described previously.[5] All isolates were tested for susceptibility against fluconazole and flucytosine, as described previously.[6]

Genotypic identification and 25S rRNA intron characterisation
All DNA isolation procedures were carried out in a class II biological safety cabinet in a different room to that used to set up reaction mixes and to that used for post-polymerase chain reaction (PCR) procedures, in order to minimise the production of false-positive results.[7] Where applicable, molecular grade water was employed (Biowhittaker Inc, Maryland, USA; LAL Grade Cat No: W50-100) to reduce contamination.

Genomic DNA from fungal culture material was extracted using an NaOH method, as described previously,[8] without using lyticase solution. Extracted DNA was transferred to a clean tube and stored at -20°C prior to PCR. Amplification of 25S rRNA was carried out, as described previously.[9] Any isolates which yielded genotype D were further characterised by PCR and sequence identification of the ribosomal internal transcribed spacer (ITS) regions, as described previously.[8] During each run, molecular grade water was included randomly as negative controls, and appropriate DNA templates from *C. albicans* and *C. dubliniensis* NCPF 3949 were included as a positive control, as appropriate.

Results

Phenotypic identification of all 65 germ-tube positive isolates was *C. albicans*. Genotypic determination is detailed in Table 1, whereby four profiles (A-D) were obtained in varying frequencies as characterised by gender, age group

Table 2. Minimum inhibitory concentration (MIC) to fluconazole and flucytosine for 65 isolates of *C. albicans* and *C. dubliniensis* isolated from patients in ICU.

	Fluconazole				Flucytosine			
Antifungal susceptibility (μg/mL)	*C. albicans* genotype Aa	*C. albicans* genotype B	*C. albicans* genotype C	*C. dubliniensis* (genotype D)	*C. albicans* genotype A	*C.albican* genotype B	*C. albicans* genotype C	*C. dubliniensis* (genotype D)
MIC range	0.25-4.0	0.25-1.0	0.25-0.5	0.25-0.5	0.25-128	0.25-0.5	0.25-0.5	0.25*
SD MIC	0.57	0.40	0.13	0.14	19.07	0.11	0.13	0*
MIC_{90}	0.55	0.57	0.39	0.41	1.22	0.39	0.39	0.25*

*only one isolate tested against flucytosine

and anatomical site of yeast isolate. There was no statistical difference ($P<0.05$) between distribution of genotypes and age, nor was there any significant difference between distribution of genotype and anatomical site of isolation.

Genotype A was the most frequently isolated type (46/65; 70.8%), followed by genotype B (18.4%), genotype C (16.2%) and genotype D (4.6%). None of the isolates were characterised as genotype E. All genotype D isolates were identified subsequently as *C. dubliniensis* by sequence analysis of rRNA gene loci.. Antifungal susceptibility to fluconazole and flucytosine for all isolates is shown in Table 2, and no statistical difference between genotype and susceptibility for each antifungal tested was found.

Discussion

In this study, a PCR primer pair designed to span the 25S rRNA gene was employed to differentiate isolates of *C. albicans* from various different anatomical sites of ICU patients into four genotypes on the basis of the amplified PCR product length, where genotype A was equivalent to a 450 bp product, genotype B an 840 bp product, genotype C were 450 and 840 bp products and genotype D a 1080 bp product (Fig. 1).[10] The classification of genotypes using this form of PCR relies on the presence of group I introns of varying sizes in the 25S rRNA.

The aim of this study was to determine a correlation between colonisation of a particular body site and *Candida* intron type. Recent data support the hypothesis that the source of yeast infection for patients in ICU is endogenous and is not acquired nosocomially.[11] Therefore, identification of site-specific intron genotypes may help to elucidate potential endogenous sites of entry, where *Candida albicans* is associated with translocation into the bloodstream.

In the present study, all 65 isolates generated a PCR amplicon that could be characterised into one of the existing genotypes (A-D) and no atypical profile types were obtained. This is the first report of the 25S intron analysis of *Candida* isolates from different sites of the body and it demonstrates a high degree of conservation with the 25S rRNA locus, where the majority of *C. albicans* isolates, regardless of anatomical site of isolation, were characterised into genotype A, which did not contain the group I intron. However, this study demonstrated that use of this genotypic assay may be useful in helping to differentiate *C. albicans* from *C. dubliniensis* isolates, without the need for sequence analysis, which may not be readily available at most primary diagnostic laboratories.

Previous studies by Soll *et al*.[12] and De Bernardis *et al*.[13] have suggested that *Candida* isolates from different anatomical sites adapt to their specific location, and more recent work has shown that expression of certain genes associated with virulence is affected by the particular niche occupied.[13] The general belief is that adaptation to anatomical location is important in colonisation, and the ability to detect such adaptation to anatomical sites depends on the use of genotypic typing schemes that have the ability to detect subtle genetic differences in strain type and/or virulence expression, which may not be totally visible by the 25S rDNA intron typing method.

In conclusion, this study demonstrates that analysis of the transposable intron region in the 25S rRNA gene, as defined by the occurrence of four distinct genotypes (A-D), is distributed equally regardless of age or anatomical site of isolation (groin, throat, etc.). Using this phylogenetic typing tool through examination of highly conserved rDNA gene loci, it would appear that there is no site-specific selective pressure that could cause an ecological shift in the frequency of rDNA intron genotypes present. At this level of analysis, this suggests that *C. albicans* intron genotypes colonise those sites of the body examined in this study equally.

Although an ecological finding such as this is interesting, it does not remove the need to find a genotypic typing method with a high discrimination index to identify the source (nosocomial or endogenous) and site of invasion of *C. albicans* among critically ill patients, which may target more hypervariable gene loci and virulence gene loci. □

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