Potential association of specific *Candida parapsilosis* genotypes, bloodstream infections and colonization of health workers' hands

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Abstract

Fungal nosocomial infections continue to be a serious problem among hospitalized patients, decreasing quality of life and adding millions of euros to healthcare costs. The aim of this study was to describe the pattern of fungi associated with the hands of healthcare workers and to genotype *Candida parapsilosis* isolates in order to understand whether their high clinical prevalence stems from endemic nosocomial genotypes or from the real emergence of epidemiologically-unrelated strains. Approximately 39% (50/129) of healthcare workers were positive for yeasts and among 77 different fungal isolates recovered, *C. parapsilosis* was the most frequent (44/77; 57%). Twenty-seven diverse genotypes were obtained by microsatellite analysis of 42 selected blood and hand isolates. Most of the isolates from hands showed a new, unrelated, genotype, whereas a particular group of closely related genotypes prevailed in blood samples. Some of the latter genotypes were also found on the hands of healthcare workers, indicating a persistence of these clones within our hospital. *C. parapsilosis* genotypes from the hands were much more heterogeneous than clinical ones, thus reflecting a high genetic diversity among isolates, which is notably unusual and unexpected for this species.

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Introduction

In the past years, a significantly increased frequency of invasive fungal infections has been repeatedly reported worldwide [1,2] and directly related to the growing numbers of patients with a variety of risk factors, including neoplastic disease, HIV infection, chemotherapy and several other clinical treatments [3]. This expanding complex population of patients raised the spectrum of fungal species causing invasive disease and often also unusual yeast pathogens have been reported infecting humans [4]. However, the most well-known causes of opportunistic mycoses include members of the genera *Candida*, *Cryptococcus* and *Aspergillus* [5], and among the species belonging to the *Candida* genus, *Candida albicans* remains the most commonly isolated agent of candidiasis [6,7]. Nevertheless, in recent years, it has been observed that there has been a dramatic increase in infections caused by other species that are commonly indicated as 'non-*albicans*' *Candida* species (NAC). Among the different NAC species, *Candida parapsilosis* has emerged as one of the most important causes of candidaemia, especially in Latin America, Asia and Europe, and it is frequently isolated from neonates in intensive care units (ICUs) [8,9]. Most of the infections are due to exogenous acquisition of the fungus, which shows a marked tendency to colonize hospital environments, including central venous catheters (CVCs) and several other medical devices [8,10].

Furthermore, *C. parapsilosis* represents the most frequently isolated fungus from the hands of healthy people and healthcare workers (HCWs), thus highlighting the importance of hand washing to prevent the horizontal transmission of this pathogen [8,10].

In this study we present data obtained in a retrospective analysis of bloodstream infections (BSIs) over a 4-year period of time in three ICUs, in an Italian University Hospital. As a matter of fact, the number of *C. parapsilosis* candidaemias that occurred between 2008 and 2012 was significantly higher than data reported in other Italian and European studies [9,11,12], thus suggesting a possible outbreak of nosocomial infection. Therefore we decided to investigate the fungal pathogens associated with the hands of HCWs to understand if our epidemiological data were affected by a defect in the hand hygiene compliance of HCWs or whether the candidaemias were due to the real emergence of epidemiologically unrelated *C. parapsilosis* strains.

Materials and Methods

The retrospective study (2008–2012) of BSIs was based on clinical records of patients admitted to three ICUs of the University Hospital in Messina: General (GICU), Cardiovascular (CICU) and Neonatal (NICU). All blood samples obtained from these wards were processed using the BACTEC 9050 system (Becton Dickinson, Milan, Italy) and positive samples were then subcultured onto Sabouraud agar with antibiotics. Yeasts were identified phenotypically using the RapID™ system (Remel Inc., Lenexa, KS, USA).

Isolation and identification of yeasts from hands of HCWs

This study was approved by the ethical committee of the University Hospital of Messina.

Between March and October 2012, samples were taken from the hands of 129 HCWs working in the three ICUs (72 GICU, 41 NICU and 16 CICU) using the broth bag method [13].

Fungal species were initially, presumptively, identified by morphological and biochemical tests and subsequently confirmed using species-specific PCR-based methods or DNA sequencing.

Molecular analysis

Sequencing of the ITS1-5,8S-ITS2 region [14] was carried out to confirm the identity of fungal species presumptively identified by phenotypic tests. All yeasts were identified in this way, except *C. albicans* and *Candida glabrata*, which were confirmed by amplification and detection of species-specific gene fragments [15,16].

Analysis of hypervariable microsatellite loci was used to evaluate genetic relatedness among 42 *C. parapsilosis* isolates [17]. All isolates were randomly selected from those of blood origin (21 isolates) and those associated with hands of HCWs (21 isolates). In addition, eight different microsatellite genotypes [18], including the allelic profile of the epidemiologically unrelated strain *C. parapsilosis* ATCC22019, were also considered and included for cluster analysis (Table 1). Before genotyping, all isolates were examined by ITS sequencing to exclude the cryptic presence of *Candida orthopsilosis* and *Candida metapsilosis* [8].

Results

During the 4-year study period, a total of 761 BSIs were observed in our three ICUs and 149 (~20%) were caused by different *Candida* species. *C. parapsilosis* was the most frequently isolated species (89/149; 59.7%) followed by *C. albicans* (33/149; 22.1%), *C. glabrata* (7/149; 4.7%), *Candida tropicalis* (6/149; 4%), *Candida lambica* (5/149; 3.3%), *Candida lusitaniae* (5/149; 3.3%) and *Candida krusei* (4/149; 2.7%).

Fifty (38.7%) out of 129 HCWs examined were positive for yeasts. The percentages of nurses, stretcher-bearers and postgraduate medicine students whose hands were colonized by yeasts were similar, ranging from 35.7 to 36%, whereas the number of physicians positive for yeasts was slightly higher (46.4%). Healthcare personnel working in CICUs were the most colonized (11/16; 68.8%), while the number of personnel working in NICUs and GICUs positive for yeasts was 34.1% (14/41) and 34.7% (25/72), respectively.

Seventy-seven isolates, corresponding to 12 different species, were found colonizing the hands of HCWs. The majority belonged to the *Candida* genus and *C. parapsilosis* was the most commonly isolated species (44/77; 57%). No *C. orthopsilosis* or *C. metapsilosis* isolates were found.

The prevalence of *C. albicans* and *C. glabrata* in these kinds of samples was 8% (6/77) and 4% (3/77), respectively, and no closely related species were recovered. However, ITS sequencing identified a number of other well-known pathogens, including unusual species that have previously been linked to nosocomial infections [20–24]. Among these, *C. tropicalis* and *C. krusei* were both recovered at rather low frequencies (1/77; 1% each), whereas the prevalence of the species, generally not associated with nosocomial infections, was quite high (22/77; ~29% in total). *Saccharomyces cerevisiae* and *Yarrowia lipolytica* were the most isolated species (6/77; ~8% each), followed by *Candida guilliermondii* (4/77; 5%), *C. lambica* (2/77; ~3%), *C. lusitaniae* (2/77; ~3%), *Debaryomyces hansenii* (1/77; 1%) and *Blastoschizomyces capitatus* (1/77; 1%).

TABLE 1. Microsatellite genotyping analysis results of selected clinical and hand *C. parapsilosis* isolates

Genotype	Microsatellites loci				Isolate code	Origin[a]
	CP1	CP4	CP6	B5		
—	244/250	306/306	292/292	132/132	ATCC-22019[b]	ATCC[b]
Nicu-1	224/244	368/368	268/320	126/126	CP-1586	Ref. [19]
					CPN001; CPN002; CPN003; CPN004	NICU
					CPaG001; CPaG002; CPaG003	GICU
					CaPC001; CaPC002; CaPC003	CICU
					CPH-108; CPH-100; CPH-112	Hands
Nicu-2	224/244	368/368	320/320	126/126	CP-4372	Ref. [19]
					CPN005; CPN006	NICU
					CPH-75B	Hands
Nicu-3	224/244	370/370	320/320	126/126	CP-4501	Ref. [19]
					CPH-114	Hands
Nicu-4	224/244	368/368	268/268	128/128	CP-4303	Ref. [19]
					CPaG006	GICU
Nicu-5	244/244	350/350	270/270	140/144	CP-3265	Ref. [19]
					CPaG007	GICU
Nicu-6	242/242	350/350	288/288	134/138	CP-8736	Ref. [19]
					CPH-40	Hands
Nicu-7	244/264	386/386	286/308	114/114	CP-8804	Ref. [19]
					CPN007	NICU
Gicu-1[c]	246/246	348/364	272/272	138/138	CPaG004[d]	GICU
Gicu-2[c]	246/246	346/355	272/272	138/138	CPaG005[d]	GICU
Cicu-1[c]	246/246	364/368	318/318	130/134	CaPC004	CICU
Cicu-2[c]	242/238	302/302	262/282	132/134	CaPC005	CICU
Cicu-3[c]	246/264	364/382	286/308	124/124	CaPC006[e]	CICU
Cicu-3	246/264	364/382	286/308	124/124	CaPC007[e]	CICU
HN-1[c]	222/264	302/302	266/286	114/116	CPH-123	Hands
HN-2[c]	250/264	364/392	308/310	120/120	CPH-125R	Hands
HN-3[c]	242/244	320/350	272/272	128/128	CPH-127B	Hands
HN-4[c]	224/244	368/368	268/336	122/122	CPH-131	Hands
HN-5[c]	224/244	302/306	258/268	122/124	CPH-144	Hands
HN-6[c]	226/266	392/392	268/268	126/130	CPH-16	Hands
HN-7[c]	244/264	364/364	268/320	124/124	CPH-36	Hands
HN-8[c]	224/244	368/368	248/320	106/106	CPH-116V	Hands
HN-9[c]	244/244	302/302	266/316	108/108	CPH-47	Hands
HN-10[c]	242/244	302/302	290/292	114/122	CPH-50	Hands
HN-11[c]	226/246	364/385	304/320	142/146	CPH-67	Hands
HN-12[c]	226/246	276/276	268/290	124/124	CPH-69	Hands
HN-13[c]	226/246	364/388	320/332	142/148	CPH-83	Hands
HN-14[c]	226/246	390/390	268/290	130/130	CPH-9	Hands
HN-15[c]	246/246	364/364	294/322	112/112	CPH-93	Hands

[a]NICU, Neonatal Intensive Care Unit; GICU, General Intensive Care Unit; CICU, Cardiovascular Intensive Care Unit. [b]This strain is from the American Type Culture Collection (ATCC).
[c]New genotypes.
[d]Same patient (GICU).
[e]Same patient (CICU).

Considerable discrepancies were observed between results obtained using phenotypic methods of identification and those achieved by molecular methods. Eleven out of 77 isolates (14%) from hands were misidentified by the RapID™ system and *C. parapsilosis* was commonly confused (7/11; ~64%) with *Candida famata*, *C. guillermondii*, *Kluyveromyces sp.*, *C. lusitaniae*, *D. hansenii* and *C. lambica*. The remaining isolates, three *Y. lypolitica* and one *S. cerevisiae*, were misidentified as *Candida zeylanoides* and *C. guillermondii*, respectively.

Results obtained by multilocus-microsatellite genotyping of 42 selected *C. parapsilosis* isolates are shown in Table 1 and Fig. 1. Twenty-seven genotypes were obtained by microsatellite analysis. Thirteen isolates out of 42 (~31%) shared the same allelic profile as the CP-1586 isolate, which corresponds to the so-called genotype Nicu-1 [18]. The majority of these clones were recovered from blood specimens (10/21; ~48%)

and only three (3/21; 14%) were obtained from hands (CPH-108 (NICU); CPH-100 (CICU); CPH-112 (NICU)) (Table 1). However, microsatellite analysis revealed the presence and diffusion of other well-known genotypes (Nicu-2, Nicu-3, Nicu-4, Nicu-5, Nicu-6 and Nicu-7), which were initially detected only in NICU patients [18]. Also in this case, although to a lesser extent than clone Nicu-1, some of them (Nicu-2, Nicu-3 and Nicu-6) were also found on the hands of HCWs (Table 1; Fig. 1).

Except for clinical isolates CaPC006/CaPC007 from CICU and CPaG004/CPaG005 from GICU, the remaining genotypes were genetically quite diverse and heterogeneous and there was no evidence of cross-transmission of these isolates (Table 1 and Fig. 1).

Most of the *C. parapsilosis* isolates from hands (15/21; ~71%) showed a new, unrelated, genotype, whereas only five new
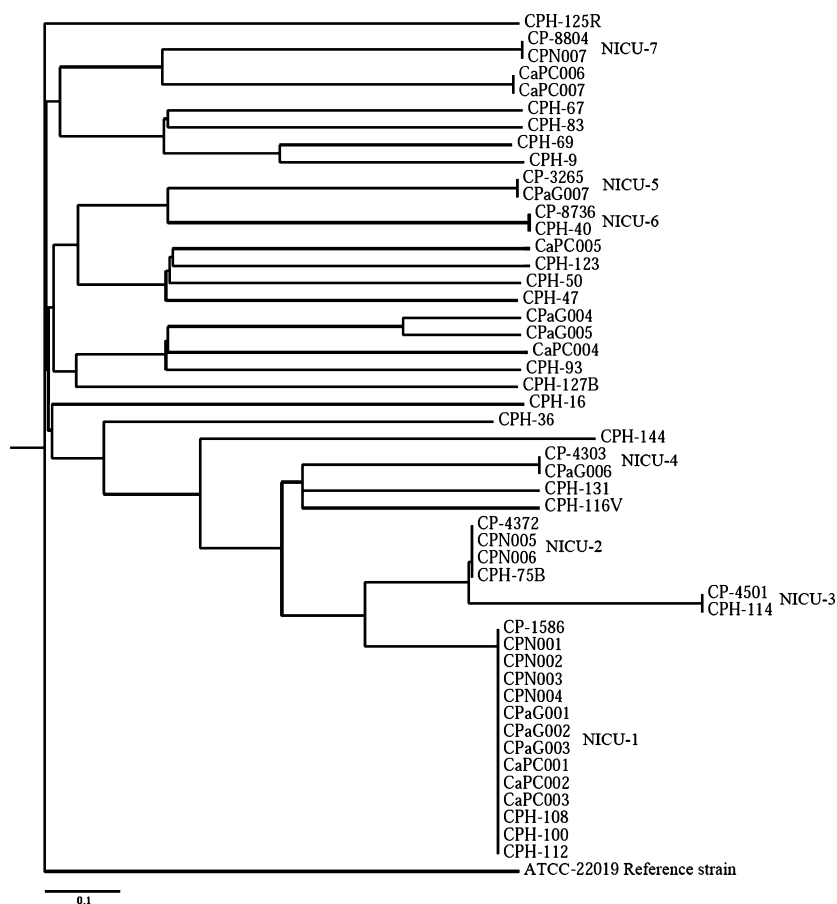
FIG. 1. Cluster analysis of microsatellite data obtained in this study. The origin of the isolates is shown in Table 1.

genotypes (5/21; ~24%) were obtained from blood isolates (Table 1).

The clinical isolates with identical (CaPC006/CaPC007) or highly related (CPaG004/CPaG005) genotypes (Fig. 1) were recovered from blood and CVC tips of the same patient (Table 1) but their genotypes were not found among those colonizing HCWs' hands (Fig. 1).

Discussion

Globally, in Italy, *C. albicans* and non-*albicans* species caused around 50% of BSIs each [12,19,25]. *C. parapsilosis* represents often the most frequently isolated species, especially in southern regions where it accounts approximately for 60% of all infections caused by NAC species [25,26]. This is perfectly in accordance with our epidemiological data, which showed that 59.7% of BSIs were caused by this species. Reasons for this epidemiological shift are multifactorial [10,27], but it is also possible that the recent European economic crisis, which involved mainly the countries of the Mediterranean area, may account, at least in part, for this phenomenon. In fact, a recent

report on European healthcare systems (Euro Health Consumer Index, 2013; www.healthpowerhouse.com) showed that Mediterranean states such as Italy, Spain and Greece still have medium-low quality hospitals with a high occurrence of nosocomial-acquired infections. Therefore inadequate financial support along with a reduction of the hospital staff (www.hope.be) could adversely affect basic infection control measures.

In this study, *Candida* species were among the most common pathogenic fungi found in HCWs' hands, where a ratio of 9.5:1 for NAC species vs. *C. albicans* was observed. Furthermore, it is also important to underline that the pattern of fungal pathogens associated with HCWs was quite heterogeneous, with several species detected.

Among *Candida* spp. isolated from hands, the occurrence of *C. parapsilosis* was exceptionally high (57%) and quite similar to that reported from clinical samples (59.7%). In addition, some *C. parapsilosis* genotypes recovered from hands were also found in blood samples, thus suggesting that the high number of infections caused by *C. parapsilosis* might be, in part, attributable to horizontal transmission of this species by hospital staff. In fact, the genotyping data obtained suggest a mechanism of persistent circulation of several epidemic clones

within our hospital. These clones, especially Nicu-1, were originally found to be prevalent in blood cultures from NICU patients [18] but microsatellite data revealed that they have also scattered sparsely throughout the rest of our hospital, thus representing a serious and persistent threat for critically ill patients.

Previous studies have already shown that single *Candida* isolates can smoulder, within the same hospital or ICU, for years and cause a number of temporally associated infections [28]. In agreement with this, and on the basis of previous and current genetic data, we can state that the Nicu-1 genotype caused hospital infections for several years [18,29]. In addition, it was reported that some genotypes may evolve over time by spontaneous variations in one microsatellite marker, thus generating clonal complexes of closely related genotypes [30]. This suggests that isolates with genotype Nicu-1, Nicu-2 or Nicu-3 could be grouped into a large clonal complex that is clearly associated with blood samples and probably spread within the hospital environment through contamination of HCWs' hands (Fig. 1). This group of isolates was responsible for the majority of BSIs in our ICUs and their presence on the HCWs' hands is consistent with previous reports that have implicated this body site as the main source of nosocomial infections [31,32]. However, *C. parapsilosis* genotypes obtained from the hands were much more heterogeneous than clinical ones, thus reflecting a high genetic diversity among these isolates, which is notably unusual and unexpected for this species [33,34]. Moreover, although Tavanti *et al.* [34] showed that different environments have no significant effect upon genetic variability of *C. parapsilosis*, our data instead revealed a peculiar tropism for hands of several genetically unrelated isolates.

These observations should encourage further studies in order to evaluate genetic diversity of *C. parapsilosis* using microsatellite analysis, which represents an excellent tool for investigations of outbreaks, even if some authors suggested that it would not be useful for genetic relatedness studies due to the inherently unstable nature of microsatellites loci over time [34].

In conclusion, this study confirms that candidaemia in ICU patients is caused predominantly by strains colonizing HCWs' hands and therefore the implementation of surveillance programmes could increase knowledge of this problem among HCWs and increase their awareness regarding the correct use of gloves and hand washing.

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Transparency Declaration

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