Molecular surveillance of healthcare-associated *Candida* infections in a rehabilitation centre for patients with severe acquired brain injuries

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Today, infections caused by fungi represent a very important and neglected public health problem as evidenced by over 1.5 million of people supposed die annually worldwide [1]. This mortality rate currently exceeds that of other serious infectious diseases, such as malaria (445.000 deaths in 2016) or HIV/AIDS (1 million deaths in 2016) even if the extent of the global burden of fungal infections appears to be considerably much wider (www.who.int; www.gaffi.org). The impact of these infections on the economy of the countries and their health systems is also enormous, with billions of euros/dollars spent each year because of the burden of healthcare-associated infections (HAIs). In Italy, *Candida* species account for almost 10% of all ICU-acquired bloodstream infections (BSI) with *C. albicans* being the most frequently encountered species followed by *C. parapsilosis* and *C. glabrata*, *C. krusei* and *C. tropicalis* [2]. However, several Italian studies have shown significant regional differences in the prevalence of pathogenic *Candida* spp., and often non-albicans species (NAC) cause ~50% of the *Candida* BSI or even outrank *C. albicans* in some hospitals, especially in those of the southern regions [3-5].

The aim of this study was to describe the genetic pattern, and population structure, of different pathogenic *Candida* species recovered from blood samples, central venous catheters (CVCs), hands of healthcare workers (HCWs) and hospital environments (air, medical equipments and various surfaces) in one of the most important hospitals of Southern Italy in the treatment of patients with severe acquired brain injuries.

Materials and Methods

This study was conducted in the IRCCS Centro Neurolesi "Bonino-Pulejo" hospital, Messina, Italy. All *Candida* isolates were initially identified using phenotypic methods and subsequently, subjected to molecular identification using species-specific PCRs and/or ITS-sequencing.

Multilocus sequence typing (MLST) method was used for *C. albicans*, *C. glabrata* and *C. tropicalis* genotyping (<https://pubmlst.org>) (Table 1-3).

Microsatellites genotyping was used to evaluate genetic relatedness among our *C. parapsilosis* isolates. Four microsatellites markers (CP1, CP4, CP6, B5) were amplified and analyzed as described by Sabino et al., 2010 [6].

Table 2. *C. glabrata* isolates and multilocus sequence typing profiles generated using seven loci of the MLST scheme.

N° Isolate (Source)	FKS	LEU2	NMT1	TRP1	UGP1	URA3	ST
6 (Environmental)	2	5	7	5	1	2	6
4 (Clinical)	2	5	7	5	1	2	6
3 (Environmental)	8	4	3	5	1	2	10
1 (Environmental)	5	7	8	7	3	6	3

Table 1. *C. albicans* isolates and multilocus sequence typing profiles generated using seven loci of the MLST scheme.

Isolates	AAT1a	ACC1	ADP1	MPIb	SYA1	VPS13	ZWF1b	DST
IRCCS12	62	12	21	1	42	194	4	3376*
IRCCS13	2	7	8	4	7	10	8	3377*
IRCCS16	70	14	8	4	7	10	8	656
IRCCS: 17; 18; 19	2	2	5	2	2	6	5	277
IRCCS20	2	3	5	2	2	6	20	3378*
ALOG1V	2	5	5	9	2	24	5	185
AD2LAV	3	3	21	1	34	30	4	3379*
B1SUAP20	70	14	21	4	7	10	8	3380*
AD5COM12	60	13	21	1	7	11	12	3381*
BINF1V	2	5	5	2	2	6	5	69
STPA7	70	14	8	4	42	3	22	3382*
STPA17	33	22	6	18	64	53	15	3383*
ASUAPLET17	62	13	21	1	7	11	12	3384*
B1S26; B4SD204	3	14	8	4	7	3	8	3385*
B3SD104VE	3	4	8	26	7	4	8	3386*
B3SD204VE	3	14	8	4	7	10	8	3387*
B4SD207	96	7	32	26	2	61	48	3388*
BINF1VE	3	5	5	2	2	6	5	184

* New DST

Results

Twenty-six blood and 28 CVCs *Candida* isolates were examined in this study. Two hundred-eight additional isolates (161 collected from ward environments and 47 from HCWs), were also included. *C. parapsilosis* (199/262; ~76%) was the most frequently isolated species followed by *C. tropicalis* (28/262; ~11%), *C. albicans* (21/262; 8%) and *C. glabrata* (14/262; ~5%). We found a total of 28 microsatellites genotypes among 66 random selected (genotyped) *C. parapsilosis* isolates but only 1 genotype was shared by clinical and HCWs/environmental isolates (data not showed). MLST analysis of *C. albicans*, showed 18 different genotypes of which 13 (~72%) were completely new to the central MLST database (Table 1). Most of these new DSTs were found to colonize mainly hospital environments.

Genotyping of *C. glabrata* isolates showed the presence of only 3 diverse genotypes (Table 2). The genotype ST6 was the most frequent and was found in both clinical and environmental isolates. For *C. tropicalis*, a total of 8 different DSTs were recovered (Table 3). Interestingly, 6 (DSTs: 747-51 and 759) of these 8 genotypes (75%) were novel to the *C. tropicalis* MLST database and one genotype (DST747) was the most frequently observed (12/28 isolates; 42.9%) followed by the genotype DST333 (6/28 isolates; 21.4%). The goeBURST analysis showed that 4 clonal clusters (CC2, CC10, CC11 and CC33) contained all the DSTs found in this study. Four genotypes (DSTs: 747, 748, 749, 759) composed a single well-defined Italian clonal cluster (CC33) with the most commonly recovered type, the DST747, as putative founding genotype (Fig. 1).

Table 3: *C. tropicalis* isolates and multilocus sequence typing profiles generated using six loci of the MLST scheme.

PT	Isolate n°	Sample	MLST loci						DST
			ICL1	MDR1	SAPT2	SAPT4	XYR1	ZWF1	
P1	IRCCS-1	Blood	17	93	29	83	138	30	747
P1	IRCCS-2	PICC	17	93	29	83	138	30	747
P2	IRCCS-3	Blood	1	44	12	7	94	22	333
P3	IRCCS-7	Blood	17	93	29	82	138	30	748
P3	IRCCS-8	PICC	17	93	29	82	138	30	748
P4	IRCCS-14	Blood	1	17	2	14	53	3	750
P5	IRCCS-15	Blood	17	93	29	83	138	30	747
P6	IRCCS-23	Blood	17	93	29	83	138	30	747
P6	IRCCS-24	PICC	17	93	29	83	138	30	747
P7	IRCCS-28	Blood	1	7	3	10	9	3	751
P7	IRCCS-29	PICC	1	7	3	10	9	3	751
P8	IRCCS-31	Blood	17	93	29	83	138	30	747
P8	IRCCS-32	PICC	17	93	29	83	138	30	747
P9	IRCCS-37	Blood	17	93	29	83	138	30	747
P9	IRCCS-38	PICC	17	93	53	83	138	30	749
P9	IRCCS-39	PICC	17	93	29	83	138	30	747
P10	IRCCS-40	Blood	1	44	12	7	94	22	333
P10	IRCCS-42	Blood	1	44	12	7	94	22	333
P10	IRCCS-43	Blood	1	44	12	7	94	22	333
P11	IRCCS-46	Blood	1	17	2	14	100	3	359
P11	IRCCS-47	PICC	1	17	2	14	100	3	359
P12	IRCCS-51	Blood	17	93	29	83	2	30	759
P12	IRCCS-52	PICC	17	93	29	83	2	30	759
--	B4BR3	DH	1	44	12	7	94	22	333
--	C1T1V	BT	17	93	29	83	138	30	747
--	ALOG1	HCW	1	44	12	7	94	22	333
--	OSS1C-R	HCW	17	93	29	83	138	30	747
--	IRXLP2	BH	17	93	29	83	138	30	747

PT= Patient; DST= Diploid sequence type assigned by *C. tropicalis* MLST database; PICC= Peripherally inserted central catheter; DH=Door handle; BT= Bedside table; HCW= Healthcare Worker; BH= Bed handles.

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Fig. 1: goeBURST clonal clusters CC2, CC10, CC11 and CC33 containing all the DSTs (in red) found in this study. For the CC2 the putative founding genotype is indicated in green. The size of each DST reflects the number of isolates with that DST in the input data set used for the analysis.

Conclusions

Recent epidemiological data showed that in Italy candidemia is more common than in other European countries [7]. In the southern regions, a dramatic increase of BSI caused by NAC species has also been recently observed [3-5] and data from this study confirmed this trend. *C. parapsilosis* and *C. tropicalis*, appear to be the most common NAC species and outrank *C. albicans* in our and other nearby Sicilian hospitals [4].

In conclusion, this study also shows that the population structure of pathogenic *Candida* species is far from being fully elucidated as its complexity increases as different categories of patients and geographic areas are examined.

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