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Epidemiological investigation of *Candida* species causing bloodstream infection in pediatric small bowel transplant recipients

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Abstract
Small bowel transplantation (SBT) can be a life-saving medical procedure. However, these recipients experience high risk of bloodstream infections caused by *Candida*. This research aims to characterize the SBT recipient gut microbiota over time following transplantation and investigate the epidemiology of candidemia in seven pediatric patients. *Candida* species from the recipients' ileum and bloodstream were identified by internal transcribed spacer sequence and distinguished to strain by multilocus sequence typing and randomly amplified polymorphic DNA. Antifungal susceptibility of bloodstream isolates was determined against nine antifungals. Twenty-two ileostomy samples harbored at least one *Candida* species. Fungemia were caused by *Candida parapsilosis*, *Candida albicans*, *Candida glabrata*, *Candida orthopsilosis* and *Candida pelliculosa*. All but three bloodstream isolates showed susceptibility to all the antifungals tested. One *C. glabrata* isolate showed multidrug resistance to itraconazole, amphotericin B and posaconazole and intermediate resistance to caspofungin. Results are congruent with both endogenous (*C. albicans*, *C. glabrata*) and exogenous (*C. parapsilosis*) infections; results also suggest two patients were infected by the same strain of *C. parapsilosis*. Continuing to work towards a better understanding of sources of infection—particularly the exogenous sources—would lead to targeted prevention strategies.

Keywords: candidemia, fungemia, microbiota, mycobiota

1 Introduction

Candida species colonize the gastrointestinal and reproductive tract and skin of most individuals and are among the most common causes of hospital-acquired bloodstream infections in the United States.[1,2] In healthy persons, carriage of *Candida albicans* is estimated at 30%-60%. [3] However, *Candida* species can also act as opportunistic pathogens that are capable of infecting a broad range of body sites, especially when host resistance mechanisms are compromised. Candidemia, when *Candida* gains access to the bloodstream, is a life-threatening condition, with mortality rate estimates of nearly 50%,[4] especially in immunocompromised patients.

Solid organ transplant (SOT) recipients present a unique combination of risk factors that may predispose them to candidemia, including prolonged administration of immunosuppressive therapies, repeated courses of broad-spectrum antibiotics, hemodialysis, various surgical procedures and presence of central venous catheters.[5,6] SBT recipients evince a higher incidence of allograft rejection than other SOT recipients, due to the high immunogenicity of the bowel, and therefore require more potent immunosuppression therapy.[7] During episodes of allograft rejection or breach of the

gastrointestinal tract during surgery, breakdown of the gut epithelial barrier occurs, which can lead to the translocation of enteric organisms from the recipient or donor bowel into the bloodstream or peritoneum.

Small bowel transplantation is considered a last-resort treatment option for pediatric patients with extreme short bowel syndrome or those suffering life-threatening complications from parenteral nutrition. Bloodstream infections are common complications following intestinal transplant and represent a major cause of morbidity and mortality among SBT recipients, with up to 70% of pediatric SBT recipients developing bloodstream infections.[8] In particular, pediatric SBT recipients experience an increased incidence of candidemia, with up to 25% of recipients developing candidemia—the most common fungal bloodstream infection in this population.[9]

Strains implicated in candidemia may be of endogenous or exogenous origin.[10] The donor and remnant recipient bowel may each harbor commensal fungi,[6] and donor organs may additionally become infected during hospitalization of the donor or during procurement and preservation of the organ.[10,11] There is increasing evidence for *Candida* infections originating from the hospital environment and personnel, contaminated implanted medical devices, or from the skin.[12-14] The ability of *Candida* to produce biofilms on catheter instruments is a common source of candidemia episodes, as these give the yeast direct access into the bloodstream upon insertion.[15]

Antifungal prophylaxis is commonly administered in the first weeks after surgery in SBT recipients. However, extensive use of antifungal prophylaxis can select for resistant *Candida* strains. For instance the recent clinical emergence of azole-resistant species (*Candida glabrata* and *Candida krusei*) is likely due to widespread use of fluconazole and other antifungal agents.[16] In addition, an overall shift towards non-*C. albicans* species commonly associated with fungemia has been observed,[17] possibly related to the extensive use of antifungal prophylaxis.

In the present work, we defined the gut mycobiota of SBT recipients and genotyped ileum and bloodstream *Candida* isolates in attempt to infer the source of infection, and determine the distribution of isolates within and between patients. Isolates were also assessed for antifungal susceptibility. Overall, the data obtained in this study will contribute to the knowledge base of *Candida* epidemiology and infections acquired by SBT recipients and will also help design future studies.

2 Materials and methods

Seven pediatric short bowel syndrome patients who underwent SBT and later developed candidemia at the University of Nebraska Medical Center (UNMC) were included in this study. The patient cohort comprised three females and four males with a mean age of 23.1 months (median: 20 months; range: 16-37 months). UNMC is a premier facility for SBT, conducting approximately 20 transplants per year. Ileostomy and blood samples (Table 1) were collected from recipients under UNMC Institutional Review Board protocol #417-02, and informed consent was received from the caregivers of all patients. Candidemia was diagnosed on the basis of at least one sample isolated in pure culture from the blood, which was then identified by the API 20 C test (BioMérieux, Marcy l'Etoile, France). Multiple ileostomy samples were collected post-transplant during hospitalization from each patient and stored at −80°C until processing.

To obtain pure culture isolates from ileostomy samples, approximately 250 µL of ileal effluent was grown at 37°C in liquid media (yeast nitrogen broth [YNB; 6.7 g/L yeast nitrogen base, 10 g/L glucose, 0.76 g/L L-asparagine, 50 mg/L chloramphenicol, 20 mg/L gentamicin sulfate], and yeast extract peptone dextrose broth [YPD; 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 50 mg/L chloramphenicol, pH 5.6]) to restore cells that may have been damaged by up to 10 years storage at −80°C with possible exposure to freeze-thaw cycles and to increase the volume available for plating. Volumes of 100 µL were plated after 24, 48 and 72 hours on YPD agar (supplemented with 50 mg/L chloramphenicol), CHROMagar Candida (Paris, France), potato dextrose agar (PDA, Oxoid; supplemented with 50 mg/L chloramphenicol) and dichloran rose bengal chloramphenicol agar (DRBC; 10 g/L glucose, 5 g/L peptone, 1 g/L monopotassium phosphate, 0.5 g/L magnesium sulfate, rose bengal 0.025 g/L, dichloran 0.002 g/L chloramphenicol 0.10 g/L). Multiple liquid and solid media were used to maximize the chances of isolating fungi. Plates were incubated at 37°C and monitored daily for 2 weeks. Multiple colonies from each plate were further isolated on the basis of color and morphology.

DNA was extracted from yeast cultures following the methods of Harju et al.[18] DNA from each ileostomy sample and pure culture isolate was selectively amplified by PCR using fungal-specific primers ITS1-F and ITS4 as described in Suhr et al.[19] To survey the gut mycobiota, DNA was extracted directly from ileostomy effluent following the protocol of Oh et al.[20]

PCR products from ileostomy effluent were cloned using the pGEM®-T Easy Vector System Kit (Promega, Lyon, France) following the manufacturer's instructions. For each sample, 25 colonies negative for β-galactosidase activity were evaluated and those with distinct *Eco*R1 patterns were sequenced on an ABI 3730xl platform at Michigan State University's Research Technology Support Facility (East Lansing, MI, USA). Fungal sequences were identified by sequence homology using nucleotide BLAST against the UNITE curated fungal ITS sequence database[21] and the curated FUNCBS database, as accessed through the Centraalbureau voor Schimmelcultures (http://www.cbs.knaw.nl/Collections/BioloMICSSequences.aspx?file=all ; accessed September 2014). Sequences were deposited as GenBank accessions #KU987836-KU987907.

All *C. albicans* and *C. glabrata* ileostomy and bloodstream isolates were typed by MLST as described previously.[22,23] After amplification of each fragment, PCR products were purified using the Wizard SV Gel and PCR Clean-up Kit (Promega, Madison, WI, USA). Subsequently, amplicons were sequenced in both directions using the same primers used for PCR. Sequences were aligned and assembled using

Table 1. Outline of patients, sample collection, species identification, RAPD and MLST genotypes

Patient	Age at transplant (mo)	Sex	Sample[a]	Days posttransplant	Ileal *Candida*[b]	Ileal fungi (non-*Candida*)	Blood isolate	RAPD genotype[c]	MLST genotype
P32	28	F	32_I_1	390	CA	Acr, EN, PR			
			32_B_1	400			CP[d]	G	n/a
P33	37	F	33_I_1	532	CA, CK	PR			
			33_I_2	579	CA[d], CK			A	2854
			33_B_1	587			CA[d]	A	2854
			33_I_3	755	CA, CG, CK[d], CT[d]				
P39	23	M	39_I_1	64	CG[d]			D	3
			39_B_1	187			CG[d]	D	3
			39_I_2	201	CG[d]			D	3
P47	20	F	47_I_1	12		EN, PR			
			47_I_2	23	CP	Deb, PR, Tri			
			47_I_3	27	CG[d], CP			E	2
			47_B_1	29			CP[d]	H	n/a
			47_I_4	30	CG[d], CP[d]	PR		CG-E, CP-G	CG-2, CP-n/a
			47_I_5	34	CG[d], CP	PR		E	2
			47_B_2	35			CP[d]	H	n/a
			47_I_6	41	CG				
			47_I_7	48	CG[d]			E	2
			47_I_8	56	CG				
			47_I_9	61	CG[d], CP			E	2
P62	16	M	62_I_1	53		PR			
			62_I_2	61	CA	Fus, PR			
			62_B_1	69			WA[d]	n/a	n/a
			62_I_3	72	CA, CT	Deb, Fus, PF, PR			
			62_I_4	103	CA, CP[d]			G	n/a
			62_I_5	138	CA PR				
P74	20	M	74_I_1	254	CA	PR			
			74_I_2	274		PR			
			74_B_1	282			CP[d]	G	n/a
			74_I_3	357	CA, CL				
			74_I_4	381	CA	EN, PR			
			74_B_2	392			CA[d]	B	2855
			74_I_5	400		AP			
			74_I_6	402		SC			
P88	18	M	88_B_1	188			CO[d]		
			88_I_1	189		PR			
			88_I_2	201	CK	EN, PR, SC			

a. Sample name given as patient number_ileostomy (I) or bloodstream (B)_sample number.

b. CA, *Candida albicans*; CG, *C. glabrata*; CK, *C. krusei*; CL, *C. lusitaniae*; CO, *C. orthopsilosis*; CP, *C. parapsilosis*; CT, *C. tropicalis*; Acr, *Acremonium*; Deb, *Debaryomyces*; Fus, *Fusarium*; Tri, *Trichoderma*; AP, *Aureobasidium pullulans*; EN, *Epicoccum nigrum*; *PF*, *Pichia fermentens*; *PR*, *Penicillium cf. roqueforti*; *SC*, *Saccharomyces cerevisiae*; WA, *Wickerhamomyces anomalus*.

c. RAPD and MLST genotypes are given for samples in pure cultured only, and are described in the text. *Candida parapsilosis* lacks an MLST, and *W. anomalus* lacks MLST and RAPD, systems.

d. Obtained in culture.

ClustalW implemented in BioEdit Sequence Alignment Editor version 7.3.2. For *C. albicans*, heterozygosities were assessed by visual inspection of the chromatograms and designated using IUPAC nomenclature. Sequences were entered into the respective MLST database (http://pubmlst.org/calbicans/ & http://cglabrata.mlst.net/), and assigned an allele profile for each locus which produced a sequence type (ST) based on the entire allelic combination

Candida albicans, *C. glabrata* and *Candida parapsilosis* isolates were also typed by RAPD using the primer Oligo 2 (5′-TCACGATGCA- 3′) as described previously.[24] *Candida parapsilosis* isolates were further typed with the primer OPE-04 (5′-GTGACATG-3′). [25] Amplification reactions were as described above for PCR except 10 ng of DNA was used. Amplicons were separated in a 1.5% agarose gel for 90 minutes and visualized.

Antifungal susceptibility was assessed using the Sensititre YeastOne YO-9 plate (TREK Diagnostic Systems, Cleveland, OH, USA), which provides antifungal susceptibility testing and minimum inhibitory concentration (MIC) determination against nine antifungals (see Table 2, below), following the manufacturer's instructions. Guidelines for breakpoint interpretations for amphotericin B were determined from CLSI M27-A[26]; for anidulafungin, caspofungin, fluconazole, micafungin and voriconazole from CLSI M27-S4;[27] for 5-flucytosine and itraconazole from CLSI M27-S3,[28] and for posaconazole from the European Committee on Antimicrobial Susceptibility Testing.[29]

3 Results

The ileal effluent of SBT recipients harbored several fungal species (Table 1). *Candida albicans* was the predominant species, detected in the ileum of four patients (57%) and 11 out of 28 ileostomy samples (40%). All ileum samples tested contained detectable fungi, and the species detected per sample ranged from 1 to 6. P33 harbored four *Candida* species in a single ileal sample. Other, non-*Candida* fungi were also detected in 60% of ileal samples, with *Penicillium roqueforti* and/or allied species (*Penicillium roqueforti, Penicillium carneum* and *Penicillium paneum* cannot reliably be distinguished on the basis of ITS sequence) detected in samples for all patients.

Species isolated from each blood culture are shown in Table 1. All bloodstream infections were caused by *Candida*. *Candida parapsilosis* was the predominant species isolated from the bloodstream (three patients). *Candida albicans* was the second most-isolated species from the bloodstream, in two patients, whereas *C. glabrata, C. orthopsilosis* and *C. pelliculosa* (=*C. beverwijkiae Wickerhamomyces anomalus*) were each isolated from one patient. P74 sustained two distinct bloodstream infections, from *C. parapsilosis* in July 2006 and from *C. albicans* in November of 2006.

All *C. albicans* (n = 3) and *C. glabrata* (n = 8) isolated in culture from the ileum and bloodstream were subjected to MLST. Twelve alleles were identified among the seven loci of *C. albicans* isolates, including two new alleles, which generated a new, unique diploid sequence type (Table S1). Fourteen polymorphic nucleotide sites were found between the two *C. albicans* sequence types. Both *C. albicans* sequence types detected in this study (ST 2854 & ST 2855) are novel isolates and have been deposited in the MLST database. In addition, two sequence types (ST 2 & ST 3), consisting of 12 alleles and 23 variable nucleotide sites, were identified among the six loci of *C. glabrata* isolates (Table S1).

For P33, ileostomy and blood *C. albicans* isolates yielded one sequence type (ST 2854). *Candida albicans* causing bloodstream infection in P74 was ST 2855, distinct from P33. *Candida glabrata* ileostomy and bloodstream isolates from P39 shared ST 3. P47, who developed candidemia by *C. parapsilosis*, had gastrointestinal colonization by *C. glabrata*. However, the *C. glabrata* isolated from this individual was ST 2, which was a distinct genotype from P39. In both species for which MLST was possible, sequence type was consistent within a patient over time, but differed between patients.

RAPD fingerprinting with primer Oligo 2 was performed on all cultured *Candida* isolates that were detected more than once (see Table 1; Figures S2-S4). Four *C. albicans* isolates generated three distinct RAPD profiles. *Candida albicans* isolates recovered from the ileostomy and bloodstream of P33 had the same RAPD profile A. The bloodstream isolate from P74 gave a distinct RAPD profile B (*C. albicans* was detected in ileostomy samples from P74 but could not be cultured). The common laboratory strain *C. albicans* SC5314 also gave a distinct pattern C. Nine *C. glabrata* isolates generated three distinct RAPD profiles. *Candida glabrata* isolates obtained from the ileostomy and bloodstream of P39 had the same RAPD profile D. *Candida glabrata* ileostomy isolates from five consecutive ileostomy samples over a 1 month period in P47 generated RAPD profile E, whereas control strain *C. glabrata* NRRL Y-65 gave a distinct RAPD pattern F. The RAPD results obtained from *C. albicans* and *C. glabrata* isolates are congruent with strain typing data by MLST (Table 1). No RAPD patterns for *C. albicans* or *C. glabrata* were shared across individuals.

Seven *C. parapsilosis* isolates generated two distinct RAPD profiles. *Candida parapsilosis* isolates from P47 showed distinct RAPD patterns between the ileum and bloodstream. The two *C. parapsilosis* blood culture isolates were collected from this patient before and after the ileostomy sample was collected. Both blood cultures generated the same RAPD pattern H. However, the ileostomy isolate showed RAPD pattern G. The two ileostomy samples prior to the first bloodstream collection harbored *C. parapsilosis*, but viable organisms could not be cultured; therefore, RAPD could not be used to identify the *C. parapsilosis* to strain. Three *C. parapsilosis* isolates, collected from the ileum of P62 and the blood of P32 and P74, gave the same RAPD pattern G. Control *C. parapsilosis* 232, isolated from cheese, also had RAPD pattern G. Since a *C. parapsilosis* MLST scheme does not exist, primer OPE-04 was also used for RAPD to confirm results obtained by primer Oligo 2. Primer OPE-04 also yielded two distinct patterns among the seven isolates, which agreed with the results from Oligo 2 RAPD (Figure S5).

The chronological carriage of *Candida* strains is shown in Figure 1. At two points, samples were collected from multiple patients during the same time period. In both cases, patients carried distinct strains from one another. *Candida parapsilosis* isolates of RAPD profile G were detected consistently over time in four patients. Isolates of all other MLST and RAPD profiles were each restricted to a single patient in the tested samples. No one genotype dominated the patient population.

The antifungal susceptibilities and MICs for the yeast isolates against nine antifungals are shown in Table 2. *Candida glabrata* isolate 39_B_1 was reported as susceptible dose-dependent to fluconazole (which was administered in a level to

Table 2. Summary of MIC values and antifungal susceptibility of bloodstream isolates[a]

	MIC (µg/mL) and susceptibility profile																	
	AMB[b]		AND[c]		CAS[c]		FCT[d]		FLZ[c]		ITZd		MCF[c]		POZ[e]		VOR[c]	
Candida species	MIC	SP	MIC	SP	MIC	SP	MIC	SP	MIC	SP	MIC	SP	MIC	SP	MIC	SP	MIC	SP
Candida albicans																		
33_B_1	1	S	0.03	S	0.03	S	0.12	S	0.5	S	0.06	S	0.008	S	0.03	S	0.03	S
74_B_2	1	S	0.03	S	0.03	S	0.12	S	4	SDD	0.12	S	<0.008	S	0.06	S	0.12	S
Candida glabrata																		
39_B_1	>1	R	0.06	S	0.25	I	<0.06	S	32	SDD	2	R	0.015	S	2	R	2	ND[f]
Candida orthopsilosis																		
88_B_1	1	S	1	S	0.5	S	0.12	S	1	S	0.12	S	0.5	S	0.12	S	0.12	S
Candida parapsilosis																		
32_B_1	1	S	2	S	0.25	S	0.25	S	1	S	0.12	S	1	S	0.06	S	0.03	S
47_B_1	>1	R	2	S	2	S	0.12	S	2	S	0.06	S	2	S	0.06	S	0.06	S
47_B_2	>1	R	2	S	2	S	0.12	S	2	S	0.06	S	2	S	0.06	S	0.06	S
74_B_1	0.25	S	1	S	0.25	S	0.06	S	1	S	0.06	S	1	S	0.03	S	0.12	S
Candida pelliculosa[g]																		
62_B_1	0.5	S	0.015	S	0.015	S	<0.06	S	2	S	0.06	S	0.015	S	0.12	S	0.06	S

Abbreviations: AMB, amphotericin B; AND, anidulafungin; CAS, caspofungin; FCT, 5-flucytosine; FLZ, fluconazole; ITZ, itraconazole; MCF, micafungin; POZ, posaconazole; VOR, voriconazole; MIC, minimum inhibitory concentration; SP, susceptibility profile, S, susceptible; SDD, susceptible dose-dependent; I, intermediate; R, resistant; ND, not determined.

a. Antifungal susceptibility determined using the Sensitire® YeastOne plate (TREK Diagnostic Systems, Cleveland, OH, USA).

b. The methodology to currently determine amphotericin B MICs for *Candida* species is inconsistent and present interpretation suggests that an MIC of >1 µg/mL is likely to be resistant.[26]

c. Guidelines for breakpoint interpretations for anidulafungin, caspofungin, fluconazole, micafungin and voriconazole are determined from CLSI M27-S4.27

d. Guidelines for breakpoint interpretations for 5-flucytosine and itraconazole are determined from CLSI M27-S3.[28]

e. Antifungal breakpoints used for posaconazole are those described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for *Candida* species.[29]

f. Current data are insufficient to demonstrate a correlation between in vitro susceptibility testing and clinical outcome for *C. glabrata* and voriconazole.[27]

g. The teleomorphic state of *C. pelliculosa* is identified as *Wickerhamomyces anomalus (Pichia anomala*).

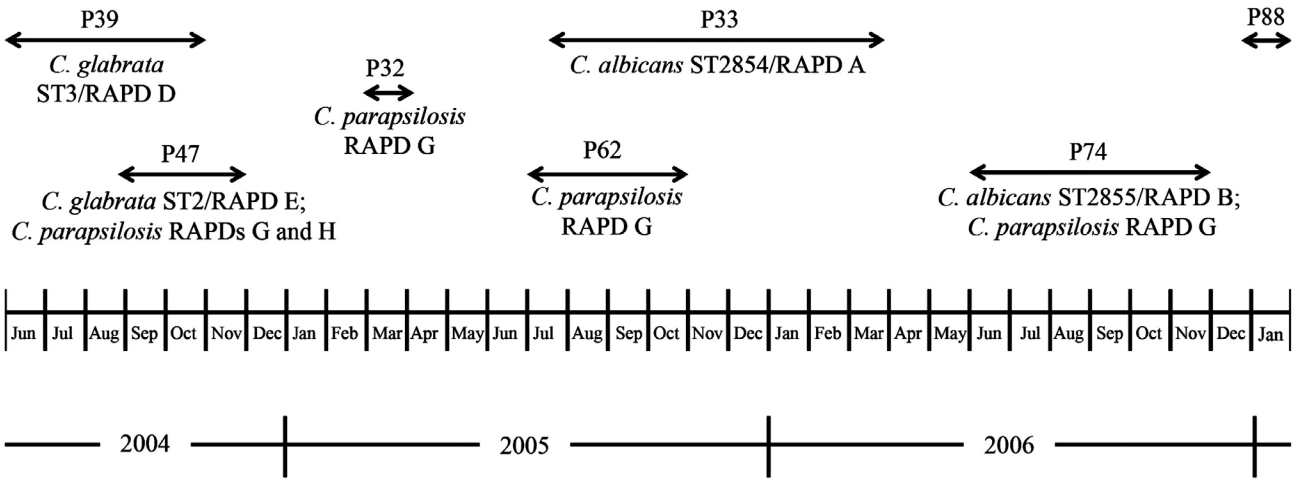

Figure 1. Chronological outline of patient sample collection. The MLST sequence types and RAPD profiles are indicated for each patient

patient considered susceptible), intermediate to caspofungin, and resistant to amphotericin B, itrazonazole and posaconazole. *Candida parapsilosis* isolates 47_B_1 and 47_B_2 were determined to be resistant to amphotericin B. *Candida albicans*, *C. orthopsilosis* and *C. pelliculosa* isolates were uniformly susceptible or susceptible dose dependent (74_B_2 for fluconazole) to all antifungals. In addition, we tested one ileal isolate of *C. krusei* (from 33_I_3; not shown). This isolate was resistant to fluconazole, showed an intermediate response to 5-flucytosine, and was susceptible dose-dependent to itraconazole. Isolates showed a wide range of MICs to fluconazole (0.5-32 μg/mL), as well as micafungin (<0.008-2 μg/mL), anidulafungin (0.015-2 μg/mL) and caspofungin (0.015-2 μg/mL), and a narrow range for 5-flucytosine (<0.06-0.25 μg/mL).

4 Discussion

This study describes SBT recipients who developed fungemia post-transplant at the UNMC from 2004 to 2007. We employed a variety of techniques to identify and characterize *Candida* isolates colonizing and infecting SBT recipients. This is the first study characterizing the fungal intestinal microbiota of SBT recipients. Comprehensive surveys of gut fungi, using next-generation sequencing methods, may report dozens of species or OTUs (operational taxonomic units) from a single fecal sample[30]; however, the majority of these are rarely detected environmental or dietary fungi that do not appear to colonize the gastrointestinal tract.[31] The fungi we detected from ileostomy samples in this study are largely those species that do have a well-established association with and role in the gut, especially *Candida* species.

Prior to 1990, the predominant species causing *Candida* infections was *C. albicans*.[32] In recent years infections caused by non-*albicans* species have increased in clinical importance.[17] Bloodstream infections in our study were most frequently caused by *C. parapsilosis* (four cases), whereas *C. albicans* was isolated in only two infections. Only P47 experienced candidemia shortly after transplant (at 29 and 35 days, with *C. parapsilosis*), accordingly, this is the only patient for whom the 2 week postoperative antifungal prophylaxis with fluconazole was anticipated to influence species isolated or susceptibility. One infection each was caused by *C. glabrata, C. orthopsilosis* and *C. pelliculosa*.

Candida pelliculosa is widespread in the environment and in food, and is an opportunistic human and animal pathogen. This yeast transiently colonizes the oropharyngeal and gastrointestinal tract of humans and it has been implicated in outbreaks in neonatal and pediatric intensive care units.[33] *Candida pelliculosa* was not identified in the intestine of P62 from whom it was isolated from the blood. Therefore, it is possible that this patient acquired this species from an exogenous source, as suspected in previous cases.[34]

We detected previous digestive tract colonization with *Candida* in six of the patients with candidemia. Our findings suggest that at least two bloodstream infections (in P33 and P39) originated from *Candida* colonizing the small bowel, although complete exclusion of environmental exposure during hospitalization is not possible at this time. Closely related isolates in the intestine and blood have been reported in other studies.[35,36] Gastrointestinal colonization is an important source of candidemia, although only a few studies using molecular typing methods have been reported and sample numbers are small.[14] Many studies only report an association between anatomic site of *Candida* colonization as a risk factor for subsequent candidemia and invasive candidiasis in hospitalized and critically ill patients.[37,38] However, it is plausible that patients in our study would be infected by endogenous *Candida* due to the invasive nature of this procedure.

P47 had different strains of *C. parapsilosis* colonizing the intestine and infecting the bloodstream, suggesting candidemia derived from a distinct source. *Candida parapsilosis* has been reported in the human oral microbiota so endogenous infection remains a possibility. Other possibilities include mutation within the patient and increase in prevalence of a previously undetectable isolate. Nucleotide sequence variation among serial isolates or as separate clones from individual patients is a known phenomenon for strains of *C. albicans*.[39] The majority of documented *C. parapsilosis*

infections are of exogenous origin; endogenous infections are rarely seen.[36] *Candida parapsilosis* is the most common fungus recovered from the hands and studies have implicated health care workers' hands as the source of nosocomial candidaemia.[40] Another possible route of *C. parapsilosis* transmission could be contaminated parenteral nutrition and intravascular devices.[41] *Candida parapsilosis* was not detected in ileostomy samples from P32 and P74, who developed bloodstream infection with *C. parapsilosis*. These two individuals could represent further cases of exogenous acquisition. In addition to P88, who had no gastrointestinal colonization with the fungus isolated from the bloodstream, *C. orthopsilosis*, may have acquired candidemia exogenously.

P74 was co-infected by two *Candida* species. *Candida parapsilosis* was the first organism recovered from the blood, but this was not detected in ileostomy samples prior to infection. *Candida albicans* was present in three ileostomy samples prior to *C. albicans* detection in the blood. The possibility exists that P74 was infected exogenously by *C. parapsilosis* and endogenously by the patient's own intestinal *C. albicans*. The inability to culture *C. albicans* from the patient's ileum precluded strain typing and thus confirmation of a possible endogenous source.

Studies have documented the ability of *Candida* isolates to persist in hospital settings and cause temporally associated infections.[42,43] In our study, temporal analysis revealed multiple species were responsible for causing bloodstream infections during the study period. At two different time points, samples collected from two patients overlapped, but the patients did not carry the same genotype. However, the same *C. parapsilosis* genotype G in four patients was documented at multiple time points. Therefore, there is potential evidence that this *C. parapsilosis* strain had a common source. However, environmental sampling would be necessary to confirm or rule out any exogenous source.

Methods used to strain-type *Candida* species have greatly advanced our knowledge of fungal epidemiology and MLST has been shown to be more discriminating than RAPD for *C. albicans*.[44] In our study, MLST detected distinct sequence types between, but not within, patients. This suggests independent, endogenous acquisition of *C. albicans* candidemia, but would need to be confirmed by full genome sequencing. RAPD patterns yielded the same conclusions as MLST for *C. albicans* and *C. glabrata* isolates. RAPD fingerprints generated by two independent primers gave the same results for *C. parapsilosis* isolates. Other RAPD primers may possibly further differentiate between our isolates; conversely, genetic variation in *C. parapsilosis* may be minimal. Other methods such as microsatellite markers may be more discriminatory for epidemiological investigations of *C. parapsilosis*,[45] but ultimately future work will have to encompass full genome sequence of well-documented, larger collections.

There is increasing concern regarding antifungal resistance in *Candida,* especially *C. parapsilosis*. In our study, two *C. parapsilosis* isolates from the same patient showed resistance to amphotericin B. In addition, the one isolate of *C. glabrata* also showed resistance to amphotericin B using the in vitro microplate susceptibility testing method. Although resistance of *Candida* species to amphotericin B is rare, the frequency of resistance to amphotericin B has been shown to be highest with *C. parapsilosis* (2.5%) followed by *C. krusei* (2.0%) and *C. glabrata* (0.8%).[46] Interestingly also, the formation of biofilms in *C. parapsilosis* did lead to a significant (P = .0004) lowering of activity for amphotericin B from 100% susceptible to 32.1% susceptible in this species.[47] However, there are no standardized methods available to perform and interpret in vitro amphotericin B susceptibility testing.[26] *Candida albicans* isolates from this study were susceptible to all antifungal agents. The *C. glabrata* strain causing candidemia in addition to amphotericin B was also resistant to itraconazole and posaconazole, intermediate to caspofungin and susceptible dose-dependent to fluconazole. This strain was also detected in the patient's ileal fluid 4 months prior to infection. One other patient harbored *C. glabrata* in the ileum, but was only infected by *C. parapsilosis*. The one *C. krusei* isolate we obtained in culture showed some level of drug resistance, notably to fluconazole; however, no *C. krusei* strain in this study was implicated in candidemia. All patients were exposed to fluconazole prophylaxis in the immediate perioperative period.

One limitation of this study was the inability to culture viable organisms from several ileostomy samples. Thus, while we know an ileostomy sample harbored a specific species, intestinal origin could not be confirmed due to the inability to strain-type. Techniques to strain-type species in mixed matrices need to be developed to avoid requiring pure cultures of isolates. Another limitation was the inability to determine whether ileal *Candida* originated in the donor or recipient bowel. Donor-derived *Candida* infections have been documented in kidney transplant recipients.[48] To date, no efforts have been made to identify *Candida* species and/or strains from the donor small bowel, or from other sources of recipient microbiota, to investigate the origin of candidemia in SBT recipients.

These findings highlight the possibility of hospitalized SBT patients acquiring candidemia both endogenously and exogenously, although the exact route of infection in these transplant patients is not clear. Knowing the source of *Candida* implicated in infection (recipient, donor or hospital acquired) will inform pretransplant interventions and treatments to improve outcomes for SBT patients. Future efforts should include collection and culturing of donor and recipient samples (including oral, colon and skin microbiota) prior to transplant, as well as continuing ileostomy sample collection throughout hospitalization. This would highlight the role (if any) of the donor microbiota and may direct prevention efforts. Targeted interventions could reduce exogenous and endogenous infections, and environmental sampling may reveal sources of infection and solutions. Reducing endogenous recipient-derived infection by means of existing prophylactics will be more difficult and will require innovative interventions.

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The authors declare that they have no conflict of interest.

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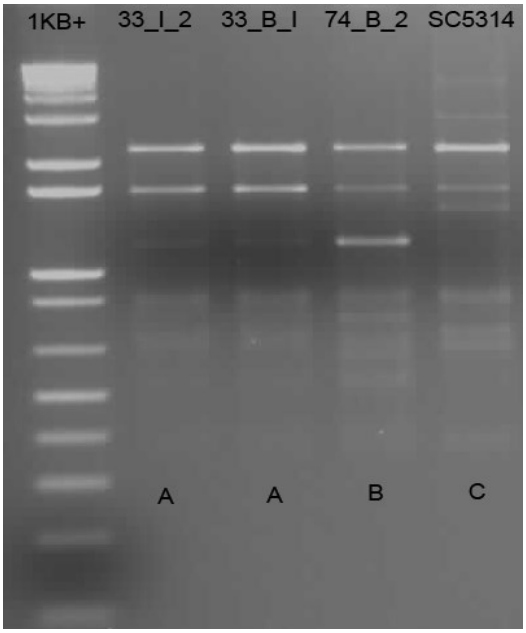
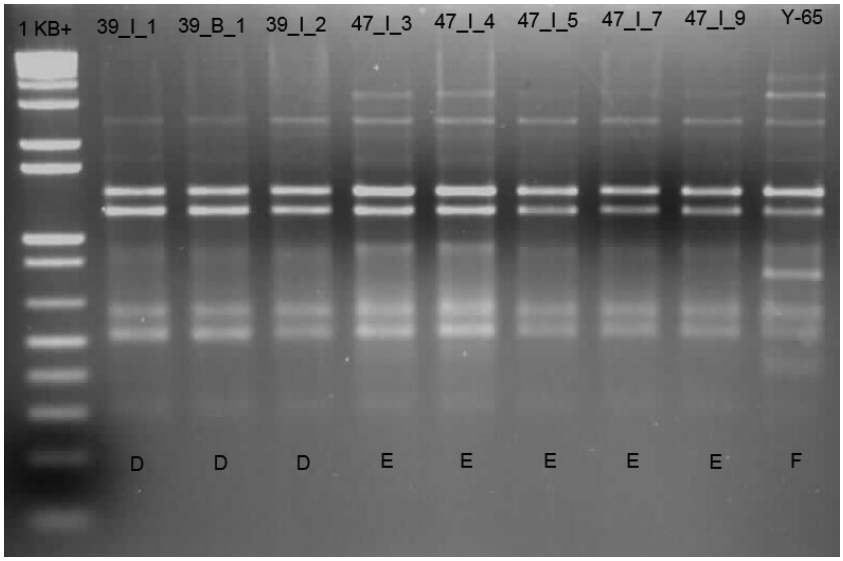
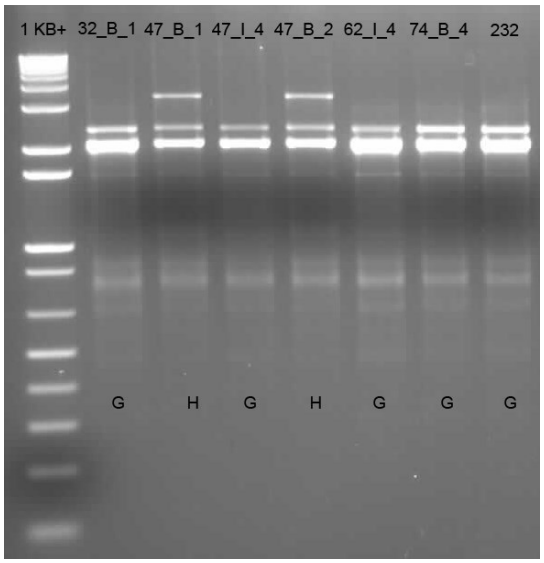
Additional Supporting Information follows.



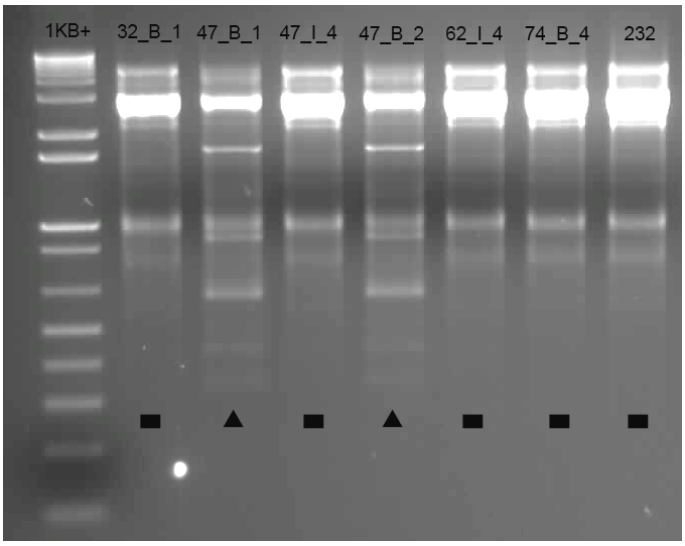
Supplemental Figure S1. RAPD fingerprint patterns of *C. albicans* isolates obtained with primer Oligo 2. Fingerprints indicated with the same letter have equal genotypes. First lane 1 KB+ molecular size marker. Laboratory strain *C. albicans* SC5314 included with isolates.



Supplemental Figure S2. RAPD fingerprint patterns of *C. glabrata* isolates obtained with primer Oligo 2. Fingerprints indicated with the same letter have equal genotypes. First lane 1 KB+ molecular size marker. *C. glabrata* NRRL Y-65 included with isolates.



Supplemental Figure S3. RAPD fingerprint patterns of *C. parapsilosis* isolates obtained with primer Oligo 2. Fingerprints indicated with the same letter have equal genotypes. First lane 1 KB+ molecular size marker. *C. parapsilosis* 232 included with isolates.



Supplemental Figure S4. RAPD fingerprint patterns of *C. parapsilosis* isolates obtained with primer OPE-04. Fingerprints indicated with the same shape have equal genotypes. First lane 1 KB+ molecular size marker. *C. parapsilosis* 232 included with isolates.

Supplemental Table S1. MLST genotypes of the *C. albicans* and *C. glabrata* isolates studied.

Candida albicans

Sample	Allele number							MLST Genotype
	AAT1a	*ACC1*	*ADP1*	*MPIb*	*SYA1*	*VPS13*	*ZWF1b*	
33_I_2	156	4	4	4	204	4	4	2854
33_B_1	156	4	4	4	204	4	4	2854
74_B_2	35	7	6	4	4	4	239	2855

Candida glabrata

Sample	Allele number						MLST Genotype
	FKS	*LEU2*	*NMT1*	*TRP1*	*UGP1*	*URA3*	
39_I_1	5	7	8	7	3	6	3
39_B_1	5	7	8	7	3	6	3
39_I_2	5	7	8	7	3	6	3
47_I_3	1	2	2	1	1	1	2
47_I_4	1	2	2	1	1	1	2
47_I_5	1	2	2	1	1	1	2
47_I_7	1	2	2	1	1	1	2
47_I_9	1	2	2	1	1	1	2