ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2016, 54, 725–732
doi: 10.1093/mmy/myw030
Advance Access Publication Date: 3 May 2016
Original Artical

Original Artical

Fungal peritonitis in patients undergoing peritoneal dialysis (PD) in Brazil: molecular identification, biofilm production and antifungal susceptibility of the agents

Juliana Giacobino[1], Augusto Cezar Montelli[1], Pasqual Barretti[2], Ariane Bruder-Nascimento[1], Jacqueline Teixeira Caramori[2], Luciano Barbosa[3] and Eduardo Bagagli[1,*]

[1]Departamento de Microbiologia e Imunologia, Instituto de Biociências, UNESP, Botucatu, [2]Departamento de Clínica Médica, Faculdade de Medicina de Botucatu, UNESP and [3]Departamento de Bioestatística, Instituto de Biociências, UNESP, Botucatu

*To whom correspondence should be addressed. Eduardo Bagagli, Department of Microbiology and Immunology, Bioscience Institute of Botucatu, UNESP, São Paulo, Brazil. E-mail: bagagli@ibb.unesp.br

Received 24 November 2015; Revised 31 March 2016; Accepted 12 April 2016

Abstract

This paper presents data on fungal peritonitis (FP) in patients undergoing peritoneal dialysis (PD) at the University Hospital of Botucatu Medical School, São Paulo, Brazil. In a total of 422 patients, 30 developed FP, from which the medical records and the fungal isolates of 23 patient cases were studied. All patients presented abdominal pain, cloudy peritoneal effluent, needed hospitalization, had the catheter removed and were treated with fluconazole or fluconazole plus 5-flucitosine; six of them died due to FP. Concerning the agents, it was observed that *Candida parapsilosis* was the leading species (9/23), followed by *Candida albicans* (5/23), *Candida orthopsilosis* (4/23), *Candida tropicalis* (3/23), *Candida guilliermondii* (1/23), and *Kodamaea ohmeri* (1/23). All the isolates were susceptible to amphotericin B, voriconazole and caspofungin whereas *C. albicans* isolates were susceptible to all antifungals tested. Resistance to fluconazole was observed in three isolates of *C. orthopsilosis*, and dose-dependent susceptibility to this antifungal was observed in two isolates of *C. parapsilosis* and in the *K. ohmeri* isolate. Biofilm production estimates were high or moderate in most isolates, especially in *C. albicans* species, and low in *C. parapsilosis* species, with a marked variation among the isolates.

This Brazilian study reinforces that FP in PD is caused by a diverse group of yeasts, most prevalently *C. parapsilosis sensu stricto* species. In addition, they present significant variation in susceptibility to antifungals and biofilm production, thus contributing to the complexity and severity of the clinical features.

Key words: peritonitis, *Candida* spp., peritoneal dialysis, biofilm, antifungal susceptibility.

Introduction

Fungal peritonitis (FP) is a serious complication in patients with chronic renal failure undergoing peritoneal dialysis (PD). Despite scientific and technological developments in the systems, these infections are still an important cause of the technical failure in PD.[1–4] Bacterial infections are responsible for about 80% of the episodes of PD-related peritonitis, compared with approximately 2% to 16% of the cases due to FP, and usually occur in patients with prior exposure to bacterial infections and antibiotics.[5–8] FP has a worse prognosis when compared to bacterial peritonitis,[4] since it might causes irreversible damage in the peritoneal membrane that results in a PD cessation that may be temporary but is frequently permanent.[4,7] In addition, FP is associated with higher rates of morbidity and mortality, ranging from 5% to 53% of cases.[9–12]

Among the pathogenic or opportunistic fungi, the main causes of FP in PD are species of the genus *Candida*.[13,14] Although *Candida albicans* is the most common species causing fungal peritonitis, in recent years, an increase has been observed in the cases attributable to *Candida parapsilosis* and other *Candida* species, as well as some uncommon yeasts species, which frequently present distinct antifungal susceptibility that requires more aggressive treatments.[15–17]

The present work aimed to identify the fungal species causing peritonitis in patients undergoing PD in a tertiary public university hospital, located in Botucatu, Brazil, South America, over a 16-year period. The biofilm production and antifungal susceptibility profiles were also studied. To the best of our knowledge, this is the first Latin American study with these goals.

Materials and methods

Sampling and patient data

This retrospective study was carried out in patients undergoing PD at the University Hospital of Botucatu Medical School, São Paulo, Brazil, between 1994 and 2010, which present FP, diagnosed by the clinical symptoms (abdominal pain or cloudy dialysate, dialysate white cell count $>100/\mu$l with at least 50% neutrophilic cells) and fungal positive culture of dialysate. Death related to peritonitis was defined according to the International Society for Peritoneal Dialysis (ISPD) guidelines, as death of a patient with active peritonitis, or admitted with peritonitis, or within 2 weeks of a peritonitis episode.[8] Exclusion criteria were incomplete information and/or occurrence of repetitive fungal infection in the same one-month period. Additional patient data and information on the local dialysis service can be obtained in Barretti et al. (2012) and Camargo et al. (2014).[18,19] All the isolates studied were maintained in the culture collection of the Department of Microbiology and Immunology, Biosciences Institute of Botucatu, UNESP. This study was approved by the local Research Ethics Committee (Of. 535/2011).

Morphophysiological identification

The isolates were initially cultured on Sabouraud dextrose agar (SDA) medium (Oxoid®) and incubated at 35°C, for 48 to 72 hours, to ensure their purity. Subsequently, each isolate was cultured on chromogenic medium (CHROMagar Candida BD®), and submitted to the auxanogram tests for carbon and nitrogen assimilation profiles.

Molecular identification

DNA was extracted from isolates cultured on SDA for 36 h at 35°C, according to McCullough et al. (2000)[20] and its concentration estimated on agarose gel with Low Mass DNA Ladder marker (Invitrogen®) and also in NanoVue (GE Healthcare®). The rDNA sequences were analyzed by PCR amplification and sequencing of region ITS1-5.8S-ITS2, using primers ITS4 and ITS5, and in inconclusive cases, also the region D1/ D2 26S of rDNA, using primers D1 and D2, described by White et al. (1990)[21] and Kurtzman and Robnett (1998)[22], respectively. The amplicons were purified using GFX PCR DNA and Gel Band Purification Kit (GE Healthcare®), according to the manufacturer's guidelines; its DNA concentration adjusted to 10 ng/ul, and then sequenced using TaqDyeDoxy Terminator Cycle Sequencing Kit (Applied Biosystems) in an automated sequencer (ABI-PRISM 377). Sequences were analyzed by BioEdit and Mega 6.0, manually edited if necessary, and then compared to the NCBI database, by the tool Blast (http://blast.ncbi.nlm.nih.gov/Blast).[23] The species identification process considered only the alignments that presented sequence identity of ≥98.5%, as proposed by Irinyi et al. (2015)[24], and with matching sequences published in peer-reviewed journals, originated from reference isolates (ATCC or CBS strains) or deposited at ISHAM-ITS database.

Biofilm production

The production and quantification of biofilm were performed according to the protocol described by Chandra et al. (2008)[25], using silicone disks (Kinner®), produced by the same material as hospital catheters. The biofilm adhering to the disks was quantified using XTT (2.3-Bis (2-methoxy-4-nitro-5-sulfophenyl)-2 H-tetrazolium-5-carboxanilide inner salt (Sigma-Aldrich®) and menadione (Sigma-Aldrich®), by the tetrazolium reduction reaction, which in the presence of biofilm produces orange formazin, whose color intensity was determined on a spectrophotometer with a filter at 492 nm to measure the cellular metabolic activity of biofilm. Positive and negative controls were represented by a strain of *Candida albicans* (ATCC 36802) and a blank reference (without the inoculum), respectively. The results were expressed as the mean of the Optical Densities (ODs), obtained in quadruplicate for each isolate, which were then classified as low, moderate, and high biofilm-forming, according to the respective terciles, which were arbitrarily estimated considering the arithmetic means of all isolates and species together, adapted of Marcos-Zambrano et al. (2014).[26]

Antifungal susceptibility

The antifungal susceptibility profile was evaluated against amphotericin B (Sigma-Aldrich®), fluconazole (Sigma-Aldrich®), voriconazole (Sigma-Aldrich®), and caspofungin (Merch Co®), by the microdilution method described by the Clinical and Laboratory Standards Institute Protocol (CLSI M27-A3 and M27-S4)[27,28] for determining the minimum inhibitory concentration (MIC). The susceptibility breakpoints for fluconazole, voriconazole and caspofungine were those established by CLSI M27-S4. In the case of amphotericin B, it was considerate S $\leq$ 2 μg/ml and R for >2 μg/ml. In the case of *K. ohmeri*, it was adopted the breakpoints values already established by CLSI to the phylogenetically related species *C. guilliermondii*. The test was validated by ATCC 22019 (*C. parapsilosis*) and ATCC 6258 (*C. krusei*) as control strains.

Statistical analysis

The nonparametric test of Kruskal–Wallis and One-Way Analysis of Variance on Ranks followed by Dunn's pairwise multiple comparison procedure were carried out for both the biofilm production (in which the dependent variable was optical density values) and the antifungal susceptibility tests (with MIC values as the dependent variable), with significance when $P < .05$.

Results

Between 1994 and 2010, in a total of 422 patients undergoing PD at the service, it was observed that 685 cases of peritonitis, of which 30 episodes (4.4%) were caused by fungi. Each patient had only one FP episode. After applying the exclusion criteria, it was here studied only the FP cases (n = 23) in which the patient medical records and the isolates were available for additional microbiological evaluations. Of the 23 cases, 14 patients were treated by continuous ambulatory peritoneal dialysis (CAPD) and nine by automated peritoneal dialysis (APD). In 20 of them, FP occurred after previous bacterial peritonitis, which were treated with antibiotics, and in three cases the primary infections were by fungus. The patient ages varied from 2 to 72 years (54.8 $\pm$ 16.8), 61% were male, 48% were diabetics. During the FP, all patients presented abdominal pain, cloudy peritoneal effluent, and needed hospitalization. Some of them also presented nausea and vomiting (65%), hypotension (26%), and fever (22%). As soon as FP was confirmed, all the patients had the catheter removed and were treated with fluconazole or fluconazole plus 5-flucitosine; however, six of them (26.1%) died due to the fungal infection, of which five were treated by CAPD and one by APD (Table 1 and Table S1). Peritoneal dialysis was discontinued in all patients.

The morphophysiological identification by means of CHROMagar medium and auxanogram tests, detected 12 (52%) isolates of *Candida parapsilosis*, 5 (22%) of *Candida albicans*, 3 (13%) of *Candida tropicalis* and 3 (13%) inconclusive isolates. Molecular identification by ITS1-5.8S-ITS2 rDNA sequencing not only corroborated the majority of the morphophysiology identifications but also correctly identified the isolates 4, 10, and 13 as *C. orthopsilosis*, which had been classified erroneously by morphophysiology as *C. parapsilosis*. In addition, the sequencing of ITS1-5.8S-ITS2 allowed the identification of the three morphophysiologic inconclusive isolates (6, 8, and 14 isolates), whose identities were also confirmed by sequencing of the D1/D2 of rDNA regions, finding the following taxa: *Kodamaea ohmeri* (isolate 6), *C. orthopsilosis* (isolate 8) and *C. guilliermondii* (isolate 14) (Table S2, supplementary material, also containing the Genbank accession numbers of the deposited sequences). Thus, it was found that of the 23 isolates studied, 9 (39%) are *C. parapsilosis*, 5 (22%) *C. albicans*, 4 (17%) *C. orthopsilosis*, 3 (13%) *C. tropicalis*, 1 (4%) *C. guilliermondii,* and 1 (4%) *K. ohmeri* (Table 1).

The biofilm production was also evaluated in the 23 isolates. Based on the average optical densities (ODs) observed, the isolates were classified into three groups showing: (i) low biofilm-forming (OD less than 0.12); (ii) moderate biofilm-forming (OD of 0.12–0.20), or (iii) high biofilm-forming

Table 1. Main findings of the patients in peritoneal dialysis that develop fungal peritonitis at the University Hospital of Botucatu Medical School, São Paulo, Brazil.

Patient Data[1]					Mycological findings and clinical outcome[2]			
Patient	Age	Sex	Diabetes	Dialysis*	Agent	Fluco[3]	Biofilm**	Death
1	43	F	no	APD	*Candida albicans*	S	High	no
2	72	M	no	CAPD	*C. parapsilosis*	S	High	no
3	70	M	yes	APD	*C. parapsilosis*	S	Low	no
4	41	M	no	CAPD	*C. orthopsilosis*	R	High	no
5	68	F	yes	CAPD	*C. parapsilosis*	S	Medium	No
6	71	M	yes	CAPD	*Kodamaea ohmeri*	DDS	High	Yes
7	55	F	no	CAPD	*C. albicans*	S	High	Yes
8	68	F	yes	APD	*C. orthopsilosis*	S	Medium	Yes
9	60	M	yes	APD	*C. tropicalis*	S	High	No
10	58	M	yes	APD	*C. orthopsilosis*	R	High	No
11	36	F	no	CAPD	*C. albicans*	S	High	No
12	43	M	no	CAPD	*C. albicans*	S	High	No
13	46	F	yes	CAPD	*C. orthopsilosis*	R	High	Yes
14	70	M	yes	APD	*C. guilliermondii*	S	High	No
15	66	F	no	CAPD	*C. parapsilosis*	DDS	Low	No
16	36	F	no	CAPD	*C. parapsilosis*	S	Low	Yes
17	56	M	no	APD	*C. parapsilosis*	DDS	Medium	No
18	66	M	yes	APD	*C. parapsilosis*	S	Low	No
19	67	F	no	CAPD	*C. parapsilosis*	S	Low	Yes
20	55	M	yes	CAPD	*C. parapsilosis*	S	Low	No
21	71	M	yes	CAPD	*C. albicans*	S	High	No
22	2	M	no	CAPD	*C. tropicalis*	S	High	No
23	42	M	no	APD	*C. tropicalis*	S	Low	No

[1]Near all patients presented abdominal pain, turbid liquid and needed hospitalization.
[2]All patients had catheter removal and were treated with Fluconazole or Fluconazole + 5 Flucitosine.
[3]Fluconazole susceptibility (S = suscecptible; R = resistant; DDS = dose-dependent susceptible).
*Automatic Peritoneal Dialysis (APD) and Continuous Ambulatory Peritoneal Dialysis (CAPD).
**Biofilm production ($P < 0.05$, significant comparison between *C. albicans* and *C. parapsilosis*).

(OD greater than 0.20). Higher biofilm production was observed in *C. albicans* when compared with *C. parapsilosis* ($P < .05$), which also presented variation in biofilm forming capacity among the isolates (Table 1 and Figure 1).

With regard to the *in vitro* susceptibility profiles, it was observed that all isolates were susceptible to voriconazole, caspofungin, and amphotericin B. *C. albicans* isolates were susceptible to all antifungals tested. In relation to fluconazole, we observed resistance in three isolates of *C. orthopsilosis*, dose-dependent susceptibility in two isolates of *C. parapsilosis* and in the *K. ohmeri* isolate (Table 2).

Discussion

Fungal peritonitis, although less frequent than its bacterial counterpart, is associated with increased morbidity and mortality, and frequently causes the interruption of peritoneal dialysis due to the necessity of catheter removal.[1–14] Although all the patient cases herein had been hospitalized and treated with antifungals, death occurred in 6/23 of them.

The correct identification of fungal pathogens by the classic and/or conventional methods, as well as by the automated procedures, has become progressively more difficult, especially due to the emergence of new fungal species that are, in some cases, still considered rare.[29,30] This is particularly true for species that require several days or weeks for growing and present poor morphological structures and difficulties in the interpretation of their auxanogram and zymogram tests. On the other hand, molecular techniques have already been proven highly efficient at providing innovative complementary procedures for the diagnosis and identification of fungal species, mainly because they are faster and offer greater sensitivity and conclusiveness than traditional phenotypic methods.[31] The present data highlight the excellent discriminatory capacity of molecular techniques, since they properly differentiate the *psilosis* isolates and also correctly define the taxa of three

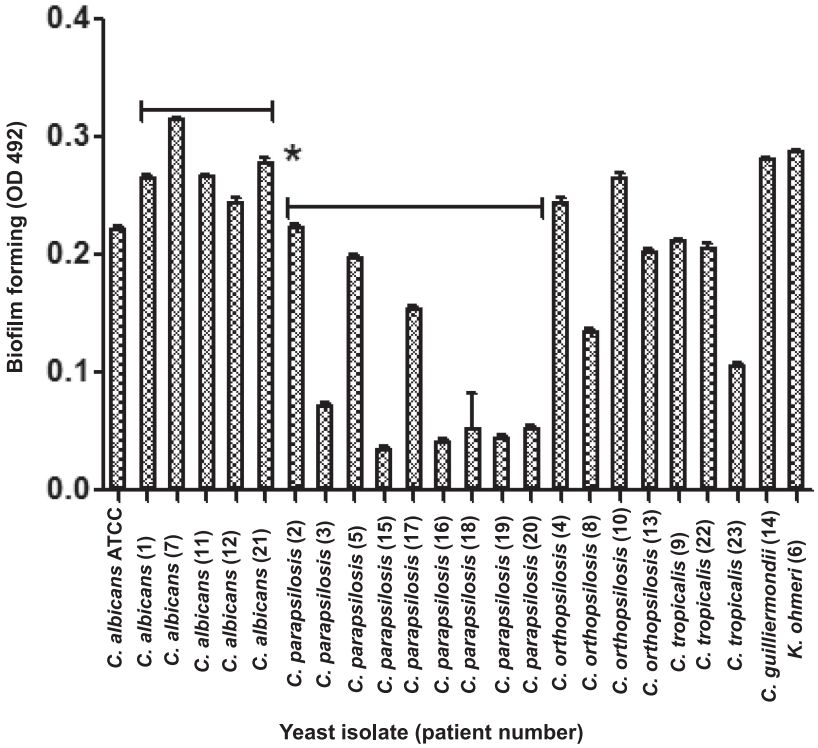

Figure 1. Biofilm production of yeast isolates obtained from patients undergoing peritoneal dialysis, at the University Hospital of Botucatu Medical School, São Paulo, Brazil, expressed by the optical densities (OD).

inconclusive isolates, previously evaluated by the traditional identification procedures. These methods also allowed the identification of a relatively rare yeast species, named *Kodamaea (Pichia) ohmeri,* which also belongs to the family *Sacharomycetae*. This species, which has been used in the food industry, in the fermentation of fruits and pickles, among others, and considered a contaminant by medical mycologists, has been sporadically isolated from important clinical cases, such as endocarditis, peritonitis, and fungemias, especially in immunocompromised patients.[32–35]

By the sequential use of morphophysiological and molecular techniques in the present study, we have observed a high prevalence of *psilosis* group species (56%), of which *C. parapsilosis* predominates with 39% versus 17% for *C. orthopsilosis*; *C. albicans* was second (22%) and *C. tropicalis* the third (13%) most frequent species. In addition, two other species were found at a lower prevalence, namely, *C. guilliermondii* and *K. ohmeri,* one case each.

The set of species herein identified as causing FP in patients undergoing PD presents some epidemiologic aspects in common with other studied regions, such as the occurrence of yeast species belonging mainly to the genus *Candida* and a tendency for species of the *psilosis* group to be predominant.[9–11,36] A study conducted over a three-year period in Jerusalem, Israel, has shown that *C. parapsilosis* was responsible for 43.8% of the all FP cases and an increase in the prevalence of *C. parapsilosis* infections in pediatric patients submitted to PD.[37] In Taiwan, in 2004, it was reported that *C. parapsilosis* was the most common pathogen causing FP, accounting for 29% of the cases, while *C. albicans* occurred in 14% of the cases.[38] Among the 10 observed cases of FP in another study, conducted over a 10-year period in Spain involving 175 chronic renal patients submitted to PD, the most common agent was *C. parapsilosis* (4/10), followed by *C. albicans* (2/10), and *C. tropicalis*, *C. glabrata*, *C. famata* and *Fusarium* sp., one case each.[39] To explain this high presence of *C. parapsilosis sensu stricto* in certain clinical samples, some authors have proposed that this species is better adapted to the human environment as a skin commensal and thus can be easily transmitted from person to person, especially through manual contact by health professionals with patients that require intensive use of medical devices such as catheters.[40] According to this hypothesis, *C. orthopsilosis* and especially *C. metapsilosis* are found in smaller quantities in these samples because they are not related to human commensalism and present ecological niches not associated with mammals.[40–42]

Table 2. Antifungal susceptibility *in vitro* of yeast species isolated from patients undergoing peritoneal dialysis, at the University Hospital of Botucatu Medical School, São Paulo, Brazil.

Yeast (n)	Antifungals (μg/ml) range MIC	S	DDS	R
Candida parapsilosis (n = 9)	Am B (1)	9	–	–
	Flu (0.125–4)	7	2	–
	Vor (0.06–0.125)	9	–	–
	Cas (0.125–1)	9	–	–
Candida orthopsilosis (n = 4)	Am B (0.5–1)	4	–	–
	Flu (0.125–32)*	1	–	3
	Vor (0.03–0.06)	4	–	–
	Cas (0.03–1)	4	–	–
Candida albicans (n = 5)	Am B (0.5–1)	5	–	–
	Flu (1–2)	5	–	–
	Vor (0.03)	5	–	–
	Cas (0.06–0.125)	5	–	–
Candida tropicalis (n = 3)	Am B (1–2)	3	–	–
	Flu (1–2)	3	–	–
	Vor (0.03–0.06)	3	–	–
	Cas (0.125–0.06)	3	–	–
Candida guilliermondii (n = 1)	Am B (2)	1	–	–
	Flu (8)	1	–	–
	Vor (0.06)	1	–	–
	Cas (1)	1	–	–
Kodamaea ohmeri (n = 1)	Am B (0.5)	1	–	–
	Flu (32)	–	1	–
	Vor (0.125)	1	–	–
	Cas (0.06)	1	–	–

n: number of isolates; AmB: amphotericin B; Flu: fluconazole; Vor: voriconazole; Cas: caspofungin; S: susceptible; DDS: dose-dependent susceptibility; R: resistant; *$P<.05$ (significant comparison between *Candida orthopsilosis* vs. *Candida parapsilosis*).

Biofilm production is the one of the most important virulence factors exhibited by yeasts, most commonly in patients using a catheter or other medical devices, and can be estimated through various methods, including XTT, which measures metabolic activity of cells forming the biofilm.[25,43] We have noted herein that isolates of *C. albicans* species were the largest producers of biofilm, whereas the isolates of *C. parapsilosis* species presented the lowest production, though with a marked variation among the isolates. Douglas et al. (2003), Ramage et al. (2012). and Silva-Dias et al (2015)[43–45] also observed greater *in vitro* biofilm production in isolates of *C. albicans* and lower production in *C. parapsilosis*. It is also important to note that high biofilm production also occurred in some isolates from non-albicans group, including *C. tropicalis* and *C. orthopsilosis* and in those considered rare species, such as *K. ohmeri* and *C. guilliermondii*, although without statistical significance, due to the low number of isolates evaluated in these groups.

In relation to the antifungals herein tested, echinocandin (caspofungin) and the voriconazole proved to be effective in all isolates of the several species, therefore confirming that these antifungals have a wide spectrum of action against these agents. The results on fluconazole draw our attention to the decreased susceptibility, especially in the isolates of *C. orthopsilosis* species, which differed statistically from those of *C. parapsilosis*, probably indicating a biological feature of this cryptic species. In addition, the widespread use of fluconazole prophylaxis against fungal infections in immunocompromised patients, and in the treatment of FP in most specialized centers may have favored the selection of strains that present resistance or decreased susceptibility to this antifungal, especially non-albicans *Candida* species.[45] The literature contains other reports indicating that *C. orthopsilosis* and *C. metapsilosis* species are less susceptible to fluconazole.[46,47] The isolate of *K. ohmeri* species presented dose-dependent susceptibility to fluconazole, corroborating previous studies that indicate a probable natural resistance of this species to fluconazole.[32–34] These findings should be an alert for the nephrologists working in PD, since fluconazole is one of the antifungals proposed for treatment of FP, according to international guidelines.[8]

Considering the data as a whole and looking to the patients' outcomes that include six fatalities, it is interesting to note the particular importance of the *psilosis* group and also the occurrence of the "rare" species *K. ohmeri*. In addition, the susceptibility to fluconazole also seems to be relevant, since this was the antifungal chosen for the treatment in all patients, while resistance and dose-dependent susceptibility were observed mainly in these critical cases.

In conclusion, the results of this study indicate that different yeast species are causing FP in patients undergoing PD, with a clear prevalence of *C. parapsilosis sensu stricto* species. In addition, by presenting variations in susceptibility to antifungals and a wide range in biofilm production levels, these yeasts might exhibit different manners of infecting and interacting with the host, thus contributing to the complexity and severity of the clinical features. The correct identification and characterization of these agents, besides providing improvements in the treatment protocols of PD-related FP, may also offer valuable clues about how to avoid new infections, thereby producing positive impacts on quality of life and survival of the patients.

Acknowledgements

We thank Terue Sadatsune, Carlos Henrique Camargo, Alessandro Lia Mondelli, and Katheryne Benine Martins for their help in providing the samples and suggestions.

This work was financially supported by FAPESP (Process: 2011/03836-7 and 2012/07741-3).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Supplementary material

Supplementary material is available at *Medical Mycology* online (http://www.mmy.oxfordjournals.org/).

References

1. Barretti P, Bastos KA, Dominguez J et al. Peritonitis in Latin America. *Perit Dial Int* 2007; **27**: 332–339.
2. Dong J, Chen Y. Impact of the bag exchange procedure on risk of peritonitis. *Perit Dial Int* 2010; **30**: 440–447.
3. Ram R, Swarnalatha G, Neela P et al. Fungal peritonitis in patients on continuous ambulatory peritoneal dialysis: a single-centre experience in India. *Nephron Clin Pract* 2008; **110**: c207–c212.
4. García-Agudo R, García-Martos P. Clinical and microbiological aspects of fungal peritonitis in peritoneal dialysis. *Nefrologia* 2009; **29**: 506–517 [in Spanish].
5. Prasad KN, Prasad N, Gupta A et al. Fungal peritonitis in patients on continuous ambulatory peritoneal dialysis: a single centre Indian experience. *J Infect* 2004; **48**: 96–101.
6. Prasad N, Gupta A. Fungal peritonitis in peritoneal dialysis patients. *Perit Dial Int* 2005; **25**: 207–222.
7. Indhumathi E, Chandrasekaran V, Jagadeswaran D et al. The risk factors and outcome of fungal peritonitis in continuous ambulatory peritoneal dialysis patients. *Indian J Med Microbiol* 2009; **27**: 59–61.
8. Li PK, Szeto CC, Piraino B et al. Peritoneal dialysis-related infections recommendations: 2010 update. *Perit Dial Int* 2010; **30**: 393–423.
9. Miles R, Hawley CM, McDonald SP et al. Predictors and outcomes of fungal peritonitis in peritoneal dialysis patients. *Kidney Int* 2009; **76**: 622–628.
10. Chang TI, Kim HW, Park JT et al. Early catheter removal improves patient survival in peritoneal dialysis patients with fungal peritonitis: results of ninety-four episodes of fungal peritonitis at a single center. *Perit Dial Int* 2011; **31**: 60–66.
11. Khan FY, Elsayed M, Anand D et al. Fungal peritonitis in patients undergoing continuous ambulatory peritoneal dialysis in Qatar. *J Infect Dev Ctries* 2011; **5**: 646–651.
12. Matuszkiewicz-Rowinska J. Update on fungal peritonitis and its treatment. *Perit Dial Int* 2009; **29** Suppl 2: S161–S165.
13. Bibashi E, Memmos D, Kokolina E et al. Fungal peritonitis complicating peritoneal dialysis during an 11-year period: report of 46 cases. *Clin Infect Dis* 2003; **36**: 927–931.
14. Levallois J, Nadeau-Fredette AC, Labbé AC et al. Ten-year experience with fungal peritonitis in peritoneal dialysis patients: antifungal susceptibility patterns in a North American center. *Int J Infect Dis* 2012; **16**: e41–e43.
15. Richardson MD. Changing patterns and trends in systemic fungal infections. *J Antimicrob Chemother* 2005; **56** Suppl 1: i5–i11.
16. Aperis G, Myriounis N, Spanakis EK et al. Developments in the treatment of candidiasis: more choices and new challenges. *Expert Opin. Investig Drugs* 2006; **15**: 1319–1336.
17. Trofa D, Gácser A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. *Clin Microbiol Rev* 2008; **21**: 606–625.
18. Barretti P, Moraes TM, Camargo CH et al. Peritoneal dialysis-related peritonitis due to *Staphylococcus aureus*: a single-center experience over 15 years. *PLoS One* 2012; **7**: e31780.
19. Camargo CH, Cunha Mde L, Caramori JC et al. Peritoneal dialysis-related peritonitis due to coagulase-negative *Staphylococcus*: a review of 115 cases in a Brazilian center. *Clin J Am Soc Nephrol* 2014; **9**: 1074–1081.
20. McCullough MJ, DiSalvo AF, Clemons KV et al. Molecular epidemiology of *Blastomyces dermatitidis*. *Clin Infect Dis* 2000; **30**: 328–335.
21. White TJ, Bruns T, Lee S et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Shinsky J et al., eds. *PCR Protocols: A Guide to Methods and Applications*. Academic Press; 1990: 315–322.
22. Kurtzman CP, Robnett CJ. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Van Leeuwenhoek* 1998; **73**: 331–371.
23. Altschul SF, Gish W, Miller W et al. Basic local alignment search tool. *J Mol Biol* 1990; **215**: 403–410.
24. Irinyi L, Serena C, Garcia-Hermoso D et al. International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database–the quality controlled standard tool for routine identification of human and animal pathogenic fungi. *Med Mycol* 2015; **53**: 313–337.
25. Chandra J, Mukherjee PK, Ghannoum MA. In vitro growth and analysis of *Candida* biofilms. *Nat Protoc* 2008; **3**: 1909–1924.
26. Marcos-Zambrano LJ, Escribano P, Bouza E et al. Production of biofilm by *Candida* and non-*Candida* spp. isolates causing fungemia: comparison of biomass production and metabolic activity and development of cut-off points. *Int J Med Microbiol* 2014; **304**: 1192–1198.
27. Clinical and Laboratory Standards Institute. *Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard*. CLSI, Document M27-A3, 2008.
28. Clinical and Laboratory Standards Institute. *Reference method for broth dilution antifungal susceptibility testing of yeasts. Fourth Informational Supplement*. CLSI, Document M27-S4, 2012.
29. Chen YC, Eisner JD, Kattar MM et al. Identification of medically important yeasts using PCR-based detection of DNA sequence polymorphisms in the internal transcribed spacer 2 region of the rRNA genes. *J Clin Microbiol* 2000; **38**: 2302–2310.
30. Leaw SN, Chang HC, Sun HF et al. Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. *J Clin Microbiol* 2006; **44**: 693–699.

31. Borman AM, Linton CJ, Miles SJ et al. Molecular identification of pathogenic fungi. *J Antimicrob Chemother* 2008; **61** Suppl 1: i7–12.
32. Yang BH, Peng MY, Hou SJ et al. Fluconazole-resistant *Kodamaea ohmeri* fungemia associated with cellulitis: case report and review of the literature. *Int J Infect Dis* 2009; **13**: e493–e497.
33. Yanghua Q, Weiwei W, Yang L et al. Isolation, identification, and antifungal susceptibility test for *Kodamaea ohmeri*: a case report on endocarditis. *J Med Coll PLA* 2010; **25**: 252–256.
34. Al-Sweih N, Khan ZU, Ahmad S et al. *Kodamaea ohmeri* as an emerging pathogen: a case report and review of the literature. *Med Mycol* 2011; **49**: 766–770.
35. Santino I, Bono S, Borruso L et al. *Kodamaea ohmeri* isolate from two immunocompromised patients: first report in Italy. *Mycoses* 2013; **56**: 179–181.
36. Chang YT, Wang HC, Wang MC et al. Rapid identification of bacteria and *Candida* pathogens in peritoneal dialysis effluent from patients with peritoneal dialysis-related peritonitis by use of multilocus PCR coupled with electrospray ionization mass spectrometry. *J Clin Microbiol* 2014; **52**: 1217–1219.
37. Yinnon AM, Gabay D, Raveh D et al. Comparison of peritoneal fluid culture results from adults and children undergoing CAPD. *Perit Dial Int* 1999; **19**: 51–55.
38. Chen CM, Ho MW, Yu WL et al. Fungal peritonitis in peritoneal dialysis patients: effect of fluconazole treatment and use of the twin-bag disconnect system. *J Microbiol Immunol Infect* 2004; **37**: 115–120.
39. García-Martos P, Gil de Sola F, Marín P et al. Fungal peritonitis in ambulatory continuous peritoneal dialysis: description of 10 cases. *Nefrologia* 2009; **29**: 534–539 [in Spanish].
40. Tavanti A, Davidson AD, Gow NAR et al. *Candida orthopsilosis* and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* groups II and III. *J Clin Microbiol* 2005; **43**: 284–292.
41. Sai S, Holland LM, McGee CF et al. Evolution of mating within the *Candida parapsilosis* species group. *Eukaryot Cell* 2011; **10**: 578–587.
42. Bertini A, De Bernardis F, Hensgens LAM et al. Comparison of *Candida parapsilosis, Candida orthopsilosis,* and *Candida metapsilosis* adhesive properties and pathogenicity. *Int J Med Microbiol* 2013; **303**: 98–103.
43. Ramage G, Rajendran R, Sherry L et al. Fungal biofilm resistance. *Int J Microbiol* 2012; **2012**: 528521.
44. Douglas LJ. *Candida* biofilms and their role in infection. *Trends Microbiol* 2003; **11**: 30–36.
45. Silva-Dias A, Miranda IM, Branco J et al. Adhesion, biofilm formation, cell surface hydrophobicity, and antifungal planktonic susceptibility: relationship among *Candida* spp. *Front Microbiol* 2015; **6**: 205.
46. Dalazen D, Zanrosso D, Wanderley L et al. Comparison of susceptibility profile among clinical isolates of oral and vulvovaginal *Candida* spp. in southern Brazil. *J Bras Patol Med Lab* 2011; **47**: 33–38 [in Portuguese].
47. Feng X, Ling B, Yang G et al. Prevalence and distribution profiles of *Candida parapsilosis, Candida orthopsilosis* and *Candida metapsilosis* responsible for superficial candidiasis in a Chinese university hospital. *Mycopathologia* 2012; **173**: 229–234.