

Yeast isolation and identification in water used in a Brazilian hemodialysis unit by classic microbiological techniques and Raman spectroscopy

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ABSTRACT

The use of poorly treated water in hemodialysis centers may lead to fungal contamination, which poses a serious threat to immunologically debilitated hemodialysis patients. This study aimed to isolate and identify yeast species in the water of a Brazilian hemodialysis center by using classic microbiological techniques and Raman spectroscopy. For 12 months, a total of 288 water samples were collected from different points of the hemodialysis treatment distribution center. One hundred and forty-six yeast species were isolated and identified in the samples that tested positive for the presence of yeasts such as *Candida parapsilosis* (100 isolates, or 68.50%), *C. guilliermondii* (17 isolates, or 11.65%), *Rhodotorula mucilaginosa* (23 isolates, or 15.75%), *R. glutinis* (three isolates, or 2.05%), and *Trichosporon inkin* (three isolates, or 2.05%). Yeast susceptibility to the antifungal fluconazole was also assayed. Only two *C. guilliermondii* isolates were resistant to fluconazole: the minimal inhibitory concentrations were higher than 64 µg/mL. The different yeast species present in the water of a Brazilian hemodialysis center call for more effective water disinfection procedures in this unit. Raman spectroscopy is an excellent tool to identify yeast species and is potentially applicable in routine water monitoring in hemodialysis units.

Key words | hemodialysis center, microbiological quality, Raman spectroscopy, water, yeasts

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INTRODUCTION

Patients with renal failure often have a compromised immune system and are more susceptible to infection by pathogens and opportunistic microorganisms, including fungi. During each hemodialysis session, renal failure patients are exposed to 25 times more water than a healthy subject drinks on a typical day, which corresponds to weekly and yearly exposure of hemodialysis patients to 400 and 20,000 L of water, respectively (Pontoriero *et al.* 2005; Nystrand 2008; Schiavano *et al.* 2014). Furthermore, the thin dialysis membrane between the patient's blood and the dialysis fluid might make direct

transfer of toxins to the bloodstream easier, adversely affecting the hemodialysis patient's outcome (Hasegawa *et al.* 2015). Therefore, knowing and monitoring the chemical and microbiological purity of the water employed in dialysis is crucial (Pontoriero *et al.* 2003).

Fungi account for a spectrum of diseases that range from minor superficial skin and mucous membrane infections to life-threatening systemic involvement of internal organs (Prasad & Gupta 2005). In recent years, fungal infections have received increasing attention, especially in hospitals

where fungi have emerged as a leading cause of hospital-acquired infections (Richardson & Lass-Florl 2008). However, literature data on the isolation and identification of these organisms in dialysis water are scarce.

The source of the water used in hemodialysis consists basically of drinking water, purified by various techniques. Origin determines water composition and quality. Brazilian hemodialysis units frequently use reverse osmosis to treat water. Typically, water from the public supply passes through mechanical filters, water softeners, charcoal filters, deionizers, and the reverse osmosis filter. Then, this water is stored in tanks for subsequent distribution through polyvinyl chloride (PVC) tubing to the whole hemodialysis unit water system (Montanari *et al.* 2009). The system tubes, tanks, and faucets can become yeast reservoirs where biofilms might form. These biofilms are extremely difficult to eradicate by chemical or mechanical means (Silva *et al.* 1996).

The American Association for the Advancement of Medical Instrumentation (AAMI 2004), the European Best Practice Guidelines for Hemodialysis (EBPG 2002), and the Directive of the Brazilian Ministry of Health (Brasil Ministério da Saúde 2004) have issued criteria for dialysis water. However, the recommendations are limited: they do not advise investigation into other microorganisms and metabolites, such as *Pseudomonas* species, mycobacteria, fungi, and mycotoxins (Carson *et al.* 1988; Pires-Gonçalves *et al.* 2008; Figel *et al.* 2013).

Potential sources of infection include the skin (through repeated skin barrier and integrity disruption due to the nature of the vascular access), the dialysis water treatment system, and dialyzer reuse (Jaber 2005). In Brazil, dialyzers are nearly 100% reused in dialysis facilities (Manfredi & Canziani 2005; Toniolo *et al.* 2016). According to resolution number 154 issued on June 15th, 2004, dialyzers and arterial/venous lines can be used for the same patient up to 12 and 20 times in the case of manual and automatic reprocessing, respectively (Brasil Ministério da Saúde 2004). The use of manual reprocessing in Brazil will only be allowed until 2018, possibly because it is difficult to reprocess dialyzers by this method. The use of automated reprocessing allows reprocessing techniques to be standardized, which minimizes potential risks and consequently reduces the risk of infection (Robinson & Feldman 2005; AAMI 2008; Brasil

Ministério da Saúde 2014; Toniolo *et al.* 2016). Unfortunately, employees often neglect the quality of water and dialyzer reuse during hemodialysis therapy, especially in developing countries. Such neglect causes significant morbidity and mortality among renal failure patients.

The time that is necessary to identify pathogens is an important determinant of infection-related mortality rates among hospitalized patients. Rapid identification techniques significantly reduce mortality and costs associated with infectious diseases (Doern *et al.* 1994; Maquelin *et al.* 2003). Most commercially available identification systems that are routinely used in hospitals rely on the microorganism's physiological and nutritional characteristics. These systems require a pure microbial culture and a large inoculum. Consequently, a turnaround time ranging from 24 h (e.g. for *Staphylococcus aureus*) to 5 days (e.g. for *Candida* species) commonly elapses between the moment the patient's material is received and the moment the identification results are presented to the clinician (Maquelin *et al.* 2003).

Vibrational spectroscopy techniques like Raman spectroscopy allow for simple, rapid, and accurate microbial identification. According to Guimarães *et al.* (2006), the Raman scattering effect is an inelastic process that occurs when a sample is illuminated with a strong monochromatic light source (such as a laser beam). In this process, energy from the incident photons is transferred to the sample molecules, which excites the molecules to high vibrational modes. Scattered photons have lower frequency than incident photons because energy is lost along the scattering process. The shift in the excitation radiation frequency corresponds to the different vibrational frequencies of the sample material molecules. Because each molecule has its own characteristic Raman spectrum, Raman spectroscopy aids determination of sample composition.

Taking account of the high risk that fungi pose to hemodialysis patients, the lack of more effective measures to disinfect dialysis water, and dialyzers reuse in Brazilian hemodialysis units, we aimed to isolate and identify yeast species that were present in a hemodialysis center located in the interior of the state of São Paulo, Brazil, by using classic methods based on culture procedures and Raman spectroscopy analysis. We also evaluated yeast susceptibility to fluconazole.

MATERIALS AND METHODS

Samples and collection points

This study was approved by the Research Ethics Committee of the University (REC) of Franca and was registered under number 023/05.

Over a 12-month period, a total of 288 water samples (Table 1) were collected in a hemodialysis unit affiliated with a public health service located in the interior of the state of São Paulo, Brazil. The water samples were collected from different points of the hemodialysis treatment distribution system represented by the supply network (I); post-osmosis room (II); dialysis rooms A (III), B (IV), and C (V); osmosis reservoir (VI); reuse rooms A (VII), B (VIII), and C (IX); and dialysis machines A (X), B (XI), and C (XII) (Table 1).

The samples were collected according to the methodology recommended by *Standard Methods* (APHA, AWWA, WEF 2005). Each sample (1,000 mL each) was collected after free flow for 3 to 5 min. The samples collected from the water supply network (I) were treated with 1 mL of 1.8% sodium thiosulfate per liter, to remove residual chlorine (*Standard Methods* APHA, AWWA, WEF 2005).

All the water samples collected from different points (1,000 mL each) were filtered through a cellulose acetate membrane (0.22- μ M pore size, Millipore, SP, Brazil) on a filtration ramp (Sartorius). The membrane was placed on a Sabouraud dextrose agar (SDA) plate (Difco) supplemented with chloramphenicol at 30 °C for 7 days. The flasks employed to collect water at the different points were sterilized in an autoclave. Only sterilized flasks were used for each collection point at the hemodialysis unit.

Organism identification

The 288 water samples were processed, and the yeast species were defined on the basis of colony morphology, germ tube test, chlamyospore formation on Tween 80 cornmeal agar (Difco), and pattern of assimilation of a variety of carbon and nitrogen sources. The results were compiled and analyzed as previously described by Kurtzman & Fell (1998). All the fungal isolates were stored at -80 °C in the microbiology laboratory of the University of Franca, SP, Brazil. Before being used, frozen stocks were recovered from storage by growth on Chromagar Candida medium (CHROMagar Microbiology, Paris, France) or SDA supplemented with chloramphenicol for Candida and non-Candida species, respectively.

Yeast identification by Raman spectroscopy

To identify the yeast isolates, the following yeasts were used as standards: *Candida parapsilosis* (ATCC 22019), *Candida guilliermondii* (IAL 112), *Rhodotorula mucilaginosa* (ATCC 9450), *R. glutinis* (ATCC 32766), and *Trichosporon inkin* (IAL 108), obtained from the American Type Culture Collection (ATCC) and the Adolfo Lutz Institute (IAL) in São Paulo. The yeast-positive samples isolated from water used in a hemodialysis center had been previously identified by phenotypic methods and processed as *C. parapsilosis*, *C. guilliermondii*, *R. mucilaginosa*, *R. glutinis*, and *T. inkin*. To identify the organisms by Raman spectrometry, the yeasts (five species) were seeded in Sabouraud (Difco) agar with chloramphenicol (Sigma) and incubated at 30 °C for 24 h. After this period, suspensions with turbidity 0.5 in the McFarland scale were prepared in sodium chloride solution (0.85%) with the aid of a sterile bacteriological handle

Table 1 | Number of samples collected at each point of a hemodialysis center water distribution system and number of yeasts counted

Collection sites	Number of samples collected at each point ^a	Number of yeasts (CFU/mL \pm SD)
Water supply network (I)	30	1.1 \pm 77.5
Post-osmosis room (II)	30	0.6 \pm 55.2
Dialysis room A (III)	30	0.9 \pm 64.2
Dialysis room B (IV)	30	1.4 \pm 102.9
Dialysis room C (V)	30	0.7 \pm 68.3
Osmosis room (VI)	30	0.1 \pm 10.3
Reuse system A (VII)	10	0.4 \pm 132.2
Reuse system B (VIII)	10	0.3 \pm 173.2
Reuse system C (IX)	10	0.01 \pm 2.1
Dialysis machine A (X)	27	2.6 \pm 526.1
Dialysis machine B (XI)	23	5.3 \pm 809.5
Dialysis machine C (XII)	28	4.4 \pm 340.3

CFU/mL, colony forming units per milliliter; SD, standard deviation.

^aTotal of 288 collected water samples (1,000 mL each sample).

(Probac, São Paulo, SP, Brazil). In a spectrophotometer, the inoculums were adjusted at 530 nm for yeast isolates (five species). Sixty successive 30- μ L increments of a suspension with approximately 1×10^6 yeasts per mL were added to a quartz cuvette containing 1.1 mL of isotonic solution. This procedure was repeated for each yeast species separately (60 repetitions), and the protocol was chosen after initial tests. Successive additions of these increments avoided Rayleigh scattering from high gradients as well as emergence of a weak Raman signal due to diluted suspension.

As previously described by Mello *et al.* (2006, 2008), the Raman spectra were collected with an OceanOptics low-resolution (approximately 15/cm) Raman spectrometer (Dunedin, FL, USA) mod. R-2001 coupled to a near-infrared 785-nm multimode diode laser (adjusted to deliver 300 mW on the sample) and to a thermoelectrically cooled 2048-element CCD array detector (to measure spectra from 200 to 2,800/cm). The instrument was wavelength-calibrated with isopropyl alcohol, and the dark current was subtracted from all the acquired spectra. Three low-resolution Raman spectra with an integration time of 30 s were acquired for each of the yeast samples evaluated in this work; the final spectrum was taken as the average of these three spectra. The Rbase version 3.0.1 (Raman Systems Inc., Watertown, MA, USA) software was used to control the spectrometer and to record the data (Mello *et al.* 2006, 2008).

Data analysis

Before multivariate statistical analyses, principal component analysis (PCA), a well-known method that reduces dimensionality in a data set, aided data reduction. The maximum number of $n - 1$ PCs was calculated (n being the number of spectra in the analysis), which typically accounted for 99 to 100% of the variation in the data set (Maquelin *et al.* 2002).

Susceptibility to fluconazole

The susceptibility of all the identified yeast species to fluconazole was tested by the agar disc diffusion method described in the National Committee for Clinical Laboratory Standards (NCCLS 2003), Clinical and Laboratory Standards Institute (CLSI) current document M44. For the

disc diffusion tests, plates containing Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) supplemented with 2% glucose and methylene blue (0.5 μ g/mL) were used. The plates were incubated in air at 35–37 °C and read at 24–48 h. The minimum inhibitory concentration (MIC) interpretative criteria used for fluconazole were those published by the NCCLS. The *Candida krusei* (ATTC 6258) and *Candida albicans* (ATCC 90028) strains, which are resistant and susceptible to fluconazole, respectively, served as quality controls (NCCLS 2003).

The MIC assays were conducted in triplicate by the broth microdilution method in 96-well microplates. A procedure adapted from the approved standard M27-A2 (NCCLS 2002) was followed. Only the two yeast isolates (*C. guilliermondii*) that were the most resistant to fluconazole as revealed by the disc diffusion test were used in the MIC assays. U-bottom microdilution plates containing 100 μ L of the twofold serial dilutions of the antifungal drugs in standard RPMI 1640 buffered with morpholinepropanesulfonic acid (MOPS) (both from Sigma Chemical Co., St Louis, MO) were inoculated with 100 μ L of each inoculum. For two yeast isolates, the inoculum were adjusted at 530 nm in a spectrophotometer, to obtain cell concentrations ranging from 0.5×10^5 to 2.5×10^5 colony-forming units (CFU) per mL. After inoculation, the microplates were incubated at 35 °C, and MICs were determined after 48 h.

RESULTS

A total of 288 water samples were collected from the hemodialysis center; 136 (47.2%) samples tested positive for yeasts. The yeasts (146) isolated from the positive samples belonged to the following species: *Candida parapsilosis* (100 isolates, or 68.50%), *C. guilliermondii* (17 isolates, or 11.65%), *Rhodotorula mucilaginosa* (23 isolates, or 15.75%), *R. glutinis* (three isolates, or 2.05%), and *Trichosporon inkin* (three isolates, or 2.05%) (see Table 2).

Regarding the collection point, isolates were detected as follows:

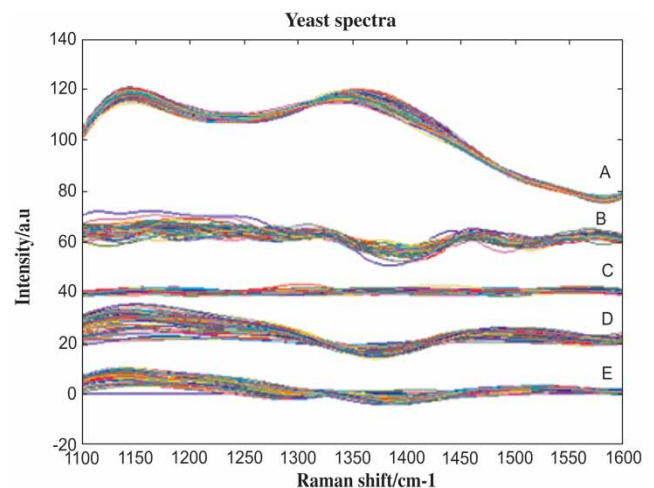
- Water supply network (I): 16 isolates (*C. parapsilosis* [eight], *R. mucilaginosa* [five], *C. guilliermondii* [two], and *R. glutinis* [one]).

Table 2 | Distribution of isolated yeast species in the water distribution system of a hemodialysis center

Collection sites	<i>Candida parapsilosis</i>		<i>Candida guilliermondii</i>		<i>Rhodotorula mucilaginosa</i>		<i>Rhodotorula glutinis</i>		<i>Trichosporon inkin</i>	
	n	%	n	%	n	%	n	%	n	%
Water supply network (I)	8	50.0	2	12.5	5	31.25	1	6.25	0	0
Post-osmosis room (II)	7	58.3	2	16.7	3	25.0	0	0	0	0
Dialysis room A (III)	8	57.2	3	21.4	3	21.4	0	0	0	0
Dialysis room B (IV)	14	77.7	3	16.7	0	0	0	0	1	5.6
Dialysis room C (V)	12	63.1	4	21.1	2	10.5	0	0	1	5.3
Osmosis room (VI)	6	54.6	2	18.2	2	18.2	1	9.0	0	0
Reuse system A (VII)	2	66.6	0	0	1	33.4	0	0	0	0
Reuse system B (VIII)	1	50.0	0	0	1	50.0	0	0	0	0
Reuse system C (IX)	1	50.0	0	0	0	50.0	1	0	0	0
Dialysis machine A (X)	12	80.0	1	6.7	2	13.3	0	0	0	0
Dialysis machine B (XI)	10	76.9	0	0	3	23.1	0	0	0	0
Dialysis machine C (XII)	19	90.6	0	0	1	4.7	0	0	1	4.7

- Post-osmosis room (II): 12 isolates (*C. parapsilosis* [seven], *R. mucilaginosa* [three], and *C. guilliermondii* [two]).
- Dialysis rooms A (III), B (IV), and C (V): 51 isolates (*C. parapsilosis* [34], *C. guilliermondii* [10], *R. mucilaginosa* [five], and *T. inkin* [two]).
- Reverse osmosis reservoir (VI): 11 isolates (*C. parapsilosis* [six], *C. guilliermondii* [two], *R. mucilaginosa* [two], and *R. glutinis* [one]).
- The smallest number of yeasts was isolated from the reuse systems A (VII), B (VIII), and C (IX): a total of seven isolates (*C. parapsilosis* [four], *R. mucilaginosa* [two], and *R. glutinis* [one]).
- The largest number of yeasts emerged in the dialysis machines A (X), B (XI), and C (XII): a total of 49 isolates (*C. parapsilosis* [41], *R. mucilaginosa* [six], *C. guilliermondii* [one], and *T. inkin* [one]) (see Table 2).

Figure 1 presents the Raman spectroscopy results. Different spectra, typical of various yeast species, were recorded. These spectra were submitted to multivariate analysis and were transformed into matrixes for analysis of the results. PCA allowed us to identify which yeasts were present in the hemodialysis center water. The spectral groups, obtained from the main information, decreased, which showed that different yeast species were present in

**Figure 1** | Representation of the Raman spectra of five analyzed yeasts: A, *R. mucilaginosa*; B, *R. glutinis*; C, *T. inkin*; D, *C. guilliermondii*; E, *C. parapsilosis*.

the water, namely *C. parapsilosis*, *R. mucilaginosa*, *C. guilliermondii*, *R. glutinis*, and *T. inkin* (Figure 2).

Susceptibility to fluconazole was assayed by the agar disc diffusion method; the 146 yeasts isolated from the hemodialysis center water samples were tested. Only two *C. guilliermondii* isolates were resistant to fluconazole. Susceptibility of the two *C. guilliermondii* isolates to fluconazole was also assayed by the microdilution assay. The two isolates presented minimum inhibitory

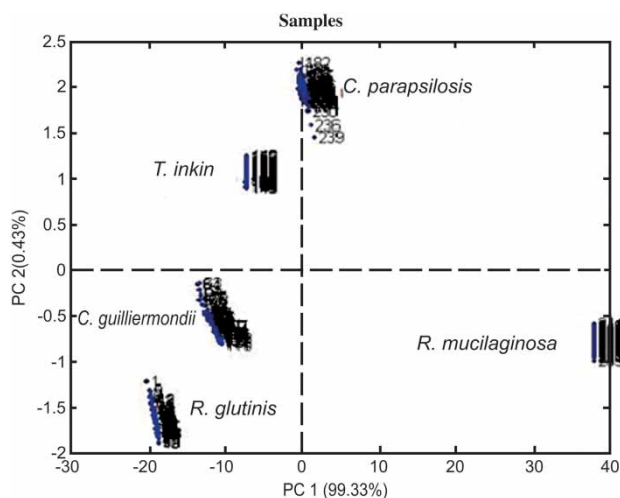


Figure 2 | Principal component analysis (PCA) of the Raman spectra of the yeasts *R. mucilaginosa*, *R. glutinis*, *T. inkin*, *C. guilliermondii*, and *C. parapsilosis*.

concentrations higher than 64 µg/mL, which confirmed their resistance.

DISCUSSION

Improper maintenance of hemodialysis water systems and dialyzer reprocessing may have adverse effects on hemodialysis patients (Borges et al. 2007; Canada et al. 2014). If the water is not treated properly, potentially harmful chemical or biological contaminants may be transferred to the patients. This exposure may lead to pyrogenic reactions and sepsis (Calderaro & Heller 2001; Varo et al. 2007; Canada et al. 2014). Dialysis patients often have compromised immune systems and are therefore more susceptible to infection by pathogens and opportunistic microorganisms, like fungi (Schiavano et al. 2014).

Over the last few years, the incidence of mycotic infections has increased progressively. Fungi that were once considered as nonpathogenic or less virulent are now recognized as a primary cause of morbidity and mortality in immunocompromised and severely ill patients (Pfaller & Diekema 2007; Deorukhkar et al. 2014). Although *Candida albicans* is the most prevalent species involved in both mucocutaneous and disseminated infections, the incidence of candidiasis due to non-*albicans* *Candida* (NAC) spp. is increasing (Sardi et al. 2013; Deorukhkar et al. 2014). This

context has created the need to identify clinically significant yeast isolates rapidly and accurately so that appropriate anti-fungal therapy can be promptly initiated. The adequacy of the initial, empirical treatment has proven to be of paramount significance in terms of morbidity and mortality in critically ill patients with an invasive *Candida* infection (Lee et al. 2002; Ibelings et al. 2005).

In this study, 136 (47.2%) of the 288 water samples collected from a hemodialysis center tested positive for yeasts. Yeasts (146) isolated from the positive samples belonged to the following species: *Candida parapsilosis* (100 isolates, or 68.50%), *C. guilliermondii* (17 isolates, or 11.65%), *Rhodotorula mucilaginosa* (23 isolates, or 15.75%), *R. glutinis* (three isolates, or 2.05%), and *Trichosporon inkin* (three isolates, or 2.05%).

Arvanitidou et al. (2000) assessed a total of 255 paired samples taken from three different points of the hemodialysis unit system (feed water, treated water, dialysate) of each of the 85 hemodialysis centers in Greece. Overall, 21 (8.2%) of the 255 samples tested positive for yeasts. Yeasts were identified in three (3.5%) tap water samples (samples A), seven (8.2%) treated water samples (samples B), and 11 (12.9%) dialysate samples (samples C). Yeast occurrence was significantly higher in dialysate samples compared with tap water samples. Our study demonstrated that the water entering the dialysis machines A (X), B (XI), and C (XII) contained more yeasts than other collection points. The results obtained in our hemodialysis center suggested that the water disinfection and dialyzer reuse procedures were not efficient and could impact patients negatively.

Pires-Gonçalves et al. (2008) analyzed 45 water samples collected from different sites of a hemodialysis unit. Forty-one fungi were isolated from dialysate samples; among these isolates, 26 (63%) were yeasts. *C. parapsilosis* and *R. rubra* were detected in 84.6% and 15.4% of these dialysate samples, respectively. All the *R. rubra* isolates were resistant to fluconazole, an efficient and little toxic drug that is frequently used in Brazil for prophylaxis and/or treatment of *Candida* spp. infections (Colombo et al. 2002). Here, the predominant species was *C. parapsilosis*, which was isolated from all the collection points. This species has emerged as a major pathogen in nosocomial candidemia in Brazilian tertiary care hospitals (Medrano et al. 2006; Pereira et al. 2010). Only two *C. guilliermondii* isolates out of the 146

isolated yeasts were resistant to fluconazole. In an important study conducted by Pfaller *et al.* (2006) on several species of different countries, the authors suggested that *C. guilliermondii* may be an emerging pathogen in Latin America but not in other regions of the world. This species clearly exhibits decreased susceptibility to fluconazole.

Another study by Pires *et al.* (2013) evidenced the ability of a considerable number of *C. parapsilosis* (34/53, 64%) isolates from a hemodialysis unit to form biofilms. Biofilms have increased levels of resistance to the most commonly used antifungal agents (Ramage *et al.* 2001; Pierce *et al.* 2008). For hemodialysis, biofilm formation represents the starting point of biofouling, resistance to antibiotic chemotherapy or disinfection, and microbial regrowth (Stewart & Costerton 2001; Ramage *et al.* 2012). In the particular case of the present study, isolation of *C. parapsilosis* in all the evaluated hemodialysis water collection sites pointed to the need for appropriate and regular monitoring of hemodialysis units by the local health authorities. Implementing measures to avoid biofilm formation in an environment where renal failure patients (who are more susceptible to fungal infection) receive treatment is essential to ensure patient safety.

Employees often neglect the quality of water and dialyzer reuse during hemodialysis therapy, which causes significant morbidity and mortality among renal failure patients. According to Ibelings *et al.* (2005), clinical microbiologists face an important challenge when they have to select a highly specific and sensitive system to identify yeasts rapidly and accurately. Conventional microbiological identification is based on an extensive series of biochemical assays that are performed after an obligatory culture period that is sufficient to obtain a biomass of 10^6 – 10^8 cells (Warren & Hazen 1999; Ibelings *et al.* 2005). This standard identification method is time-consuming with an unavoidable turn-around time of 48–96 h. Another faster alternative would be to use vibrational spectroscopic techniques (Raman and infrared spectroscopy), which yield molecule-specific spectra. When applied to complex biological samples, such as cells or tissues, the spectra are a summation of the signal contributions from all the molecular species, and therefore reflect the overall molecular composition of a sample. Such spectra are highly suitable to identify bacteria and yeasts fast because they are

reproducible and distinct for different bacterial and fungal species (Naumann *et al.* 1995; Timmins *et al.* 1998; Tintelnot *et al.* 2000; Udelhoven *et al.* 2000; Ibelings *et al.* 2005).

In our study, we used Raman spectroscopy together with a statistical approach (PCA) to identify the yeast species that were present in our hemodialysis center water. We detected the following species: *C. parapsilosis*, *R. mucilaginosa*, *C. guilliermondii*, *R. glutinis*, and *T. inkin*. In a study conducted by Ibelings *et al.* (2005), the authors used Raman spectroscopy to identify clinically relevant *Candida* spp. in peritonitis patients. Thirty-one (35%) of 88 specimens tested positive for *Candida* spp. The authors isolated 30 (55%) specimens from intensive care unit (ICU) patients and identified one (5%) specimen in general surgery ward patients. According to the authors, Raman spectroscopy offered an accurate and rapid alternative to identify yeasts. An important feature of Raman spectroscopy is the possibility to detect microbial cells (bacteria and yeast) rapidly, which is essential to prevent infection and provides near-instant characterization compared with alternative biochemical tests (Harz *et al.* 2009; Downes & Elfick 2010). Low cost and highly accurate strain identification are other advantages of Raman spectroscopy for yeast identification (Rodríguez *et al.* 2013). Hence, this technique could constitute an excellent tool to identify bacteria and yeasts in water from hemodialysis centers, providing early diagnosis and reducing morbidity and mortality rates.

CONCLUSION

Raman spectroscopy is an excellent tool to identify yeast species and is potentially applicable in the routine monitoring of water in hemodialysis units, providing early diagnosis and reducing morbidity and mortality rates in hemodialysis patients. Two potentially pathogenic *C. guilliermondii* isolates presented resistance to fluconazole, suggesting that immunocompromised patients treated at the center where these isolates were detected are at risk of contamination. Therefore, local health authorities must monitor water in hemodialysis units on a regular basis. It is also necessary to implement more efficient water disinfection procedures and to reduce dialyzer reuse in this hemodialysis center.

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