BRIEF COMMUNICATION
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Filamentous fungi in water used in hemodialysis treatment: a persistence challenge for health surveillance

Fungos filamentosos na água usada no tratamento de hemodiálise: um desafio persistente para vigilância em saúde

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ABSTRACT

Introduction: Fungi usually are resistant to disinfection and has pathogenic potential; however, they are not legally controlled in hemodialysis water (HW). **Objective:** Filamentous fungi (FF) were evaluated in HW from a tertiary hospital in southeast of São Paulo state (Brazil) and is a reference medical center providing service to millions of people every year. **Method:** A total of 84 water samples were collected in seven hydraulic system points during a period of one year and FF was identified by macroscopic and microscopic features. **Results:** FF were detected in all points ranging from 1 to 334 CFU/100 mL. Nine genera along the hydraulic system were identified, including *Acremonium*, *Alternaria*, *Aspergillus*, *Beltrania*, *Bipolaris*, *Cladosporium*, *Exophiala*, *Fusarium*, and *Penicillium*. **Conclusions:** Most fungi are ubiquitous and some cause human infection and mycotoxins producers. The various genera found reveal possible failures in treatment and distribution system, either through inadequate hygiene practices or infusion of these microorganisms into the pipes. FF detection is needed bearing mind immunological vulnerability of patients under hemodialysis treatment requiring continuous health surveillance actions.

KEYWORDS: Hemodialysis Center; Fungi; Water Quality; Microbiological Assessments

RESUMO

Introdução: Os fungos geralmente são resistentes à desinfecção e têm potencial patogênico. No entanto, eles não são legalmente controlados na água de hemodiálise (AH). **Objetivo:** Os fungos filamentosos (FF) foram avaliados na água de hemodiálise de um hospital terciário no sudeste do estado de São Paulo (Brasil), um centro médico de referência que atende milhões de pessoas por ano. **Método:** Um total de 84 amostras de água foram coletadas em sete pontos do sistema hidráulico durante o período de um ano e os FF foram identificados por características macroscópicas e microscópicas. **Resultados:** Foram detectados FF em todos os pontos, variando de 1 a 334 CFU/100 mL. Foram identificados nove gêneros ao longo do sistema hidráulico, incluindo: *Acremonium*, *Alternaria*, *Aspergillus*, *Beltrania*, *Bipolaris*, *Cladosporium*, *Exophiala*, *Fusarium* e *Penicillium*. **Conclusões:** A maioria dos fungos é onipresente e alguns deles causam infecção humana e produzem micotoxinas. Os vários gêneros encontrados revelam possíveis falhas no sistema de tratamento e distribuição, seja por práticas de higiene inadequadas ou por infusão desses microrganismos nas tubulações. A detecção dos FF é necessária, tendo em vista a vulnerabilidade imunológica dos pacientes em tratamento de hemodiálise, o que exige ações contínuas de vigilância em saúde.

PALAVRAS-CHAVE: Centro de Hemodiálise; Fungos; Qualidade da Água; Avaliações Microbiológicas

INTRODUCTION

End-stage kidney disease (ESKD) is a highly prevalent condition in the population, mainly caused by diabetes, glomerulonephritis, hypertension, and cystic kidney disease that requires intensive healthcare for improve patients' quality of life[1]. With the prospect of ensuring better living conditions, most ESKD patients (> 70%) are initially treated with hemodialysis (HD) and exposed to more than 300 L of water weekly[1,2]. Thus, water used in HD undergoes rigorous treatment processes to prevent chemical and microbiological contaminants from causing clinical complications. It has been described that aluminum, copper, nitrate, and others chemicals can cause anemia, neurological disorders, bone pain, and vomiting in patients undergoing HD treatment[3]. Bacteria and endotoxins can cause febrile reactions and sepsis; however, technological improvement of water treatment systems make the clinical complications mentioned rare[1].

In Brazil, the resolution RDC n. 11, of March 13th, 2014 established by Brazilian Health Regulatory Agency (Anvisa) determine those chemical substances must be evaluated semiannually, and total coliforms, heterotrophic bacteria, and endotoxins monthly, all of them with their respective maximum allowed values[4]. An outbreak of acute liver failure due to cyanotoxins following HD treatment has already been reported in Brazil[5]. Other microbiological agents are not legally controlled; however, it is known that fungi are morphologically more resistant to different disinfection processes than bacteria, and the investigation of these microorganisms becomes relevant whereas their pathogenic potential and opportunism features. A recent evidence report fungus causing bloodstream infections being associated with HD water contamination due to inadequate disinfection processes[6]. Here, we investigated the occurrence of nine filamentous fungi (FF) in different hydraulic system points in a Brazilian HD center.

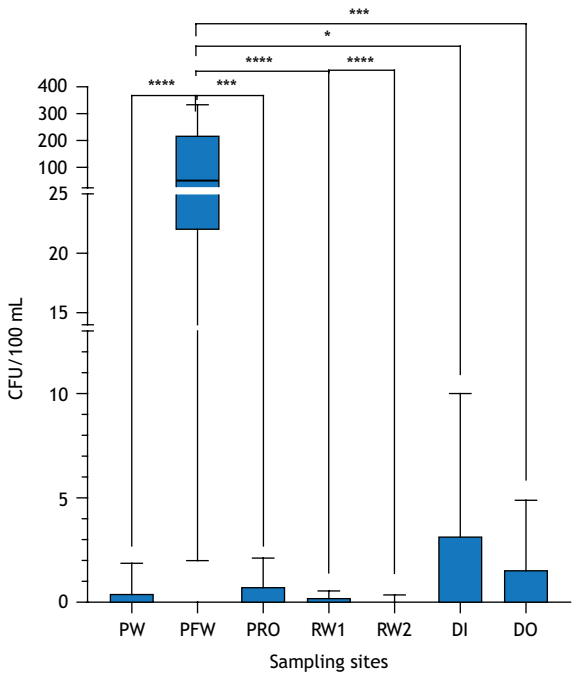
METHOD

Dialysis center under study located in a tertiary hospital in southeast of São Paulo state, Brazil. That is a reference medical center providing service to millions of people every year from all over Brazil and during the study it had capacity to attend 18 patients simultaneously. Water samples were monthly collected, along one-year previous COVID-19 pandemic (2019–2020), in seven hydraulic system points [a) potable water inlet; b) pre-filtration water; c) post reverse osmosis; d) dialysate inlet; e) dialysate outlet; f) reuse water tank #1; and g) reuse water tank #2]. Water samples (100 mL) were filtered through a vacuum system with cellulose membrane (47 mm, 0.45 µm), according to Standards Methods for the Examination of Water and Wastewater[7]. Afterwards, the membrane was incubated in Sabouraud Dextrose Agar (SDA) culture medium supplemented with chloramphenicol (30 mg/100 mL) at 25°C for a period of 7 to 10 days. After that, FF growth was evaluated, expressing the result in colony forming units per 100 mL (CFU/100 mL).

To FF identification, two techniques based on macro- and microscopic features were employed[8]. In this regard, we performed the giant colony technique on SDA culture medium to assessment the macroscopic characteristics, and the microculture technique on Potato Dextrose Agar culture medium to evaluate the microscopic characteristics after staining with lactophenol cotton blue[8]. After identifying the FF, the number and diversity of fungi at each sampling point during the course of the HD water was counted, and the Kruskal-Wallis test was applied to verify statistical significantly difference (p-value < 0.05) between FF levels among sampling points in GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

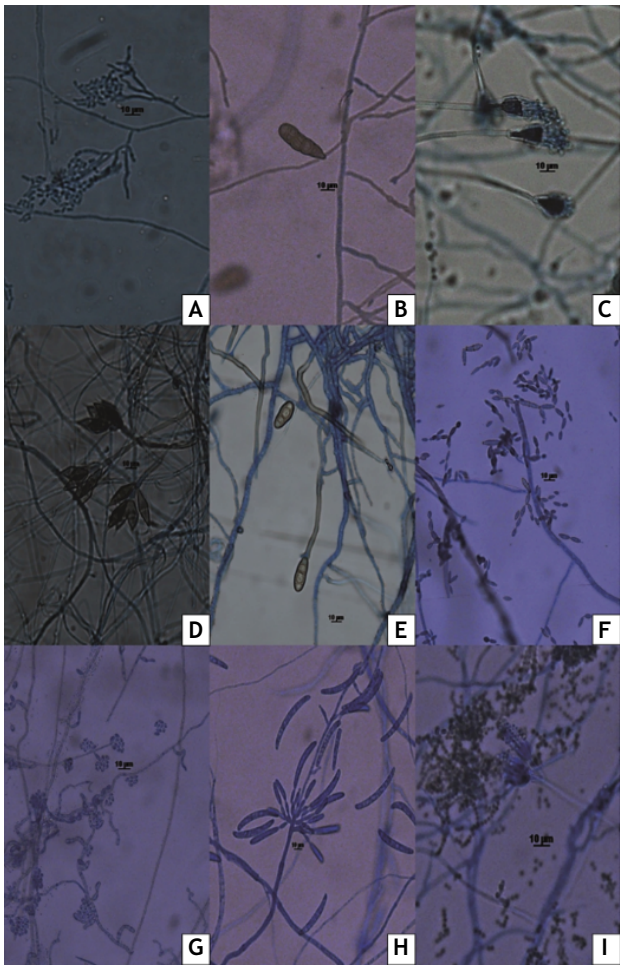
RESULTS AND DISCUSSION

A total of 84 water samples were evaluated and FF were detected in all sampling points in concentrations ranging from 1 to 334 CFU/100 mL. A significantly difference of FF level could be observed along hydraulic system as depicted in Figure 1. FF were detected in all of the pre-filtration water samples in high concentrations ranging from 2 to 334 CFU/100 mL. The pre-filter is composed of three units – sand filter, activated coal, softener – and the high concentrations found can suggest unsuitable hygiene practices or inadequate periodicity of cleaning procedures, since low concentrations were



Source: Elaborated by the authors, 2023.
PW: Potable water inlet; PFW: Pre-filtration water; PRO: Post reverse osmosis; RW1: Reuse water tank #1; RW2: Reuse water tank #2; DI: Dialysate inlet; DO: Dialysate outlet.
Asterisk represents a statistical significance difference: * p-value < 0.05, *** p-value < 0.005, **** p-value < 0.0001.

Figure 1. Concentrations (CFU/100mL) of filamentous fungi in hemodialysis water collected from a tertiary hospital in Brazil.



Source: Elaborated by the authors, 2023.

Figure 2. Filamentous fungi identified from hemodialysis water along hydraulic system. A, *Acremonium* sp; B, *Alternaria* sp; C, *Aspergillus* spp; D, *Beltrania* sp; E, *Bipolaris* sp; F, *Cladosporium* spp; G, *Exophiala* spp; H, *Fusarium* spp; I, *Penicillium* spp.

detected in water before the pre-filtration process. Therefore, this compartment of hydraulic system may represent a hotspot for microbiological agents.

FF concentrations post reverse osmosis decreased significantly (p-value < 0.005), as well as found by Pires-Gonçalves et al.[9], evidencing the high performance of reverse osmosis in water treatment. Dialysate inlet sampling point also stands out since 58.3% of samples were positive. Similar studies were performed showing an FF levels variability in dialysate[10,11]. Scientific evidences and our results highlighting the occurrence of such microorganisms in dialysate which may represent a concern regarding its contact with patient's blood.

Nine genera along the hydraulic system were identified, including *Acremonium* sp, *Alternaria* sp, *Aspergillus* spp, *Beltrania* sp, *Bipolaris* sp, *Cladosporium* spp, *Exophiala* spp, *Fusarium* spp, and *Penicillium* spp (Figure 2). This FF were also identified in studies conducted by different researchers' groups[8,9,10,11,12]. *Exophiala* spp (*n* = 1,280 isolates) was the main genus found, being exclusively detected at the pre-filtration sampling point (Table). This fungus is widely found in the environment and it has shown a high genetic similarity between isolates from deionized water used for preparing solutions and from infected patients in a hospital[13]. Aquatic dissemination of FF reinforces the necessity of appropriate techniques for HD water disinfection to avoid clinical intercurrences in patients, given their pathogenic potential in persons with immunological vulnerability.

Cladosporium spp and *Aspergillus* spp were found in four sampling points (Table). *Cladosporium* spp was detected in potable water inlet, post reverse osmosis, and mainly on dialysate inlet (n = 31) and dialysate outlet (n = 14). The similar results were also found by Schiavano et al.[10] and the relevance are associated to potential of *Cladosporium* sp to cause allergy, cutaneous and subcutaneous diseases, and occasionally deep infections[14]. In addition, *Cladosporium* is resistant to water chlorination and

Table. Total isolates of filamentous fungi detected in hemodialysis water along the hydraulic system from dialysis unit at tertiary hospital.

Filamentous fungi	Sampling sites						
	PWI	PFW	PRO	RW1	RW2	DI	DO
Acremonium sp	-	-	-	-	-	-	1
Alternaria sp	-	-	-	-	-	-	1
Aspergillus spp	1	-	4	-	1	5	-
Beltrania sp	-	-	-	1	-	-	-
Bipolaris sp	-	-	1	-	-	-	-
Cladosporium spp	4	-	4	-	-	31	14
Exophiala spp	-	1,280	-	-	-	-	-
Fusarium spp	-	-	-	-	-	1	1
Penicillium spp	-	-	-	1	-	1	2

Source: Elaborated by the authors, 2023.
PWI: Potable water inlet; PFW: Pre-filtration water; PRO: Post reverse osmosis; RW1: Reuse water tank #1; RW2: Reuse water tank #2; DI: Dialysate inlet; OD: Dialysate outlet.

can remain in the hydraulic system for long periods[15]. *Aspergillus* was also found in different points of hydraulic systems (Table) and among them highlight dialysate inlet regarding close contact with patients under HD treatment, and due to *Aspergillus* to be a medically important fungal genera considering the toxically potential of the mycotoxins (e.g., aflatoxins, ochratoxins). We also point out *Fusarium* and *Penicillium* detection taking into account their historical of humans' infections and their ability to be mycotoxin producers too[16].

CONCLUSIONS

The obtained data evidence wide distribution of FF in hydraulic system of the dialysis unit under study. The various genera found, which are ubiquitous, reveal possible failures in treatment and distribution system, either through inadequate hygiene practices or infusion of these microorganisms into the pipes. Like bacteria, some species of fungi have ability to produce biofilm which represents a challenge for suitable water disinfection. The FF occurrence and persistence throughout the year may pose risks to patients undergoing HD treatment, considering its potential as pathogenic agents. In this sense, health surveillance actions such as periodic assessment of water quality and facilities are essential for patient safety.

Furthermore, future studies are needed to verify the feasibility of including fungal determination into guidelines that ensure good operational practices in dialysis centers due to FF higher persistence to disinfection measures applied, when compared to bacteria, a commonly indicator useful to guarantee the microbiological water quality. Thus, potential improvements in the treatment and distribution system of the water may ensure greater safety for patients under dialysis treatment. The partnership with dialysis unit managers in this study ensured that the hospital's technical team had access to our data to establish improvements in the water treatment of this health service. From our results, we suggest that further studies be conducted to associate the occurrence of fungi in HD water with clinical stage of patients under HD treatment.

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Authors' Contribution

Suzuki MN, Zagui GG, Segura-Muñoz S – Conception, planning (study design), acquisition, analysis, interpretation and writing of the work. Machado CS, Reis AS, Machado GP, Meschede MSC, Santos DV, Martins CHG and Andrade LN – Analysis, interpretation of results and writing of the paper. All authors approved the final version of the paper.

Conflict of Interest

The author declares no conflict of interests.