Physical-chemical and microbiological characterization of water destined to hemodialysis

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ABSTRACT

This research evaluated the physical-chemical and microbiological parameters of water and dialysate in four distinct hemodialysis units located in the southeast region of Brazil. The physical-chemical parameters evaluated were pH, electric conductivity, turbidity, alkalinity, free chlorine, nitrate, fluoride, chloride, sulfate, sodium, potassium, calcium, and magnesium ion concentrations. Microcystin was also quantified. The microbiological parameters evaluated were the detection of total coliform, total heterotrophic bacteria count (THB), and the isolation and identification of microorganisms in pre-reverse osmosis treatment and post-reverse osmosis treatment water samples and dialysate. The nitrate, fluoride and THB levels found in the water samples may present risk to the patient under hemodialysis treatment. Microcystin was detected in one of the potable water samples. Microorganisms were identified throughout the hemodialysis of the entire water treatment system, with *Ralstonia* sp. being the most frequent. The presence of emergent pathogenic bacteria highlighted in this study highlights the necessity of microbiological monitoring of water destined for hemodialysis.

Keywords: dialysate, hemodialysis, water treatment.

Caracterização físico-química e microbiológica da água destinada a hemodiálise

RESUMO

O objetivo da pesquisa foi a avaliação de parâmetros físico-químicos e microbiológicos da água e dialisato em quatro unidades de hemodiálise localizadas na região sudeste do Brasil. Foram avaliados os parâmetros físico-químicos: pH, condutividade elétrica, turbidez, alcalinidade, cloro livre, concentração dos íons nitrato, fluoreto, cloreto, sulfato, sódio, potássio, cálcio e magnésio, também foi realizada a quantificação de microcistinas. Os parâmetros microbiológicos realizados foram a detecção de coliformes totais, quantificação de

bactérias heterotróficas totais (BHT) e o isolamento e identificação de microrganismos em amostras de água pré, pós-tratamento por osmose inversa e dialisato. Os níveis de nitrato, fluoreto e BHT encontrados nas amostras de água podem apresentar risco ao paciente em hemodiálise. Foi identificada em uma amostra de água potável a presença de microcistinas. Microrganismos foram identificados ao longo do sistema de tratamento de água para hemodiálise, sendo a *Ralstonia* sp. a mais frequente. A presença de bactérias patogênicas emergentes detectadas neste estudo aponta a necessidade do monitoramento microbiológico da água para hemodiálise.

Palavras-chave: dialisato, hemodiálise, tratamento de água.

1. INTRODUCTION

Chronic kidney disease (CKD) is characterized by progressive nephron loss as a result of irreversible lesions that gradually reduce the global renal function, it is estimated that 10% of the global population suffers from CKD (Vos *et al.*, 2016) and, in Brazil alone, more than 120 thousand patients were reported to be receiving dialysis treatment (Sesso *et al.*, 2017). Hemodialysis is the most common treatment for CKD performed worldwide, it consists of an artificial process of blood filtration, in which the exchange of substances, such as electrolytes and glucose, between blood and dialysate is performed through a semipermeable membrane (Carvalho *et al.*, 2022). In this process, usually carried out three times a week per patient, approximately 400 liters of water are used per session, which lasts an average of four hours (Pontoriero *et al.*, 2003).

The source of the water used by the hemodialysis units is the city's water supply system. In each unit, there is a water treatment system specific for hemodialysis. This basically consists of a pre-treatment with filters (sedimentation, softener and activated carbon) followed by a reverse osmosis (RO) membrane treatment. After the RO, the dialysate water is stored and distributed to the unit in a continuous flow, to reach the semipermeable membrane that exchanges substance with the blood. However, this membrane permits low molecular weight contaminants to reach the bloodstream, which can cause severe poisoning and adverse effects. One strong example of the relationship between water quality and public health is a case of chemical cyanotoxin contamination of dialysis water which occurred in the city of Caruaru, PE in 1996, which caused the death of 60 patients (Pouria *et al.*, 1998).

Microbiological contaminants can also be found in water, and their presence is often associated with failures of the dialysis water treatment and distribution system (Ferreira *et al.* 2015; Ferreira *et al.* 2020). Among the most frequent, gram-negative bacteria (Okunola and Olaitan, 2016; Anversa *et al.*, 2022; Chaoui *et al.*, 2022), that may be associated with infections in hemodialysis patients (Tejera *et al.*, 2016; Thet *et al.*, 2019), can be highlighted. These bacteria can also represent a serious threat to patients due to their ability to form biofilms and develop antibiotic resistance.

In Brazilian legislation, the RDC 11 (ANVISA, 2014), dated March 13th, 2014, determines the limit concentrations of contaminants in dialysis water services. Water for hemodialysis centers comes from the city's water supply system, and it should meet the quality standards established by Portaria MS 2914, dated December 12th, 2011 and RDC N° 11, dated March 13th, 2014 regarding the standards for potable water.

Despite established guidelines, studies conducted in Brazil reveal dissatisfaction levels regarding certain parameters of water quality intended for hemodialysis, such as high levels of heterotrophic bacteria, elevated concentrations of endotoxins (Almodovar *et al.*, 2018; Hilinski *et al.*, 2020; de Jesus *et al.*, 2022), and the significant presence of aluminum (de Jesus *et al.*, 2022). Additionally, some studies report the presence of fungi in these waters, posing potential risks to the health of hemodialysis patients (Montanari *et al.*, 2018; Oliveira *et al.*, 2018;

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Anversa *et al.*, 2021; Calumby *et al.*, 2023).

Considering the importance of hemodialysis and the increasing estimation of new hemodialytic patients, it becomes imperative to control the quality of treatment, since water is the major component of the procedure. Therefore, the objective of this research was to evaluate the physical-chemical and microbiological parameters of water and dialysate in four distinct hemodialysis units in the southeast of Brazil.

2. MATERIAL AND METHODS

2.1. Hemodialysis Centers Characterization

The water and dialysate samples destined to hemodialytic procedures were obtained from four hemodialysis units located in the metropolitan area of Espírito Santo state. Together, the four hemodialysis units provide 110 beds for treatment and care for around 400 patients every month. From this total, Unit A holds 22 beds and 45 patients while Units B and C provide 34 and 22 beds respectively and receive an average of 100 patients each. Unit D offers 35 beds and treats 180 patients.

2.2. Sampling

Water sampling was conducted in six points of each hemodialysis unit water treatment system (Figure 1). The first two points were collected before the RO (Pre - RO), in the entrance of potable water supply (P1) and after softener and deionizer filters (P2). After the RO, the samples were collected at three points: water reservoir (P3), water used in the capillary reuse room (P4) and return of treated water to the loop (P5). The dialysate used in the hemodialysis machine was collected at Point 6 (P6). For microbiological analyses, the water samples collected at Point P1 were added to a 1.8% sodium thiosulfate solution.
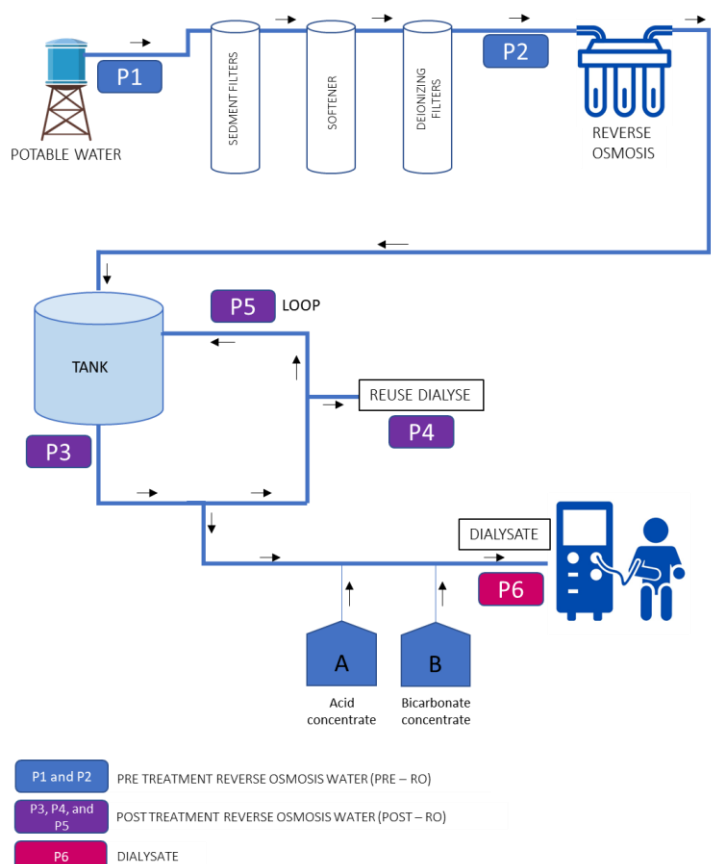

Figure 1. Representation of the sampling points of water in the treatment system used by the hemodialysis units.

A total of 75 samples of water were collected, of which 45 were Pre-RO samples, 30 Post-RO samples and 15 samples of dialysate from the water treatment system of the hemodialysis unit, from 2015 to 2017 (Table 1). Dialysis Unit A does not have any physical structure (faucet device) that allowed the collection of water in the loop point (P5).

Table 1. Description of water and dialysate samples performed in the hemodialysis units between January, 2015 and July, 2017.

Hemodialysis units	Sampling date	Water samples (n)		Dialysate samples (n)
		Pre - RO	Post - RO	
A	07/01/15 29/01/15 25/03/15 24/09/15 08/06/16 27/07/16	18	12	6
B	28/10/15 05/07/16 09/11/16	9	6	3
C	30/08/16 16/11/16 20/03/17	9	6	3
D	13/07/16 20/09/16 13/07/17	9	6	3
Total		**45**	**30**	**15**

A total of 2 liters of water were collected across sampling points P1 to P5. From this total, 1 liter was stored in polyethylene flasks for physical-chemical parameter analysis and the remainder was stored in sterile 500 mL amber glass flasks for microbiological analysis. The sampling points were sanitized with 70% alcohol and the sampling process was performed after 1 to 2 minutes of free water flow. The dialysate was collected at P6 with the aid of two sterile syringes attached to the hemodialysis machine through a connector. The sample obtained from the first syringe was disposed of and the second syringe was used to collect 1 liter of dialysate, which was stored in sterile amber glass flasks. All samples were collected in duplicate and kept refrigerated at a temperature below 10°C.

2.3. Physical-chemical parameter analysis
The pH and electric conductivity were measured by DIGIMED DM22 and CONDUCTIVITY METER-CD850 through the potentiometric method. The turbidity was determined by HACTCH TURBIDIMETER through a nephelometric method. The alkalinity was defined following the procedures from Method 2320 B, Titrimetric APHA *et al.* (2005) and residual chlorine was determined with the Hanna® kit, according to the manufacturer's guidelines. The determination of concentration of nitrate, fluoride, chloride, sulfate, sodium, potassium, calcium, and magnesium ions was determined through the colorimetric method, with the IC BASIC PLUS 883- METROHM® equipment and use of columns METROSEP A Supp 5 – 150 x 4.0 mm e METROSEP C 4 – 150 X 4.0 mm, for anions and cations, respectively.

2.4. Microcystin analysis
The detection and quantification of microcystin was obtained through ELISA, using the

Microcystin Plate Kit (Beacon®), according to the manufacturer's guidelines.

2.5. Bacteriological Analysis
Total Coliform and *Escherichia coli* detection

The analysis of total coliforms and *Escherichia coli* was carried out using the technique of chromo-fluorogenic substrate, with a presence/absence detection system (P/A), according to the methodology described in Cassini *et al.* (2013).

Total heterotrophic bacteria (THB) quantification

The total heterotrophic bacteria (THB) quantification was achieved through sowing of one fraction of the water sample by *pour plate* in a casein agar growth medium, incubated for 48 hours at $35 \pm 0,5$°C (Anvisa, 2010). After the incubation, the count of colony forming units (CFU) was performed with colony-count equipment (Phoenix cp 600).

Isolation and Identification of isolated microorganisms

A 5 mL volume of water or dialysate sample was added to 50 mL of casein broth and incubated for a maximum of 48 hours at $35 \pm 0,5$°C, and then, with the aid of an inoculating loop, a fraction of 10 µL was collected from the growth medium, sowed in TSA and incubated $35 \pm 0,5$°C for 48 hours. The growing microorganisms were peaked to mediums TSA, MacConkey and Cetrimide by draining, in order to obtain pure colonies for the biochemical identification test.

The identification of species of isolated microorganisms was performed through mass spectrometry by matrix-assisted laser desorption ionization (MALDI-TOF MS) using a *Microflex LT* mass spectrometer and *Biotyper* 3.3 software (*Bruker Daltonics*, Bremen, Alemanha), according to manufacturer's guidelines.

3. RESULTS AND DISCUSSION

3.1. Physical-chemical parameters and water quality

The Pre-RO water samples presented pH levels close to neutrality, turbidity under $4 \pm 0,5$ NTU and residual chlorine between 0,5 and 0,2 mg L^{-1}, values that are appropriate according to the dialysis water parameters described in RDC 11/2014. The ion concentration of nitrate and fluoride (Figure 2) dissolved in the Pre-RO and Post-RO water samples were also found to be within the parameters described in MS 2914/2011 for potable water and RDC 11/2014 for hemodialysis water.

The maximum concentration of nitrate in drinking water allowed, by MS 2914/2011, is 10 mg/L. The highest concentration of nitrate was $12 \pm 0,3$ mg L^{-1} in samples collected in dialysis Unit C, followed by $8,5 \pm 0,2$ mg L^{-1} in Unit B and $6 \pm 0,1$ mg L-1 in unit D and 1 mg L-1 in Unit A (Figure 2-I). Elevated levels of nitrate in potable water have been linked to several complications, such as methemoglobinemia, colorectal cancer, thyroid diseases, and neural tube defects (Ward *et al.,* 2018). The relation between the ingestion of water with nitrate and blood methemoglobin presence has been observed even in concentration levels below the recommended values (Zeman *et al.,* 2011).

The RDC 11/2014 defines the maximum nitrate value allowed as 2 mg L^{-1} of N-nitrate as a quality standard for hemodialysis water. The excess of N-nitrate in post-RO was observed in dialysis units B and C (Figure 2-II), with $2,4 \pm 0,05$ and $4,6 \pm 0,11$ mg L^{-1}, respectively. The symptoms related to water contamination by nitrate for hemodialytic patients are hypotension, nausea, vomit, and diarrhea. Although there were reports in the literature that the toxic concentration for hemodialysis patients is around 21 mg L^{-1} (Layman-Amato *et al.*, 2013), Suzuki *et al.* (2019), found nitrate levels in the dialysis water below the standards established by RDC 11/2014 were correlated to anemia in hemodialysis patients.
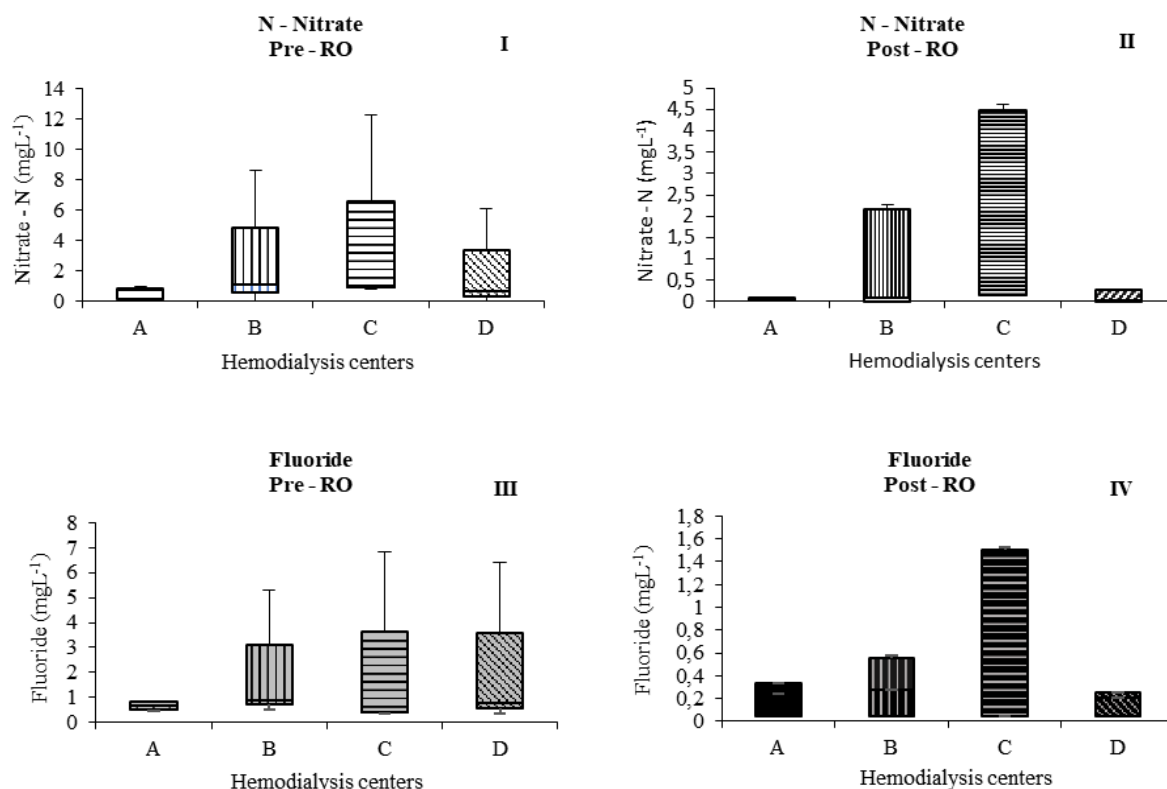
Figure 2. Concentration of ions pre reverse osmosis treatment (pre-RO), nitrate (I) and fluoride (III) and post reverse osmosis treatment (post-RO), nitrate (II) and fluoride (VI) in the hemodialysis units located in the metropolitan area of Espírito Santo, Brazil.

Regarding fluoride ions, the concentrations observed in the pre-RO samples were higher than the 1,5 mg L^{-1} defined for potable water in the MS 2914/2011. The highest concentrations of fluoride observed were approximately $6,8 \pm 0,17$ mg L^{-1} in Unit C, followed by $6,4 \pm 0,16$ mg L^{-1} in unit D and $5,3 \pm 0,13$ mg L^{-1} in unit B (Figure 2-III). Yousefi *et al.* (2019) have associated the fluoride concentration in potable water with hypertension.

We have found levels of fluoride above the legislated limit in post-RO samples in the Units B and C, $0,6 \pm 0,01$ mg L^{-1} and $1,5 \pm 0,03$ mg L^{-1}, respectively (Figure 2-IV). This value represents more than seven times the recommended limit established by the RDC 11/2014, which is 0,2 mg L^{-1}. Concentrations above 1,0 mg L^{-1} may cause bone diseases in hemodialytic patients. Furthermore, the reaction of fluoride and aluminum results in aluminum fluoride, which is highly toxic to the renal system (Silva and Moreira, 2009; Payne and Curtis, 2021).

3.2. Detection of microcystin

The presence of microcystin was detected in only one of the pre-RO water samples from Unit A, with a concentration of 4,13 µg L^{-1}, although the analysis of the water after the activated carbon step showed the removal of the microcystin, within the limitations of the detection method. Almeida, *et al.* (2016) observed that activated carbon has a lower removal rate for microcystin in concentrations above $3,83 \pm 0,36$ µg L^{-1}. The amount of toxin detected by this study is close to this limit of removal, thus increasing concerns regarding patient's health in the event of the persistence of such toxin in the water treatment system, reaching the patients undergoing the hemodialysis process.

Hilborn *et al.* (2013) reported a case where 44 patients were exposed to dialysate contaminated by microcystin in Rio de Janeiro, Brazil, in 2001. The concentration of microcystin in potable water was 0,4 µg L^{-1} and an average of 0,33 ng mL^{-1} in exposed dialytic patient's blood serum (Hilborn *et al.*, 2013). Therefore, due to the low retention of the

microcystin by the activated carbon filter and the concentration close to the limit found in this study, constant monitoring for the presence of this toxin is crucial to evaluate the treatment quality of dialysis water, in order to avoid patients' future exposure to microcystin.

3.3. Bacteriological parameters

None of the water samples presented coliforms, being within the parameters established by RDC 11/ 2014, which requires the absence of coliforms in 100 mL of sampling volume.

The quantification of total heterotrophic bacteria varied from non-detectable levels to approximately $2 \times 10^3 \pm 80$ CFU mL^{-1} (Figure 3). The water samples were obtained from various points of the dialysis water treatment system, from the entrance to dialysate. The RDC 11/2014 defines the maximum allowed values as 100 CFU mL^{-1} and 200 CFU mL^{-1} for water used in hemodialysis and dialysate, respectively. In this research, around 30% of the samples of post-RO water and 13% of dialysate samples presented unconforming parameters regarding the THB recommended.
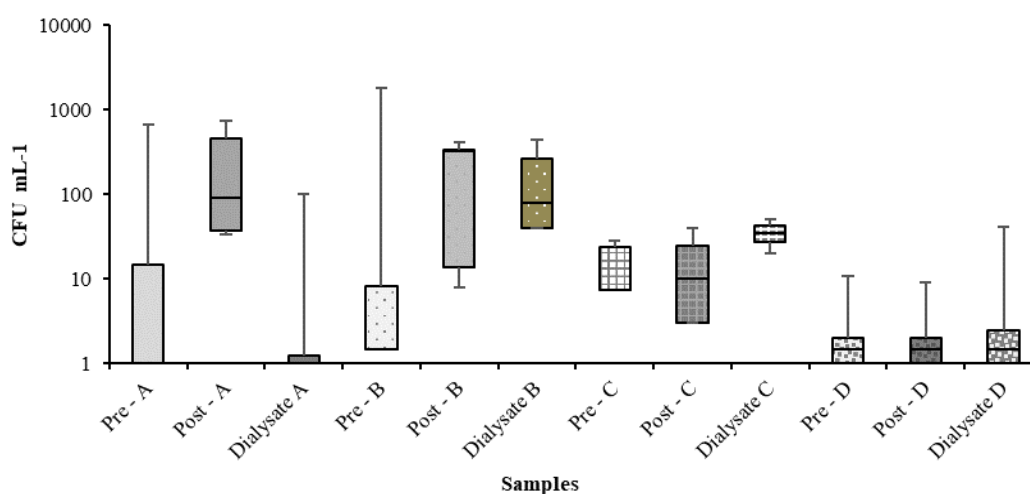

Figure 3. Distribution of maximum values, geometric mean, and minimum quantity of total heterotrophic bacteria (THB) per colony forming unit/mL (CFU/mL) expressed in logarithmic scale in water samples from the pre-RO sampling points and post-RO sampling points in hemodialysis Units A, B, C and D, located in the metropolitan area in Espírito Santo, Brazil.

Comparatively, studies in the southeastern region of Brazil, which assessed a larger number of hemodialysis units, a more extended period, and a greater number of samples, indicated higher non-compliance rates, ranging from 27% to 63% (Hilinski *et al.*, 2020). In contrast, other investigations, also encompassing a broader sampling, reported lower rates, ranging from 5% to 8% (Almodovar *et al.*, 2018). In Rio de Janeiro, the research conducted by de Jesus *et al.*, 2021, revealed a non-compliance rate of 30% for THB in water samples intended for hemodialysis. These studies underscore concerns regarding the microbiological quality of water and emphasize the importance of continuous and meticulous monitoring to ensure the safety of patients undergoing hemodialysis treatment.

During the hemodialysis water treatment, chlorine was removed, facilitating the development of bacteria. The removal of composts during the water treatment may cause irregularities in the hydraulic circuit and facilitate the formation of biofilm (Hoenich and Levin, 2003). The deionizers are another site for microbiological contamination. Bacteria contamination may occur due to the capacity of resin, especially anionic, to connect to organic matter and, thus, allow the proliferation of bacteria (Coulliette and Arduino, 2013). The RO is an advanced water treatment that allows the removal of low molecular weight components, with 99,9% efficiency, allowing the passage of bacteria to the permeate, which might be associated

to the integrity of the membrane or the sealing system (Fujioka *et al.,* 2018; 2019). Wang *et al.* (2022), reported bacteria growth and biofilm production in water permeated by RO after a disinfection process.

3.4. Isolated microorganisms in water samples from the hemodialysis units

Eight species of bacteria and one species of fungus were isolated in the water and dialysate (Table 2). Most of the bacteria species that were isolated belong to the nonfermenting gram-negative category, with the exception of *Serratia marcescens,* which is part of the fermenting gram-negative group. The gram-negative bacteria, especially the nonfermenting ones, are the most common microbiological contaminant found in hemodialysis water (Chen *et al.,* 2017; de Jesus, *et al.*, 2017; Anversa *et al.*, 2022; Chaoui *et al.,* 2022).

Table 2. Microorganisms isolated in the water from pre reverse osmosis (pre-RO) treatment samples, post reverse osmosis (post-RO) treatment samples and dialysate samples in the hemodialysis units located in the metropolitan area of Espírito Santo, Brazil.

Water sample	Hemodialysis Units			
	A	B	C	D
Pre - RO	*Brevundimonas aurantiaca*	-	*Ralstonia pickettii*	-
Post - RO	*Ralstonia insidiosa*	*Ralstonia pickettii*	*Ralstonia pickettii*	-
	Burkholderia vietnamiensis	*Ralstonia insidiosa*	*Ralstonia insidiosa*	
	Herbaspirillum sp	*Moraxella sp*		
Dialysate	-	*Nesterenkonia lacusekhoensis*	-	*Ralstonia pickettii*
		Serratia marcescens		
		Candida orthopsilosis		

In the study conducted by Anversa *et al.* (2022) in hemodialysis units in São Paulo, Brazil, MALDI-TOF methodology was employed, resembling the approach used in the present research. Distinctively, filtration treatment was incorporated into the water samples. The results revealed the presence of the genera *Herbarpirillum*, *Brevundimonas*, *Ralstonia*, and *Burkholderia*, aligning with the findings of the current study.

Also, de Jesus *et al.* (2022) studying hemodialysis units in Rio de Janeiro, Brazil, employing biochemical methods for bacterial identification, corroborated the findings of Anversa *et al.* (2022). It is noteworthy that the common presence of the species *Pseudomonas aeruginosa*, widely documented in various Brazilian studies using biochemical techniques (Lima *et al.*, 2005; Borges *et al.*, 2007; de Jesus, *et al.*, 2017; 2022); MALDI-TOF (Anversa *et al.*, 2022); and molecular biology identification methods (Montanari *et al.*, 2009), was not detected in the present investigation.

The *Ralstonia pickettii* species was present in all hemodialysis units studied. *Ralstonia* sp may be found in different types of treated water for supply, such as hospital environment, water for distribution, industrial usage and even in ultrapure water, thus being capable of surviving in low nutrient environments (Ryan and Adley, 2014; Vaz-Moreira *et al.*, 2017). Furthermore, this species has been proven capable of developing biofilm in PVC pipe systems (Dombrowsky *et al.*, 2013). Water used in hemodialysis is low in nutrient concentration and is distributed through a PVC pipe system. *Ralstonia* sp has been described in hemodialysis water by Chen *et al.* (2017) and Anversa *et al.* (2022).

The *Ralstonia* sp is considered an emerging opportunistic pathogen (Ryan and Adley, 2014) and cases of infections caused by this bacterium in hemodialytic patients have been

reported (Tejera *et al.,* 2016; Thet *et al.,* 2019). Thet *et al.* (2019) reported bacterial infection outbreaks in seven patients from a hemodialysis center, four of them being identified as infected with *R. pickettii*. The authors related the outbreak with water contamination, especially in the water reservoir, due to the high THB count, and highlighted the potential risk of reusing the dialyzers.

Five other bacteria identified in this work (*Serratia marcescens, Burkholderia vietnamiensis, Brevundimonas aurantiaca, Moraxella* sp e *Herbarspirillum* sp.) also were been reported in the literature on contaminated hemodialysis water. Novosad *et al.* (2019) reported a multicentric outbreak in hemodialytic patients caused by *Serratia marcescens.* An outbreak of bacteremia in chronic renal patients was caused by *Burkholderia* sp., and the source of contamination pointed out was lack of proper disinfection of the water system and lack of maintenance of membrane filters, which may have triggered biofilm formation in pipes. (Rocha *et al.*, 2021). The *Brevundimonas* sp. has been reported as an emerging opportunistic pathogen, with reference to infection cases in several hospitalized patients (Ryan and Pembroke, 2018). A case of peritonitis caused by *Moraxella osloensis* has been described by Adapa *et al.* (2018) in a peritoneal dialysis patient. One fatal case by *Herbaspirillum seropedicae* was described by Suwantarat *et al.* (2015), in which the bacteria were present in the hemodialysis catheter. This study is the first record found in the literature of the presence of *Nesterenkonia lawsekhoensi* in a hemodialysis unit water treatment system. It is a gram-positive bacterium that has not been associated with infections in hemodialytic patients so far.

Candida orthopsilosis was the only fungus isolated in the present study. The presence of fungus in hemodialysis water samples was reported in Okunola and Olaitan, (2016); Montanari *et al.* (2018), Oliveira *et al.* (2018), Boyce *et al.* (2021) and Calumby *et al.* (2023). The frequency of yeast was higher in dialysate samples, when compared to other samples of water from the hemodialysis treatment system. Furthermore, the development of biofilm in dialysis solution was observed (Oliveira *et al.,* 2018). Cases of infection caused by *Candida* sp in hemodialytic patients have been described by Ourives *et al.* (2016) and Shu *et al.* (2017).

4. CONCLUSION

The monitoring of water quality in hemodialysis centers is mandatory in order to evaluate and assure safety for patients undergoing hemodialytic procedures. In this study, the water used in hemodialysis did not conform to the values established by RDC 11/2014 for concentration levels of N-nitrate in Units B and C, and fluoride for all the units evaluated. Furthermore, microorganisms were found in samples from potable water to dialysate. Emergent pathogenic bacteria, such as *Ralstonia* sp. and *Brevundimonas* sp., identified in this study, demonstrate concern and risk to the health of hemodialytic patients. Therefore, the monitoring of water quality and the identification of microorganisms support the decision-making process of Health Surveillance and Health Services in order to assure appropriate water treatment and distribution for the maintenance of hemodialysis services, avoiding possible health issues for patients who are already at risk.

5. LIMITATIONS OF THE STUDY

The study primarily focused on the overall water quality in hemodialysis units but did not thoroughly investigate potential sources of contamination in treatment systems, such as the integrity of reverse osmosis membranes, filter effectiveness and potential biofilm formation. A more in-depth analysis of these components would be crucial to pinpoint specific areas requiring improvement. Furthermore, the research was confined to four hemodialysis units, potentially limiting the generalization of results. Including a broader and more diverse set of units could enhance the external validity of the study. The identification of microorganisms

based on mass spectrometry (MALDI-TOF MS) may not encompass all microbial species at the strain level. The incorporation of molecular detection techniques, such as PCR, could increase sensitivity in identifying fungi and other pathogens, providing a more comprehensive analysis of microbial diversity.

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