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# Microbiological evaluation of water used in hemodialysis services in the city of Rio de Janeiro from 2016 to 2018

## Avaliação microbiológica da água utilizada nos serviços de hemodiálise na cidade do Rio de Janeiro nos anos 2016 a 2018

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## ABSTRACT

**Introduction:** The quality of water in hemodialysis services is critical in avoiding health risks in patients with renal insufficiency. **Objective:** To monitor the microbiological quality of treated water samples from hemodialysis services in the city of Rio de Janeiro. A total of 480 water samples were collected for microbiological analysis and 192 for endotoxin testing from 96 hemodialysis clinics between 2016 and 2018 by the Sanitary Surveillance of the city of Rio de Janeiro. **Method:** Methodologies described in the Brazilian Pharmacopoeia, DRC nº 11 2014 and Consolidation Ordinance nº 5, 2017. **Results:** Twenty percent of the microbiological analysis samples showed a high number ofHeterotrophic Plate Count Bacteria (> 100 UFC/mL) and 24% of the endotoxin search (LAL) presented values above the recommended value (> 0.25 EU/mL). Seventy-eight percent of the clinics (75/96) were unsatisfactory, 41 for high BH and 34 for LAL. A total of 563 isolates were identified by confirmatory biochemical tests as: *Pseudomonas aeruginosa* (35.5%), *Burlkolderia cepacia* (21.3%), *Stenotrophonomas maltophilia*, (19.1%), *Acinetobacter baumannii* (15.1%) and *Ralstonia pickettii* (9.0%). **Conclusions:** Our results allow us to conclude that systematic monitoring of water quality in hemodialysis services is essential to provide safety and prevent health problems patients.

**KEYWORDS:** Hemodialysis; Microbiological Evaluation; *Pseudomonas aeruginosa*; Quality

## RESUMO

**Introdução:** A qualidade da água nos serviços de hemodiálise é fundamental para evitar riscos à saúde de pacientes com insuficiência renal. **Objetivo:** Monitorar a qualidade microbiológica de amostras de água tratada de serviços de hemodiálise, na cidade do Rio de Janeiro. Foram coletadas 480 amostras para análise microbiológica (cinco pontos de coleta) e 192 para pesquisa de endotoxinas (dois pontos) nas inspeções de 96 clínicas de hemodiálise, entre 2016 e 2018, pela Vigilância Sanitária do município do Rio de Janeiro. **Método:** Metodologias descritas na Farmacopeia Brasileira, na RDC nº 11/2014 e na Portaria de Consolidação nº 5/2017. **Resultados:** Vinte por cento de amostras do ensaio microbiológico apresentaram número elevado de bactérias aeróbias (> 100 UFC/mL) e 24% das amostras da análise de endotoxinas (LAL) apresentaram valores acima do preconizado (> 0,25 EU/mL). Setenta e oito por cento das clínicas (75/96) foram insatisfatórias, 41 por apresentarem alta contagem de bactérias heterotróficas e 34 pela detecção de endotoxinas pelo LAL. Foram identificados 563 isolados por testes bioquímicos confirmatórios, *Pseudomonas aeruginosa* (35,5%), *Burlkolderia cepacia* (21,3%), *Stenotrophonomas maltophilia*, (19,1%), *Acinetobacter baumannii* (15,1%) e *Ralstonia pickettii* (9,0%). **Conclusões:** Nossos resultados nos permitem concluir que o monitoramento sistemático da qualidade da água nos serviços de hemodiálise é essencial para proporcionar segurança e evitar agravos à saúde de pacientes.

**PALAVRAS-CHAVE:** Hemodiálise; Avaliação Microbiológica; *Pseudomonas aeruginosa*; Qualidade

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## INTRODUCTION

Renal replacement therapy or hemodialysis is a fundamental treatment for patients with chronic or acute kidney failure, which occurs when the kidneys are unable to remove waste from cellular metabolism or to perform their regulatory functions[1].

Data from the last Brazilian Society of Nephrology (SBN) census, from 2017, showed that about 126,000 Brazilian citizens underwent hemodialysis in 747 dialysis facilities in the country,65% of which located in the Southeast region[2].

Hemodialysis is a procedure through which a device filters and cleans the blood by excreting toxic metabolites, a function of the kidney, which may be performing it insufficiently or not performing it at all[3]. This process occurs in the dialyzer (hemodialysis device). This piece of equipment contains a semipermeable membrane, in which there is an anti-parallel flow of the patient blood and the dialysis fluid (dialysis solution). In the dialyzer, the migration of substances between the two systems occurs. After diffusion, the purified blood returns to the patient[4,5,6].

Water is essential for hemodialysis therapy, both in fluid production and in the reuse of dialyzers. During a hemodialysis treatment session, nearly 120 liters of purified water are used, mixed in adequate proportions to the polyelectrolyte solution for hemodialysis. Therefore, water quality is critical to avoid additional risks to patient health[7].

The treatment of water for hemodialysis is more rigorous than that of drinking water and requires an additional purification system in which the water used must be potable, have a controlled concentration of metals such as aluminum, fluorine, mercury, copper, among others, and be free of certain substances like bacterial endotoxins[8,9].

There are water purification systems for hemodialysis composed of different treatment combinations to ensure the quality of the resulting water. The pre-treatment point concerns the water supplied by the public sanitation agencies to the city clinics and hospitals, which upon entering the treatment system will be filtrated with sand and activated charcoal to remove substances and particles from the water that can damage the purification equipment. Then, through reverse osmosis and ion exchange, particles, salts, ions and bacteria that may be circulating in the treatment system are filtrated[10].

Despite the multiple barriers of the treatment system capable of removing microorganisms from the water, there is still the risk of bacterial contamination[6]. Prevention of water contamination requires knowledge of the source of the problem within the treatment line. Purified water predominantly contains heterotrophic bacteria from the aquatic environment like the ones from the Pseudomonadales class, which may grow in water circuits and hemodialysis devices and subsequently contaminate the dialysis solution[7].

Bacteremia is one of the main reasons for morbidity and mortality in hemodialysis patients and has been attributed to different causes[11]. The infection through the vascular access is the most common form due to inadequate care with the catheter[12,13]. However, some studies have shown a direct relation between bacteremia cases, through the isolation of these microorganisms from the purified water, possibly due to defects in membrane integrity or the use of contaminated water in the reprocessing of dialysis devices[14,15].

The microbiological aspect of the water treatment was taken into account when a study demonstrated that the high count of bacteria in the dyalisate was responsible for the pyrogenic reactions and bacteremia in patients undergoing dialysis sessions[16]. Studies have shown that gram-negative bacteria endotoxins can penetrate the semipermeable membrane of the dialyzer and cause pyrogenic reactions in patients on hemodialysis[16,17].

The importance of microbiological quality of water in hemodialysis services is evident. In this study, we analyzed the entire water treatment system, from entrance to use, in several hemodialysis clinics. This procedure was essential to obtain information to guide the technological development of processes and to propose corrective measures by the Health Surveillance, aiming at minimizing the risks for the patients. The verification of the microbiological quality of treated water samples from hemodialysis services showed the importance of a water monitoring program of hemodialysis facilities to avoid health problems in patients with kidney failure.

## METHODS

Samples of treated water for hemodialysis were collected in health units with hemodialysis services located in the city of Rio de Janeiro, Brazil, from 2016 to 2018.

We collected 611 samples from different points of the water treatment system: pre-filter (network entrance); post-osmosis; reuse; loop and dialysis solution; with an approximate volume of 200 mL in sterile flasks. We added 0.1 ml of 1.8% sodium thiosulphate solution (for chlorine elimination) for each 100 ml of water in the flasks used to collect the samples from the entrance point of the supply network (pre-filter). We collected the samples for quantification of endotoxins in apyrogenic flasks, kept them at a temperature below 10° C and analyzed them on the same day of collection.

The current legislation for evaluation of treated water in hemodialysis services is the Resolution of the Collegiate Board of Directors (RDC) n. 11, of March 13, 2014, that establishes as microbiological limits: absence of *Escherichia coli* in 100 mL; total aerobic bacteria count of 100 Colony Forming Units (CFU)/mL at the point of dialysis solution 200 CFU/mL and action level of 50 CFU/mL; a maximum concentration of endotoxin of 0.25 Endotoxin Unit (EU)/mL[8].

For the water entering the network (before the treatment), the microbiological limits are: absence of *E. coli* and total coliforms in 100 mL and bacterial count of 500 CFU/mL according to Consolidation Ordinance n. 5, of September 28, 2017[18], that establishes the procedures and responsibilities for the control and surveillance of the quality of water for human consumption and its potability standards.

In the present study, we performed the following tests: total aerobic bacteria count, total coliform research and quantification of endotoxins using the method of the *Limulus* Amebocyte Lysate (LAL), described in the Brazilian Pharmacopoeia[9], and phenotypic identification of microorganisms isolated in the water samples, performed according to the methodology described by Jorgensen and Pfaller[19].

### Heterotrophic bacteria count

We used the method of plate counting by depth (in duplicate), using the trypticase soy agar medium (TSA). We diluted one mL of the sample in nine mL of trypticase soy broth (TSB), with pH 7.3 ± 0.2 at 25° C. We added one mL of the dilution to 20 mL of the TSA medium, melted and cooled to 45-50° C. After solidification, the medium was incubated at 32.5° C ± 2.5° C for 48 h. After the incubation period, we did the bacterial count[9].

### Total coliform count

We added 100 mL of the sample to a flask containing 50 mL of Presence-Absence (AP) broth in triple concentration that was homogenized and incubated for up to 48 hours at 32.5 ± 2.5° C. After the incubation period, we transferred a cutoff of the culture with growth and acid and/or acid and gas to a tube containing 10 mL of brilliant green bile lactose broth with Durhan tube and incubated it for up to 48 h at 32.5° C ± 2.5° C. The presence of gas inside the Durhan tube confirms the presence of total coliforms[9].

### Phenotypic identification of microorganisms isolated from water samples

To investigate the isolated microorganisms, we performed phenotypic identification tests for each distinct colony. We performed tests of glucose fermentation-oxidation, mobility, oxidase, fluorescein, pyocyanin, catalase, growth at 42° C ± 1° C and in MacConkey agar, carbohydrates use and gas production, aminoacid decarboxylation, growth in different sodium chloride concentrations, incubation at different temperatures, citrate, methyl red and Voges Proskauer, urea, DNAse, phenylalanine, casein decomposition, lecithin and starch, pigments, indole, metallic sheen in eosin methylene blue agar (EMB) according to Levine, all described in Jorgensen e Pfaller[19].

### Determination of endotoxin concentration

The analyses for the presence and concentration of endotoxins were performed using the LAL assay, only in post-osmosis and reuse samples. We collected the samples in apyrogenic flasks.

For the interpretation of the results, we considered the microbiological limits recommended by RDC n. 11/2014[8] and Consolidation Ordinance n. 5/2017[18].

## RESULTS AND DISCUSSION

During the monitoring period, we surveyed 92 clinics and/or hospitals with hemodialysis services and collected 480 samples for microbiological assays and 192 for endotoxin testing. All facilities were located in Rio de Janeiro.

Among the samples, 80% (384/480) presented satisfactory results for bacterial counts. All samples from the pre-osmosis point (network entrance) were satisfactory and their results were compared to the limits recommended by the Consolidation Ordinance n. 5/2017[18]. The results by collection point, including post-osmosis, reuse, loop and dialysis solution revealed that 31% of the unsatisfactory samples (heterotrophic bacteria counts) were from the loop step, followed by the dialysis solution (Figure 1). The possible formation of biofilms may have enabled the dissemination of these microorganisms at different collection points, especially at the point of dialysis solution that is the direct point of the machine, in which the hemodialysis procedure of the patient will be performed. However, the results for total coliforms and *E. coli* in all samples were negative.

Regarding the endotoxin concentration, of the 192 samples collected from the post-osmosis and reuse points, 76% (146/192) presented satisfactory results (Figure 2A). The results of the reuse and post-osmosis points revealed a higher frequency of unsatisfactory samples at the reuse point (Figure 2B). These data are very important because they signal the presence of microorganisms, since the detection of endotoxins occurs after bacterial lysis and may be even higher in the absence of viable microorganisms.

All samples with microbial growth had their colonies isolated and identified in terms of genus and species (Table), although RDC n. 11/2014[8] only requires the absence of total coliforms without, however, requiring the identification of possible contaminants.

The most frequently isolated microorganism in the samples was *P. aeruginosa*, an extremely versatile Gram-negative bacterium found in soil and water and very common in hospital infections, mainly in immunocompromised patients. *P. aeruginosa* is able to adhere to various materials, contaminating catheters, fans, prostheses and contact lenses. Because of its high resistance to antibiotics and great arsenal of virulence factors, the infections caused by it are difficult to control[17].

The presence of *P. aeruginosa* with a higher frequency was also commented on the study by Peresi et al.[20]. They revealed that, from 2000 to 2009, 43 (8.5%) of the 508 dialysis water samples were contaminated by *P. aeruginosa* and less frequently by other pathogens. In RDC n. 11/2014 the search for *P. aeruginosa* was not included, but the *Pseudomonas* genus is the most frequently isolated in water treated for dialysis, dialysis solution and

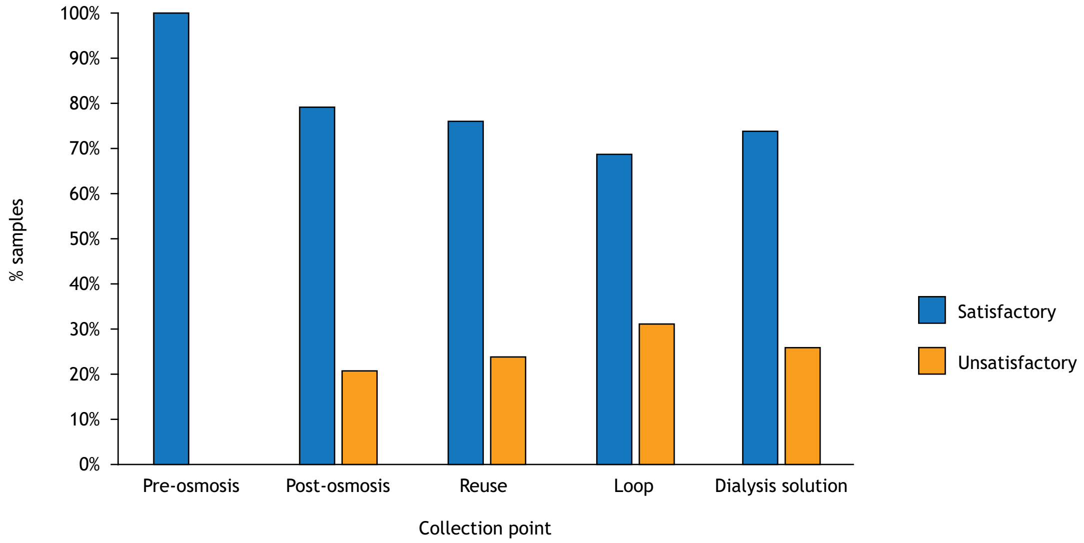


**Figure 1.** Percentage of satisfactory or unsatisfactory samples at the different collection points according to the heterotrophic bacteria count test.

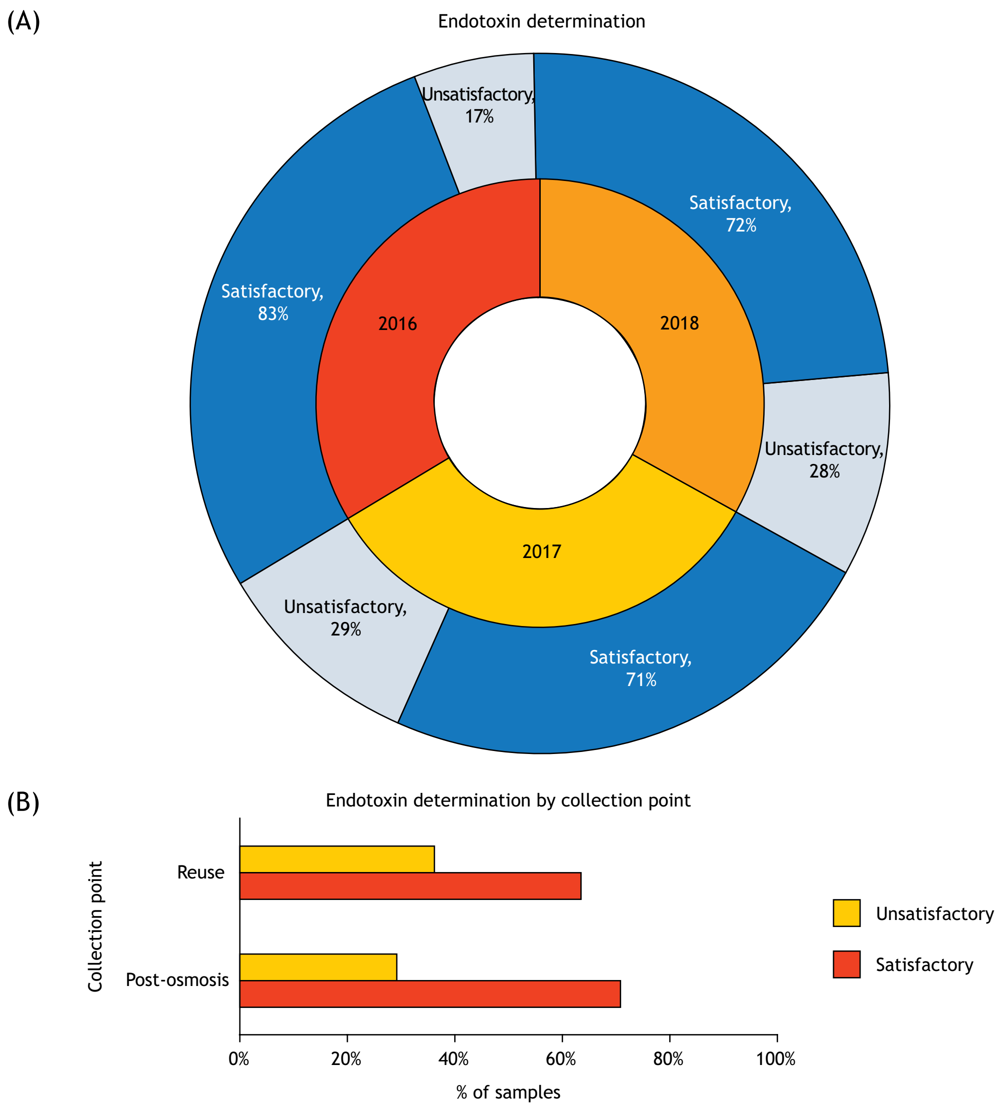


**Figure 2.** Classification of water samples from post-osmosis and reuse points compared to endotoxin determination (A) Total distribution of samples between 2016 and 2018; (B) Arrangement of the samples between the collection points.

**Chart.** Microorganisms identified in the samples per collection point between 2016 and 2018.

| Microorganism | Isolation points | Number of isolates |
|---|---|---|
| *Pseudomonas aeruginosa* | Post osmosis, reuse, loop and dialysis solution | 35.5% (n = 200) |
| *Burkholderia cepacia* | Post osmosis, reuse, loop and dialysis solution | 21.3% (n = 120) |
| *Stenotrophonomas maltophilia* | Reuse, loop and dialysis solution | 19.2% (n = 108) |
| *Acinetobacter baumannii* | Reuse, loop and dialysis solution | 15.1% (n = 85) |
| *Ralstonia pickettii* | Reuse and dialysis solution | 9.0% (n = 50) |
| **Total isolates** | | 563 |

dialysate, and its presence is related to the presence of bacterial endotoxins and the formation of biofilms, thus posing health risks to patients who require treatment[21,22].

Biofilm is a community structure of microbial cells protected by a polysaccharide or protein matrix that is synthesized by cells and adheres to either inert or living surfaces. This matrix is composed essentially of water and extracellular polymeric substances. The formation and development of biofilms start with the adhesion of the cells to the surface, maturation, during which they produce the polymers, cellular reproduction and detachment, when the release of biofilm cells can occur and these cells can return to their planktonic state[23].

The search for *P. aeruginosa* in water treated for hemodialysis is already recommended by the American[24] and Brazilian[9] pharmacopoeias. Therefore, it is important to include it in the current Brazilian legislation for the quality control of water used in dialysis services[21,22].

A study about the microbiological quality of the water intended for hemodialysis showed that bacterial contaminants in samples from different points in a clinic were identified as belonging to the same bacterial species from post-osmosis to the dialysis solution[25].

The bacteria isolated in this study showed common characteristics: they were Gram-negative rods, non glucose fermenters, very associated to hospital infections due to their ability to adhere to objects and sites, resistance to antimicrobials and their opportunistic action in immunocompromised patients. *Stenotrophonomas maltophilia*, for example, is associated with pneumonia or bacteremia, commonly endocarditis, mastoiditis, peritonitis, meningitis, endophthalmitis and infections of soft tissues, surgical wounds and urinary tract[17,18].

*Acinetobacter baumannii is more commonly related to infections involving the respiratory tract (endotracheal tubes or tracheostomy); urinary tract and wounds (including catheter sites) that may progress to septicemia. Burkholderia cepacia is associated with the "cepacia syndrome", a very frequent septic condition in cystic fibrosis patients, characterized by a decline in pulmonary function, with subsequent bacteremia and, in many cases, death*[17,18].

The results found in the endotoxin analysis reinforce the microbiological data found, because in all situations that showed bacterial growth in the post osmosis and reuse points there was also the presence of endotoxin above the threshold established by the current legislation[8,18].

The presence of endotoxins in water causes several acute physiological responses that can cause fever, chills, headache, malaise, myalgia, nausea, yawning, dialyzer coagulation. These depend on several factors, like endotoxin concentration, sensitiveness and the general condition of the patient, and long-term complications like cachexia and amyloidosis[26].

There is a strong correlation between endotoxin concentration and bacteria in the dialysis solution and the presence of typical pyrogenic reaction symptoms (endotoxemia)[26,27]. Bacterial concentration above 200 CFU/mL usually determines enough endotoxin level to cause clinical symptoms because, at high concentrations, the endotoxin may cross dialyzer membranes with minimal rupture or even intact membranes. High concentrations of endotoxins in the blood or cerebrospinal fluid may be fatal because of the complications.[26,27].

Because of phenotypic plasticity and survivalability, mainly of *P. aeruginosa*, water disinfection processes during treatment must be judicious in order to meet the safety levels required by the legislation. Prevention of water contamination requires knowledge about the source of the problem within the treatment line and the correct use of disinfection procedures. Bacterial contamination in water treatment and distribution systems can lead to the formation of biofilms that may persist at different points in the treatment system and develop greater resistance to disinfection procedures[28].

Monitoring services performed by the National Institute for Quality Control in Health (INCQS) along with the Health Surveillance agency exist since 1999 and play an important sanitary role. Today, in general, these services are much better than at the beginning of the monitoring, when the majority microbiological analyses, about 60%, were inadequate. Today, contamination cases are still reported, but are often related to other administrative problems of the services[25].

Because of the importance of the control of the water treated for hemodialysis, current legislation considers the need for it to follow the same criteria as the water for dilution of medications. Therefore, according to the Brazilian Pharmacopoeia, it must follow the standards of medications. This often generates controversy among the laboratories licensed by the Health

Surveillance and causes the release of reports with false negative results, which may pose risks to the population served by these services[9].

Accordingly, the microbiological monitoring of water for hemodialysis, performed by the INCQS, seeks to comply with the action level and improve the quality of this water, pointing out changes that are necessary in the treatment system of health facilities in order to avoid water contamination and bacteremia in patients.

With this study, we hope that there will be collaboration for future reviews on existing specific legislation in order to avoid failures that may harm the patients served by these services. Furthermore, we verified that professionals in both the analytical and health surveillance areas must be continuously trained, so that the services provided in health inspection can meet the necessary quality standards.

## CONCLUSIONS

This study evaluated the microbiological quality overview of the water used in the hemodialysis services in Rio de Janeiro, in partnership with the Health Surveillance of the city. Overall, the results were similar in all points of analysis, emphasizing that control and monitoring must be performed throughout the treatment system.

Among the bacterial genera isolated in the unsatisfactory samples, the most prevalent was *P. aeruginosa*, which highlights the importance of its investigation and warrants its inclusion in current legislation, considering its occurrence in hospital infections and as an opportunistic pathogen in patients treated at hemodialysis services.

Although efforts are currently under way to ensure the safety of patients undergoing invasive procedures like hemodialysis and the number of cases of bacteremia and its complications has decreased, the control of water quality and of the services rendered must be a public health priority.

The results of this study reinforce the importance of a continuous program of water monitoring in the hemodialysis services, because, despite so many years of intervention by the monitoring agencies, we still have unsatisfactory results and pathogenic microorganisms that can cause health problems to patients who require hemodialysis to stay alive.

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## Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.