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MICROBIOLOGICAL ANALYSES OF WATER FROM HEMODIALYSIS SERVICES IN SÃO LUÍS, MARANHÃO, BRAZIL

José de Ribamar Oliveira Lima[1,2]*; Sirley Garcia Marques[2]; Azizedite Guedes Gonçalves[1]; Natalino Salgado Filho[1]; Paulo Cruz Nunes[1]; Hernandes Sousa Silva[3]; Silvio Gomes Monteiro[1]; Jackson Mauricio Lopes Costa[1,4]

[1]Universidade Federal do Maranhão, São Luis, MA, Brasil; [2]Hospital Presidente Dutra da Universidade Federal do Maranhão, São Luís, MA, Brasil; [3]Fundação Josué Montelo, São Luís, MA, Brasil; [4]Centro de Pesquisas Gonçalo Moniz, FIOCRUZ, Salvador, BA, Brasil.

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ABSTRACT

Rigorous control of water quality in hemodialysis services is extremely important in order to guarantee a better quality of life of the patients submitted to this treatment. The lack of adequate water monitoring has caused the death of various patients in the past. The objective of the present study was to determine the physicochemical and bacteriological characteristics of water used by hemodialysis services in hospitals of the city of São Luís, Maranhão, Brazil. Bacteriological analyses included the membrane filter method for the determination of total coliform bacteria, the Cult-Dipcombi-TTC-agar method for heterotrophic bacteria and the limulus amebocyte lysate method for the determination of endotoxins. Eighteen water samples obtained from three hospital units, six samples per hemodialysis service, collected directly at the pre- and post-treatment points, were analyzed. Microorganisms were detected in the water used by the hemodialysis services in two of the three hospital units (B and C) studied. No contamination with heterotrophic bacteria was observed in pretreatment samples, while endotoxin production was detected in 100% of the samples. In post-treatment samples, heterotrophic bacteria were detected in 66.6% of the samples and endotoxins in 33.3%. The microorganisms identified in unit B were *Burkholderia cepacia*, *Alcaligenes xylosoxidans*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. In unit C *Flavimonas oryzihabitans*, *Ralstonia pickettii* and *Burkholderia cepacia* were identified. A significant correlation was observed between the presence of endotoxins and the physicochemical characteristics of water such as turbidity and conductivity. These data indicate that two of the three hospital units studied should revise the control of their hemodialysis water system.

Key words: water, microbiology, hemodialysis, contamination

INTRODUCTION

Water is the main component of the human body and, without any doubt, of all living organisms. However, various microorganisms present in water can cause diseases in humans, leading to infectious, toxigenic and parasitic processes (14). In view of the environmental degradation caused by the high rate of pollution as a result of the ecological imbalance on the planet, and because water is an essential element to life, active environmental management and a greater control of the quality of water resources are required (6).

In the case of water contamination, patients with chronic renal failure are more susceptible than the general population due to the dialytic treatment to which they are submitted, but the prognosis and future of these patients have undergone marked modifications with the latest technology employed

*Corresponding Author. Mailing address: Centro de Pesquisas Gonçalo Moniz-FIOCRUZ, Rua Valdemar Falcão, 121, Brotas. 40295-001, Salvador, BA, Brasil. Telefax: (+5571) 3568782 Ext. 261. E-mail: jcosta@cpqgm.fiocruz.br; ribamarjr@hotmail.com

when compared to the survival of the general population (9,11,16).

The water used in the hemodialysis process should be recognized as a vital product since its direct user is certainly an immunocompromised individual who is susceptible to any opportunistic process. Although microorganisms are known to grow in certain fluids associated with dialysis equipment, microbiological contamination has not been taken seriously in the projects of systems developed for dialytic therapy (12,14).

In the water treatment system adopted by hemodialysis services, water should be at least drinkable, with reverse osmosis and/or deionization being the most widely used techniques to achieve the quality recommended by the current federal legislation (Decree 82, Ministry of Health/GM/January 3, 2000), and should be constantly monitored to respect the maximum levels of contaminants permitted, thus guaranteeing the health of the patient (2,10).

Although Gram-negative bacteria and nontuberculous mycobacteria are the most frequent biological contaminants, the possibility of other types of contaminants should be borne in mind such as cyanobacteria, which have been emphasized as other microorganisms important in the control of dialysis quality after the death of more than 50 patients at a dialysis center in the town of Caruaru, Pernambuco, Brazil, in 1996. Cyanobacteria produce a set of potent toxins, including hepatotoxins, neurotoxins and skin irritants (12,16).

The blood of patients with chronic renal failure submitted to hemodialysis is exposed upon contact with the dialysis membrane to about 1,500 liters of water per month, with uremic patients under hemodialysis treatment being exposed to water volumes ranging from 18,000 to 36,000 liters per year (14,16).

Based on the above considerations and taking into account the different factors that interfere with the quality of water used in dialytic processes and the possible risk factors for the health of hemodialysis patients, the objective of the present study was to determine the physicochemical and bacteriological characteristics of water used by hemodialysis services in hospitals of the city of São Luís.

MATERIALS AND METHODS

An exploratory study was conducted at three hemodialysis services of the public and private network in the municipality of São Luís, capital of the State of Maranhão, Brazil, which comprises a population of approximately 870,028 inhabitants (5). The hemodialysis centers were identified as units A, B and C, and water used for hemodialysis was collected monthly during the period from May 2002 to January 2003 according to the guidelines established by the Brazilian Ministry of Health (2).

The water treatment system employed by these services consisted of filtration using sand and coal filters, deionization with cationic and anionic resins, and reverse osmosis. The system received water from the local supply network and from a deep well distributed by an authorized company.

Eighteen water samples were directly collected from the dialytic pre- and post-treatment points monthly over a period of nine months, with six samples being obtained from each service.

The Health Ministry guidelines establish the following bacteriological tests for treated water used for the preparation of dialysis solutions: total coliform count, determination of the number of heterotrophic bacteria and endotoxins, with the maximum value permitted being the absence of coliforms in 100 mL, 200 CFU/mL and 1 ng/mL, respectively, which should be confirmed by control analysis.

Total coliform bacteria were determined by the membrane filter technique, which is based on the filtration of 100 mL water through a membrane filter (HA cellulose ester) measuring 47mm in diameter with 0.45-µm porosity. The membrane was then transferred to a Petri dish containing selective and differential culture medium (Endo LES agar) and incubated at 35 ± 0.5ºC for 24h (1). Typical and atypical colonies were recorded.

The Cult-Dipcombi-TTC-agar method (Merck) was used for the quantification of heterotrophic bacteria since this is the method employed for the analysis of hemodialysis water in the institution where the study was carried out without restrictions of local sanitary surveillance. One milliliter of the water sample was seeded onto a slide which was then placed into a flask and incubated in a vertical position in a bacteriological oven at 27 to 30ºC for 48h. After incubation, counts were performed by comparing the density of the colonies formed according to manufacturer recommendations and the result is reported as number of bacteria/mL (1).

After verification and counting, all isolated colonies (m-Endo and Cult-Dipcombi-TTC-agar) were reisolated on MacConkey and blood agar and incubated in a bacteriological oven at 35 ± 0.5ºC for 24 h. After growth of the microorganism, a bacterial inoculum was standardized in 0.45% physiological saline using the turbidimeter recommended by the Vitek system according to a scale for Gram-negative bacteria. The inoculum was aspirated onto a Gram-negative identification card and the card was read with an automatized system (Vitek-CC4, bioMerieux). Endotoxins were determined by the limulus amebocyte lysate method (Bio Whittaker), with a sensitivity of 0.125 EU/mL, corresponding to 0.025 ng/mL (15).

Simultaneously to the microbiological tests, the following parameters were determined: apparent water color by visual comparison with potassium chloro platinum standards, free residual chlorine by the DPD method using the Microquant kit (Art. No. 1.14978, Merck), turbidity by measuring the amount of scattered light with a nephelometer (AP-2000 turbidimeter), conductivity using a Digimed DM-3 apparatus, temperature, and pH.

The data regarding bacterial growth (selective medium for total coliforms), heterotrophic bacteria and endotoxins in the

pre- and post-treatment situations were analyzed by Cochran's Q-test. These variables were correlated with the physicochemical and organoleptic characteristics of water using the nonparametric Kendall tau test (7,8). The level of significance was set at 5% for all tests.

RESULTS

Table 1 shows the quantitative results of total coliform bacteria, heterotrophic bacteria and endotoxins, as well as the physical and organoleptic characteristics of drinking water used for hemodialysis established by the Ministry of Health Guidelines. These properties include apparent color, temperature, turbidity, pH and free chlorine which should be analyzed when the water leaves the treatment system for dialysis, and conductivity or resistance which is monitored continuously by an instrument that compensates temperature variations.

Microorganisms were detected in the water used for hemodialysis in units B and C. *Burkholderia cepacia* was isolated from pre- and post-treatment samples of unit B and from post-treatment samples of unit C. Growth of *Alcaligenes xylosoxidans*, *Pseudomonas aeruginosa* and

Table 1. Quantitative results of total coliform bacteria, heterotrophic bacteria and endotoxins, and physicochemical and organoleptic characteristics of water from the three hemodialysis services (São Luís, Maranhão, Brazil).

		A		B		C	
		Pre	Post	Pre	Post	Pre	Post
Total coliforms (m-Endo)/100 ml	1st sampling	AG	AG	AG*	AG*	AG	AG*
	2nd sampling	AG	AG	AG	AG*	AG	AG*
	3rd sampling	AG	AG	AG	AG*	AG	AG*
Heterotrophic bacteria (TTC-agar)/ml	1st sampling	AG	AG	AG	10^3	AG	10^3
	2nd sampling	AG	AG	AG	10^4	AG	10^3
	3rd sampling	AG	AG	AG	10^4	AG	10^4
Endotoxins (EU/ml)	1st sampling	> 0.125	<0.125	> 0.125	> 0.125	> 0.125	<0.125
	2nd sampling	> 0.125	<0.125	> 0.125	> 0.125	> 0.125	<0.125
	3rd sampling	> 0.125	<0.125	> 0.125	> 0.125	> 0.125	<0.125
Apparent color (< 15 HU)	1st sampling	15	4	4	4	9	4
	2nd sampling	10	4	4	4	14	4
	3rd sampling	10	4	4	4	4	4
Temperature (ºC)	1st sampling	30	30	29	29	28	28
	2nd sampling	29	29	29	29	30	30
	3rd sampling	31	31	29	29	30	30
Turbidity (< 5 TU)	1st sampling	4.20	0.29	0.05	0.02	1.11	0.15
	2nd sampling	1.94	0.25	0.15	0.12	4.07	0.36
	3rd sampling	3.69	0.19	0.18	0.05	2.14	0.22
Conductivity	1st sampling	128.5	4.73	801	107.7	62.9	9.23
	2nd sampling	110.0	4.73	834	110	66.9	8.19
	3rd sampling	75.8	4.48	701	32	66.3	11.81
PH (6.5-8.5)	1st sampling	6.34	4.26	7.12	5.09	7.9	6.9
	2nd sampling	6.47	4.73	7.17	5.6	8.0	6.7
	3rd sampling	7.01	7.08	7.01	6.02	7.75	7.11
Free chlorine (> 0.2 to < 2.0 mg/l)	1st sampling	1.0	0.0	0.0	0.0	0.1	0.0
	2nd sampling	1.0	0.0	0.0	0.0	0.1	0.0
	3rd sampling	1.0	0.0	0.0	0.0	0.5	0.0

HU – Haven unit; TU – turbidity unit; AG – absence of growth; EU – endotoxin unit; CFU – colony-forming units.
* Presence of atypical colonies (>100 CFU/mL).

Stenotrophomonas maltophilia was observed in post-treatment samples from unit B and *Flavimonas oryzihabitans* and *Ralstonia pickettii* were detected in post-treatment samples from unit C (Table 2).

No growth of total coliform bacteria was observed in pre- or post-treatment water samples. Heterotrophic bacteria were identified in 11.1% of samples and endotoxins in 100%. Among post-treatment samples, 66.6% were positive for heterotrophic bacteria and 33.3% for endotoxins. Growth of *Burkholderia cepacia* and *Ralstonia pickettii* was observed on the selective medium chosen for the isolation of total coliform bacteria (Endo LES agar), and *Alcaligenes xylosoxidans*, *Flavimonas oryzihabitans*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* grew on medium chosen for the isolation of heterotrophic bacteria (TTC-agar) (Table 3).

Statistical analysis only revealed a significant correlation between post-treatment endotoxins and the physical characteristics turbidity and conductivity (-0.707 and 0.628, respectively), which were both significant at the 5% level. Cochran's Q-test for bacterial growth (medium selective for total coliform bacteria), heterotrophic bacteria and endotoxins in the pre- and post-treatment situations was significant at the 5% level in all cases.

Table 2. Bacteria isolated from dialysis system (pre- and post-treatment) water at three hemodialysis units in São Luís, Maranhão, Brazil.

Bacteria isolated	A		B		C	
	Pre	Post	Pre	Post	Pre	Post
Alcaligenes xylosoxidans	-	-	-	+	-	-
Burkholderia cepacia	-	-	+	+	-	+
Flavimonas oryzihabitans	-	-	-	-	-	+
Pseudomonas aeruginosa	-	-	-	+	-	-
Ralstonia pickettii	-	-	-	-	-	+
Stenotrophomonas maltophilia	-	-	-	+	-	-

Pre and Post = before and after treatment, respectively; (-) = absent; (+) = present.

DISCUSSION

Hemodialysis is an important alternative treatment for patients with chronic renal failure, increasing the quality of life of these individuals and becoming for some the hope of life in view of the irreversibility of the disease and during the waiting period for a renal transplant. Monitoring water quality during hemodialysis is one of the main concerns of health care workers, since contaminations can lead to serious consequences for the patients.

In the present study, we assessed the quality of water used at three hemodialysis centers in the municipality of São Luís, where microorganisms associated with water contamination were identified in two of the services analyzed.

Exposure to bacteria and endotoxins of patients submitted to dialytic treatment is clearly associated with short-term complications causing organic alterations such as fever, shivering, discomfort, myalgia, nausea, yawning, and coagulation of the dialyzer, but also leads to long-term consequences such as migraine and amyloidosis, in addition to contributing to subdialysis (4,15).

Monitoring of the physicochemical and microbiological parameters of water used in the hemodialysis process, as well as the water treatment procedures themselves, is the determining factor to guarantee its quality. In this respect, different treatment steps should be performed using specific systems whose size is calculated based on the volume and characteristics of the water that supplies the dialysis system according to the regulations of the Ministry of Health (2).

The microorganisms associated with the contamination of water used during pre- and post-treatment in the units analyzed

Table 3. Percentage of samples positive of total coliform bacteria, heterotrophic bacteria and endotoxins in hemodialysis water (São Luís, Maranhão, Brazil).

Sample type	No. of samples	Total coliforms Endo LES agar (%)[1]		Atypical colonies Endo LES agar (%)[1]		Heterotrophic bacteria TTC-agar (%)[2]		Endotoxins (%)[3]	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Pretreatment	9	0	100	11.1	88.9	0	100	100	0
Post-treatment	9	0	100	66.6	33.4	66.6	33.4	33.3	66.7
		$P < 0.05$		$P < 0.05$		$P < 0.05$		$P < 0.05$	

Maximum value permitted: [1]absence in 100 ml; [2]200 CFU/ml; [3]1 ng/ml.
$\alpha = 5\%$.

were Gram-negative bacilli, in agreement with other reports (11,12,15,16). In the study by Santos *et al*. (15), about 90% of the bacteria found were Gram-negative, with a clear predominance of the genus *Pseudomonas*, which was able to multiply rapidly even in sterile water, reaching high concentrations (>100,000 CFU/mL) in less than 48 h. In dialysis solutions, this bacterial growth might be even faster due to the presence of glucose and bicarbonate, thus producing high toxin levels.

The microorganisms identified in pre- and post-treatment samples were heterotrophic bacteria, with the percentage being higher percentage in post-treatment samples, a finding similar to those reported by others (9,12).

No growth of total coliform bacteria was observed on selective medium (Endo LES agar); however, atypical colonies were isolated which were subsequently identified as heterotrophic bacteria, demonstrating the inadequate conditions of the water used. Among these bacteria, *Burkholderia cepacia* was detected in two of the units analyzed, with this microorganism being associated with cystic fibrosis, causing respiratory infection that can lead to irreversible tissue damage (13).

Growth of heterotrophic bacteria on Cult-Dipcombi-TTC-agar was expected for the pretreatment sample of unit B in view of their growth on Endo medium, but this was not the case. Probably, the small amount of sample (1 mL) was responsible for this lack of growth.

Endotoxins represent the main lipid component of the outer membrane of Gram-negative bacteria and are released into the surrounding medium during bacterial multiplication and even more so when the bacteria die. Both endotoxins and their fragments are highly stable substances, and their chemical degradation requires the use of strong acids or bases, or a process of pyrolysis (15). The fact that the units studied here used chlorine in the water reservoirs before dialytic treatment may explain why the pretreatment samples collected at units A, B and C tested positive for endotoxins in the absence of heterotrophic bacteria.

The presence of *Pseudomonas aeruginosa* was only observed in one of the units, this being a bacterium recognized as one of the most important etiological agents of nosocomial infections, which easily spreads to other immunocompromised patients (9,17).

With respect to the water's physicochemical properties, free chlorine levels were found to be below the value permitted by Decree MS No. 518, March 25, 2004 (>0.2 to <2.0 mg/L) in three pretreatment water samples from unit B and two pretreatment water samples from unit C. In unit A, where free chlorine levels were within the permitted values, no bacterial growth was observed. Free chlorine and its derivatives (dioxides, hypochlorite and chloramines) are added to natural water to eliminate microorganisms and/or to oxidize certain undesired ions such as iron and manganese (16).

Endotoxins are the major lipid components of the outer membrane of Gram-negative bacteria, which are released into the circulating medium during multiplication and after bacterial death, with a good correlation being observed between endotoxin and bacterial concentration in the dialysate and the presence of pyrogenic reactions in the patients (11,15).

In the present study, endotoxins were identified in 100% of the pretreatment samples of all units analyzed and in 33% of post-treatment samples, with the corresponding values being above the sensitivity of the test (0.125 EU/mL), and the presence of endotoxins alerting to the need for a more rigorous monitoring and control of all steps of the process of hemodialysis water treatment (15).

Due to its drinking water status, water monitoring during pretreatment is regulated by ministerial decree, which establishes for the human organism a limit of tolerance of cyanobacteria, bacteria frequently found in water used for human consumption. These guidelines establish that every time their number exceeds 20,000 cells/mL (biovolume: 2 mm^3/L) in the source water at the capture point, weekly analysis of cyanotoxins is required in water leaving the treatment plant and water entering (water meter) the hemodialysis clinics (3).

Due to the complexity of the water treatment systems, the monitoring of hemodialysis services requires qualified professionals for operation and maintenance in order to guarantee adequate application of chemical products for disinfection of the piping system, thus eliminating chemical residues and bacterial contamination that may infect patients.

The presence of bacteria in two of the three units analyzed in São Luís, Maranhão, indicates the need for adequate monitoring by local health authorities in order to guarantee better control of the water treatment systems supplying hemodialysis centers.

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RESUMO

Análises microbiológicas da água dos serviços de hemodiálise em São Luís, Maranhão, Brasil

A necessidade de um controle rigoroso no serviço de hemodiálise tornou-se algo de extrema importância para garantir uma melhor qualidade de vida aos pacientes submetidos a este tratamento, uma vez que, a falta de controle de qualidade da água, tem levado a óbito vários pacientes. Este estudo teve como objetivo avaliar as características físico-químicas e bacteriológicas da água utilizada pelos serviços de hemodiálise em hospitais da cidade de São Luís. A metodologia utilizada para

as análises bacteriológicas foi: método da membrana filtrante para determinação de coliformes totais, método Cult-Dipcombi-TTC-ágar para bactérias heterotróficas e método limulus amebocyte lysate para endotoxinas. Foram analisadas dezoito amostras de água em três unidades hospitalares, sendo seis amostras provenientes de cada serviço de hemodiálise, colhidas diretamente dos pontos pré e pós-tratamento. Quanto à presença de microrganismos na água utilizada pelos serviços de hemodiálise, observou-se positividade nas unidades hospitalares B e C. Não foi encontrada contaminação por bactérias heterotróficas nas amostras examinadas no pré-tratamento, entretanto a contaminação por endotoxinas foi em 100% das amostras. Nas amostras pós-tratamento encontrou-se para bactérias heterotróficas 66,6% e endotoxinas 33,3%. Os microrganismos identificados na unidade hospitalar B foram: *Burkholderia cepacia, Alcalígenes xilosoxidans, Pseudomonas aeruginosa e Stenotrophomonas maltophilia.* Na unidade C, foram identificados: *Flavimonas oryzihabitans, Ralstônia pickettii e Burkholderia cepacia.* Houve uma correlação significativa entre a presença de endotoxinas e características físico-químicas da água tais como: turbidez e condutividade. Estes dados revelaram que duas das três unidades hospitalares avaliadas necessitam rever o controle do sistema de água de hemodiálise.

Palavras-chaves: água, microbiologia, hemodiálise, contaminação

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