


Research Article

Study of the Microbiological Quality of Water for Hemodialysis After Implementation of a New Treatment System at the University Hospital of Yaoundé

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Abstract

Controlling the microbiological quality of water in hemodialysis centers is essential to avoid complications in hemodialysis patients that may be caused by microorganisms. The aim of this study was to determine the microbiological quality of water after the installation of a new water treatment system in the hemodialysis department of the Yaoundé University Hospital Center. A total of sixteen (16) samples were taken every two weeks at sites A (network inlet), B (filter outlet/osmosis inlet), C (osmosis outlet) and D (loop return) between May and July 2023. Microorganisms were isolated after filtration of 100 ml of water through a nitrocellulose membrane, microporosity 0.22 µm, then deposited on Tryptone Glucose Extract Agar (TGEA) medium and incubated at room temperature between 17 and 22 °C for 7 days. After subculturing on different media, the pure microorganisms were identified by their cultural characteristics and marketed biochemical galleries. The compliance threshold was below 100CFU/ml. Of the samples analyzed, 56% (9/16) were declared non-compliant (>100UFC/ml) versus 43% (7/16) compliant (<100CFU/ml). Only samples from the fourth series were all compliant at points A, B, C and D. Of the microorganisms identified, five (5) species were Gram-negative bacilli, including *Acinetobacter baumannii*, *Pseudomonas luteola*, *Burkholderia cepacia*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. Gram-positive cocci were all coagulase-negative *Staphylococcus* and yeasts were *Candida* spp. The most frequently isolated bacterial genera were *Pseudomonas* (29.17%), *Staphylococcus* (25%), *Acinetobacter* (16.67%), *Stenotrophomonas* (12.50%), *Candida* (12.50%) and *Burkholderia* (4.17%). In this study, although the samples from the fourth series of sampling were all compliant at the various sampling points, the high rate of non-compliance and the detection of a variety of microorganisms demonstrate the need to reinforce the disinfection system in the hemodialysis water treatment circuit.

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Received: 5 July 2024; Accepted: 22 July 2024; Published: 6 August 2024



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Keywords

Water, Hemodialysis, Contamination, Microorganisms, Disinfection

1. Introduction

The most common modality of renal replacement therapy worldwide is hemodialysis (89%) [1]. This is a procedure in which a dialysis machine and a special filter called an artificial kidney or dialyzer are used to cleanse the blood of impurities [2].

In the course of an average week of hemodialysis, a patient may be exposed to 300-600 liters of water, providing multiple opportunities for exposure to waterborne pathogens [3]. The water used for treatment comes from the mains and is therefore not sterile [4]. Patients undergoing hemodialysis are vulnerable because of their weakened immunity, and contaminants such as microorganisms, endotoxins, peptidoglycans and bacterial DNA fragments present in the water and resulting dialysate can cross the dialyzer membrane, stimulate cytokine production and trigger a rise in acute-phase reagents. These can give rise to acute intra-dialytic complications such as fever, acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), chills, hypotension, headaches, nausea and cramps [6]. Controlling the quality of the water used in hemodialysis is therefore a major concern, due to the various contaminations of microbiological origin that can have serious repercussions for hemodialysis patients. Despite advances in extrarenal purification techniques, the quality of water and associated dialysis solutions have been implicated in adverse patient outcomes [7].

In Europe, a microbiological quality control of dialysate from several hemodialysis centers in Germany showed a high concentration of microorganisms and endotoxins above the standard (<100 CFU/ml and <0.25EU/ml) set by the Association for Advancement of Medical Instrumentation (AAMI) [8]. They have set standards to avoid any risk of contamination, as in 1996, when an epidemic of bacteremia occurred in a hemodialysis center in Campinas, in the Brazilian state of São Paulo [9]. Following this episode, water and dialysate samples were collected at various sites in the hemodialysis system for monitoring. At the first collection, 80% of samples showed *Pseudomonas aeruginosa* and *Burkholderia cepacia*, two Gram-negative bacteria, while at the second collection, 100% of samples showed identification with both bacteria [9]. In Cameroon, studies carried out in 2015 by *Gueguim et al* on hemodialysis water showed microbiological contamination, with 83.3% non-compliance at the Centre Hospitalier Universitaire de Yaoundé [10]. These results led to the installation of a new hemodialysis water treatment system in the CHUY hemodialysis unit. The

aim of this study was to investigate the microbiological quality of hemodialysis water after installation of the new system.

2. Materials and Methods

2.1. Sample Collection and Transport

2.1.1. Collection

Water sampling was carried out under rigorous aseptic conditions. After disinfecting the sampling sites with a hydro-alcoholic solution, we let the water run for 1 to 2 minutes to ensure that the sample was not contaminated by traces of the disinfectant, then collected it in sterile single-use bottles [11].

2.1.2. Transport

The vials were clearly labelled and transported immediately to the laboratory, accompanied by a form containing all the necessary information (date, time and place of collection) for immediate analysis.

2.2. Microbiological Analysis

2.2.1. Isolation and Quantification of Microorganisms

To count the number of microorganisms suspended in the water sample, a 100 ml volume of water sample was filtered through a nitrocellulose membrane with microporosity (0.22 µm). The filter was then placed on Tryptone Glucose Extract Agar (TGEA) and incubated at room temperature (17-22 °C) for 7 days [11]. The number of colonies found was expressed as the mean colony-forming units (CFU/mL) [11]. Sterile water for injection (SWFI) was used as a control.

2.2.2. Microorganism Identification

After Gram staining, pure isolates were transferred to Chapman, Mueller Hinton, MacConkey and Sabouraud agars and incubated at 37 °C for 24 to 48 hours.

Microorganisms were identified using commercially available biochemical strips: API 20NE strip for identification of bacteria classified as non-fermentative, API 20E strip for identification of enterobacteria, mannitol, catalase, co-

agulase and DNase tests for identification of staphylococcus species.

2.3. Data Processing and Analysis

Data were collected, processed and analyzed using Excel (version 2010). Results were presented in the form of tables, graphs or narratives.

3. Results

3.1. Colony Count After Culture

To check whether the number of germs present in CHUY's hemodialysis water met international standards, colonies isolated on membrane filters were counted at each sampling point and expressed as CFU/ml.

Table 1. Colony counts after membrane filtration.

Number of colonies UFC/ml for each sample after 7 days				
	Sample 1 May 11, 2023	Sample 2 May 29, 2023	Sample 3 June 12, 2023	Sample 4 June 26, 2023
Point A	PC	PC*	PC*	PC*
Point B	PC	PC	PC*	PC*
Point C	PC	PC	PC	PC*
Point D	PC	PC	PC	PC*

Point A: Network inlet; Point B: Filter outlet/Osmosis inlet; Point C: Osmosis outlet; Point D: Loop return;
PC: Presence of colonies >100CFU/ml (non-compliant); PC*: Absence of colonies <100CFU/ml (compliant)

The results for the first sample were above the standard on all points (>100UFC/ml); Two weeks later, a second sample was taken and only non-conformity was observed on points B, C and D (>100UFC/ml); The analysis of the third sample showed non-conformity on points C and D (>100UFC/ml); And the fourth sample had all points conforming to the standards in force (<100UFC/ml).

3.2. Compliance After Cultivation

According to [Figure 1](#), 56.25% of samples were non-compliant, with colony counts well above the norm (>100 CFU / mL), while only 43.75% of samples were compliant (<100 CFU / mL).

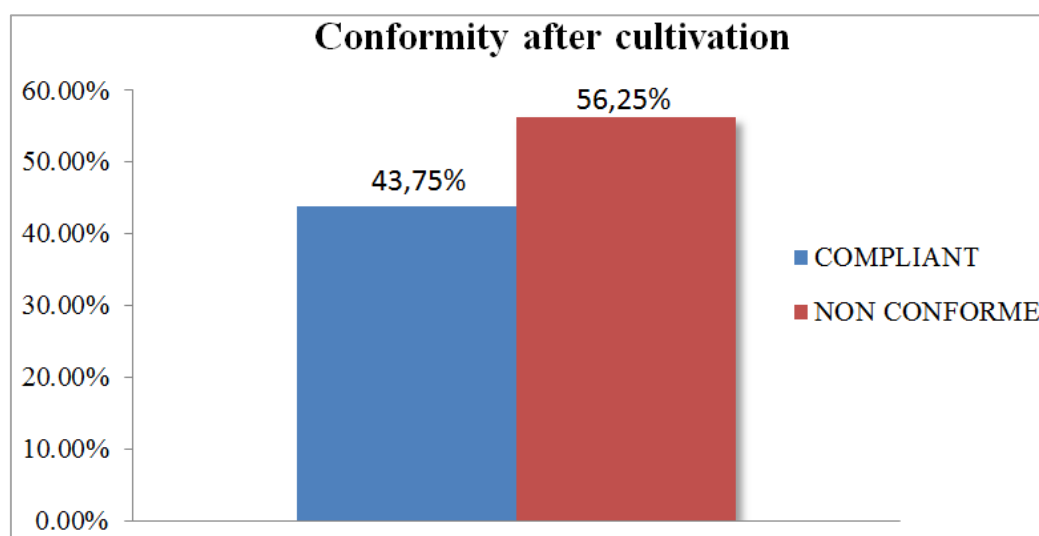


Figure 1. Percentage of compliance after culture.

3.3. Colony Identification After Culture

Gram staining and fresh state observation were carried out to determine the type of germ present at each sampling point. Table 2 below shows the results obtained. Our results show that we mainly isolated gram-negative bacilli, then gram-positive cocci and finally yeasts.

Table 2. Type of bacteria at each sampling point.

Type of germs	Levy 1/ May 11, 2023	Sampling 2/ May 29, 2023	Sampling 3/ June 12, 2023	Sampling 4/ June 26, 2023
	A B C D	A B C D	A B C D	A B C D
Gram- bacilli	++++	++++	++++	++++
Gram+ cocci	----	----	++--	++++
Yeast	----	---+	++--	++--

(+) = present (-) = absent

3.4. Microorganisms Isolated in Hemodialysis Water

The results show a variation in the germs isolated, depending on the type of sample taken. In the first sampling, *Acinetobacter baumannii* was more frequently isolated, followed by *Pseudomonas aeruginosa* in the second sampling. On the other hand, *Staphylococcus coagulans negativa* was most frequently isolated in the third sampling.

Table 3. Microorganism species isolated from hemodialysis water.

Type of isolated germs	Levy 1/ May 11, 2023	Sampling 2/ May 29, 2023	Sampling 3/ June 12, 2023	Sampling 4/ June 26, 2023
	A B C D	A B C D	A B C D	A B C D
<i>Acinetobacter baumannii</i>	++++	----	----	----
<i>Pseudomonas luteola</i>	++--	-+--	----	----
<i>Burkholderia cepacia</i>	--+-	----	----	----
<i>Pseudomonas aeruginosa</i>	----	-+++	---+	----
<i>Stenotrophomonas maltophilia</i>	----	--+-	----	-+-+
<i>Coagulase-negative Staphylococcus</i>	----	----	++--	++++
<i>Candida sp</i>	----	---+	++--	++--

(+) = Presence (-) = Absence Point A: Network inlet; Point B: Filter outlet/Osmosis inlet; Point C: Osmosis outlet; Point D: Loop return

3.5. Distribution of Bacterial Genera

The figure below shows the representation of the germs isolated. The genus *Pseudomonas* accounts for 29.17% of germs isolated, *Staphylococcus* 25%, *Acinetobacter baumannii* 16.67%, *Stenotrophomonas maltophilia* 12.50%, *Candida spp* 12.50% and *Burkholderia cepacia* 4.17%.

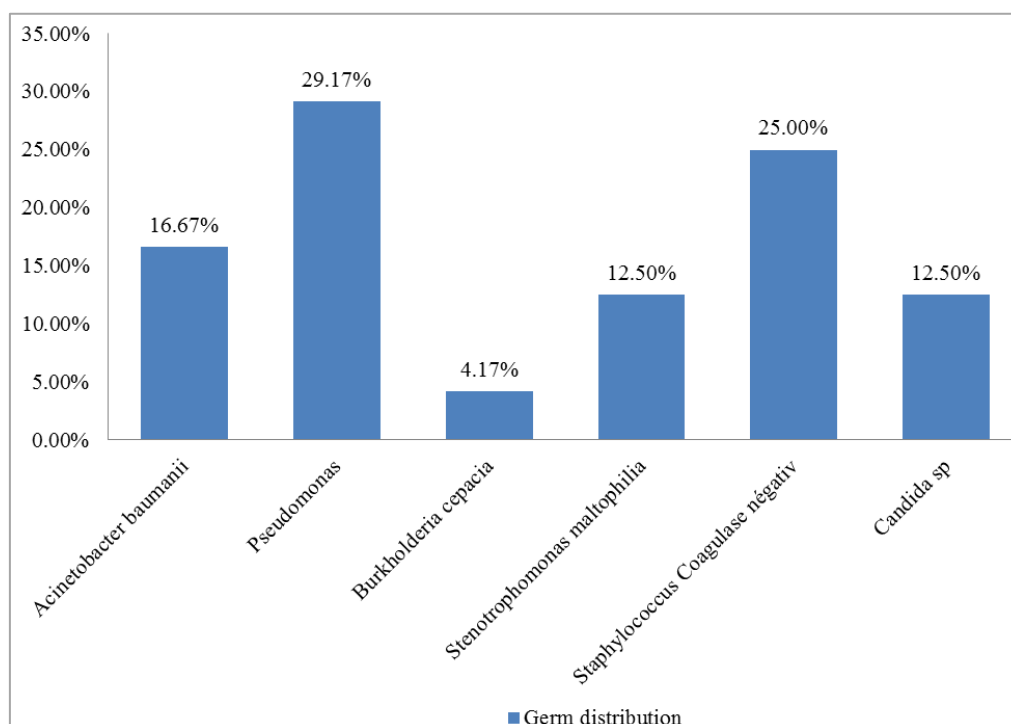


Figure 2. Bacteria isolated by genus.

4. Discussion

Every week, hemodialysis patients are exposed to around 400 l of water used for the production of dialysis fluids which, albeit with the interposition of an artificial semi-permeable membrane, come into direct contact with the bloodstream [12]. The best possible treatment of water intended for dialysis is therefore of paramount importance, and recurrent maintenance of treatment devices is essential to reduce or eliminate the load of possible contaminants. Disinfection should be carried out at least once a week, using appropriate and effective products. During our expedition to the CHUY hemodialysis department, the system was treated weekly with a solution of hydrogen peroxide and citric acid, added at the system inlet. This solution is used as a water disinfectant because of its effectiveness in combating impurities and micro-organisms.

Contamination of dialysis water has been a problem in hemodialysis departments for several years, due to the presence of contaminants such as bacteria and endotoxins that can come into contact with blood and present risks for hemodialysis patients. Indeed, contamination by microorganisms >100UFC/ml can lead to septicemia and even endotoxemia.

The study showed bacterial loads ranging from 10 to 1000UFC/ml, which is higher than international standards (<100UFC/ml). These results differ from those of Oumokhtar et al [13] published in 2013, carried out in a hemodialysis center in the city of Fez, Morocco, with a bacterial load

meeting standards of 8.74 ± 5.52 CFU/ml. But similar to the results of Heidarieh et al [14] announced in 2016 and carried out in five hospitals in a developing country (one in Tehran, and the others in Alborz), which reported variant loads of 50 to 1000 CFU/ml.

The sampling points were all non-compliant at the first sampling, but compliance was observed as early as the second sampling for point A, at the third sampling for points A and B, and compliance for all points at the fourth sampling. The low microbial load at point A (network inlet) can be explained by the use of a disinfectant (hydrogen peroxide) and a descaler (citric acid) at this site by the maintainer of the CHUY hemodialysis water treatment room, with a treatment frequency of once a week. Point B (filter outlet/Osmosis inlet) has a filter designed to retain particles suspended in the water. To block microorganisms, this filter had a porosity of 1 μm . It is recommended to have a filter with a porosity of 0.2 μm , so it would be possible that it acts much more as a support for bacterial growth, hence the high contamination observed.

The high level of contamination at point C (Osmosis outlet) indicates the installation of a biofilm, which explains the results at point D (loop return), which also show the development of a biofilm. This situation could reflect a failure in the maintenance of the disinfection system used to purify the water distributed in the hemodialysis circuit. In fact, it has been shown that the presence of biofilm on pipes leads to rapid bacterial growth after just a few hours of disinfecting the water system [15].

Microbiological analysis of the water revealed the presence of various types of micro-organisms. Among these, a number of Gram-negative bacilli (64%), Gram-positive

cocci (24%) and yeasts (12%) were isolated. These results are very similar to those of Gueguim *et al* [10] performed in the hemodialysis department of CHUY and published in 2016 with the presence of Gram-negative Bacilli (50%) and Gram-positive Cocci (33.3%). Similarly, those of Al-Haik *et al* [16] performed at the Artificial Kidney Center in the town of Mukalla published in 2020 with (85.7%) Gram-negative Bacilli and (14.2%) Gram-positive Cocci. Several Gram-negative Bacilli were isolated from the water distribution system, with a clear predominance of the *Pseudomonas* genus. As the *Pseudomonas* genus is able to multiply rapidly even in sterile water, they can reach high concentrations (>100,000 CFU/ml) in less than 48 hours in dialysis solutions, and this bacterial growth could be even more rapid due to the presence of glucose and bicarbonate in dialysis solutions, producing high levels of toxin responsible for infections in hemodialysis patients [17].

The high concentration of bacteria found in hemodialysis water also suggests a high level of endotoxins. Endotoxins are low-molecular-weight proteins (2,000 to 20,000 Da) that can cross dialyzer membranes and enter the patient's bloodstream, stimulating circulating immune cells across the membrane to produce sepsis symptoms or a pyrogenic reaction [7].

The germs most frequently isolated from hemodialysis water were *Pseudomonas* (29.17%), *Staphylococcus* (25%), *Acinetobacter* (16.67%), *Stenotrophomonas* (12.50%), *Candida* spp (12.50%) and *Burkholderia* (4.17%). These results are similar to those of Cădric Gueguim *et al* [18] carried out in the hemodialysis department of Douala General Hospital and published in 2022, with *Pseudomonas* dominating at 38.5% and *Staphylococcus* spp at 23%, and the studies by Laila Chaoui *et al* [19] published in 2022 at the Mohammedia hemodialysis center in Morocco, with *Pseudomonas* (25.53%) and *Staphylococcus* spp (17.02%) dominating. In the study by Sang-Hyun Park *et al* [20], on the other hand, the genus *Staphylococcus* spp was the most isolated at 30.15%, followed by the species *Sphingomonas paucimobilis* at 21.42%.

Studies by Okunola and Olaitan [21] published in 2016 in three hemodialysis centers in Nigeria revealed that the most frequently isolated bacteria in hemodialysis water belonged to the genus *Pseudomonas* (55%), and those by Jesus *et al* [22] carried out in the hemodialysis department of the city of Rio with 35.5% of genus *Pseudomonas* isolated. In our study, the frequency of *Pseudomonas luteola* species (12.5% (3/24)) and *Pseudomonas aeruginosa* (16.6% (4/24)) is worrying, as these predominant opportunistic pathogens can cause infections in dialysis patients. Thus, it is important to consider their presence as biofilms in dialysis units, and also to prevent their existence as biofilms [23].

5. Conclusion

The aim of this study was to investigate the microbiological quality of haemodialysis water in order to ensure patient safety by minimising the risk of infection and complications

associated with dialysis water contamination. Microbiological analyses of the first, second and third water samples showed the presence of microorganisms and a high level of contamination in excess of international recommendations (>100 CFU/ml). Although the last sample showed a microbial load in compliance at all points (<100 CFU/ml), it would be important to reinforce the weekly disinfection system at the various points in order to facilitate the elimination of biofilms and guarantee a better quality of life for patients receiving this treatment.

Abbreviations

CHUY	Yaoundé University Hospital
TGEA	Tryptone Glucose Extract Agar
CFU	Colony Forming Unit

Conflicts of Interest

The authors declared no conflicts of interest.

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