



(RESEARCH ARTICLE)



Microbiological analysis of hemodialysis water at the Douala General Hospital, Cameroon

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Abstract

Rigorous control of the microbiological quality of water in hemodialysis services is important because the immune system of patients with chronic renal failure is weakened. The objective of this study was to determine the microbiological quality of water for hemodialysis in the hemodialysis department of the hemodialysis center of Douala General Hospital in order to improve the disinfection strategy. Twelve water samples were collected each month at different sites of the hemodialysis circuits A (inlet of filters), B (Outlet of filters / inlet of Reverse Osmosis (RO) device) and C (outlet of the RO device / close to the generator) between November 2015 and February 2016 to be analyzed. The bacteria were isolated after filtration of 100 ml of water at each site through nitrocellulose membrane with 0.45 µm microporosity deposited on the surface of the Tryptone Glucose Extract Agar (TGEA) and then incubated at room temperature (20 to 22°C) for 7 days. After transplanting to different environments, pure bacterial isolates were identified by their cultural characters and marketed biochemical galleries. The colony count was well above the required international standards (>100 CFU / ml), for the hemodialysis water with a percentage of 50% (6/12) of non-compliance. Among the bacteria identified, nine (07) were Gram-negative bacilli including *Pseudomonas fluorescens* and *Klebsiella pneumoniae* subsp *ozaenae*, three (03) Gram-positive bacilli all *Bacillus* sp and three (03) Gram-positive cocci all of coagulase-negative staphylococci. The most frequently isolated bacterial genera were *Pseudomonas* (38.5%), *Klebsiella* (15, 5%), *Bacillus* (23%) and *Staphylococcus* (23%). The detection of a variety of bacteria in the hemodialysis water in this study indicates the need for regular and appropriate monitoring of water for hemodialysis by the hemodialysis center of Douala General Hospital to ensure a better quality of life for patients undergoing this treatment.

Keywords: Water; Hemodialysis; Microbiology; Contamination; Disinfection

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1. Introduction

Water is the main component of the human body and, no doubt, of all living organisms. However, various microorganisms present in water can cause diseases in humans, which leads to infectious, toxigenic and parasitic processes¹. Given the environmental degradation caused by the high rate of pollution linked to the ecological imbalance of the planet and the vital nature of water, the active management of the environment and the quality control of water resources are needed². In the case of water contamination, the patients with chronic kidney disease are more vulnerable than the general population because of the dialysis treatment to which they are subject^{1,3}. Hemodialysis is one of the chronic renal failure treatment modalities that requires pure water for the preparation of the dialysate. The blood of patients with chronic renal failure undergoing hemodialysis is exposed in contact with the dialysis membrane to about 1500 liters of water per month, thus a volume of water ranging from 18 000 to 36 000 liters per year^{4,5}. Patients treated for End Stage Renal Disease (ESRD) by three weekly sessions of 4 hours, are exposed in just three years of treatment to a larger volume of water that a person with normal renal function would during his entire life⁶.

Urban water contains contaminants that induce proinflammatory cytokinic responses and consequently harm the health and the quality of life of the patient. It must for this purpose be treated to be suitable for the preparation of the dialysate. An impure water is unsafe to prepare dialysate; it could contain bacteria and endotoxins that are associated with acute and chronic complications of dialysis such as fever, discomfort, dialyzer clotting, nausea, migraine, amylose and the increased risk of cardiovascular disease occurrence^{7,8}. Monitoring the microbiological quality of water for hemodialysis is thus one of the main concerns of health professionals, since contamination can have serious consequences for patients.

Although microorganisms are known to grow in certain fluids associated with dialysis equipment, microbiological contamination has not been taken seriously in the developed system's projects for dialysis treatment after the death in 2013 of 11 patients at a Dialysis Center in Cameroon⁹. Gram-negative bacteria and nontuberculous mycobacteria are the most common biological contaminants from the dialysis system, the possibility of other types of contaminants such as Cyanobacteria should be kept in mind as likely to harm the health and quality of life of hemodialysis patients in Cameroon.

Based on the above considerations and taking into account the various factors that interfere with the quality of the water used in dialytic processes and potential risk factors for the health of hemodialysis patients in Cameroon, the objective of this study was to determine the bacteriological characteristics of water used for hemodialysis in the hemodialysis department of the Douala General Hospital in order to validate the efficiency of the disinfection procedure and if necessary modify the strategy of disinfection (frequency, product, concentration, time of contact) to obtain permanently a good quality of hemodialysis water.

2. Material and methods

2.1. Collection and transport of samples

2.1.1. Collection

Samples of water collected were carried out under stringent aseptic conditions. After disinfecting the sample sites with a water-alcohol solution, we let the water run 1 to 2 minutes so that the sample is not contaminated by traces of the disinfectant, then collected in disposable sterile bottles¹⁰.

2.1.2. Transport

After sampling, the bottles were clearly labeled and transported immediately to the laboratory, accompanied by a form containing all the necessary information (date, time and collection site) and rapidly analysed.

2.2. Bacteriological analysis

2.2.1. Bacteriological isolation and quantification

In order to count the number of bacteria suspended in the water sample, a volume of 100 ml water sample was filtered through a membrane with micro porosity (0.45 µm). The filter was then placed on TGEA (Tryptone Glucose Extract Agar) and incubated at room temperature (20 to 22°C) for 7 days¹⁰. The number of colonies found were expressed as the mean of the Colony Forming Units (CFU / mL)¹⁰. Sterile water for Injection (**SWFI**) was used as control.

2.2.2. Bacteria Identification

After the completion of Gram staining, pure bacterial isolates were transplanted on Chapman, Mueller Hinton, MacConkey, blood and chocolate agar and incubated at 37 ° C for 24 to 48 hours.

Bacteria Identification was carried out using commercially available biochemical galleries: API 20NE gallery for identifying bacteria classified nonfermentative, API 20E gallery for identification of enterobacteria, mannitol tests, catalase, coagulase and DNase for the identification of staphylococcal species.

2.3. Processing and data analysis

Data was collected, processed and analyzed using the Excel software (2010 version). The results were presented in tables, graphs, or narrative.

3. Results

3.1. Counting the colonies after culture

To see if the number of germs that may be present in the CHUY water for hemodialysis meets international standards, the colonies were counted at each sampling point and expressed in CFU / mL.

Table 1 Colony counts after membrane filtration

| Number of colonies in CFU/ml for every collection/month | | | | |
|---|--------------------------------|--------------------------------|-------------------------------|--------------------------------|
| | Collection 1 /November 2015 | Collection 2/ December 2015 | Collection 3/ January 2016 | Collection 4/ February 2016 |
| Point A | PC | PC | PC | PC |
| Point B | PC | PC* | PC* | PC* |
| Point C | PC | PC* | PC* | PC* |

Point A = inlet of filters (systemic water); **Point B** = outlet of filters /inlet of RO device; **Point C** = outlet of the RO device / entry to the generator, PC = Presence of colonies (>100 CFU/mL), PC* = Presence of colonies (<100 CFU/mL).

We can see from Table I that the number of colonies is far above the norm (>100UFC / mL) only for collections carried out in November 2015 at points A, B and C. For the samples taken in December 2015, January and February 2016, only the count of colonies at point A was higher than the standard (>100UFC / mL). This suggests that the monthly disinfection of the circuit carried out after the first sampling was efficient.

3.2. Conformity after culture

We observe from Figure 1 that 50% of samples were non-compliant with the number of colonies well above the norm (>100 CFU / mL), whereas 50% of samples were compliant (<100CFU / mL).

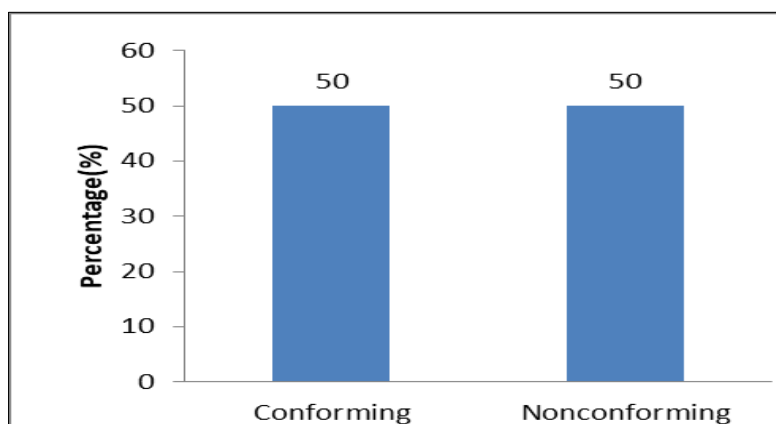


Figure 1 Percentage of conformity after culture

3.3. Identification of colonies after culture

Gram staining was performed to determine the type of bacteria present in each sampling point. Table II below shows the results obtained.

Table 2 Bacteria type as a function of each sampling point

| Type of Bacteria | Collection 1/ November 2015 | | | Collection 2/ December 2015 | | | Collection 3/ January 2016 | | | Collection 4/ February 2016 | | | Total/ Percentage |
|------------------|--------------------------------|---|---|--------------------------------|---|---|-------------------------------|---|---|--------------------------------|---|---|----------------------|
| | A | B | C | A | B | C | A | B | C | A | B | C | |
| Gram - Bacilli | + | - | - | + | + | - | + | + | - | + | + | - | 7 (54%) |
| Gram + Bacilli | + | + | + | - | - | - | - | - | - | - | - | - | 3 (23%) |
| Gram + Cocci | + | + | + | - | - | - | - | - | - | - | - | - | 3 (23%) |

(+) = Presence (-) = Absence

It is clear from our results that the Gram-negative bacilli were more isolated, than the Gram-positive bacilli and Gram-positive cocci.

3.4. Isolated microorganism in water for hemodialysis

It is observed that the bacteria isolated vary depending on the sample. Table III below shows the results obtained.

Table 3 Microorganisms species isolated in water for hemodialysis between November 2015 and February 2016

| Isolated Bacteria | Collection 1/ November 2015 | | | Collection 2/ December 2015 | | | Collection 3/ January 2016 | | | Collection 4/ February 2016 | | |
|---|--------------------------------|---|---|--------------------------------|---|---|-------------------------------|---|---|--------------------------------|---|---|
| | A | B | C | A | B | C | A | B | C | A | B | C |
| <i>Bacillus sp</i> | + | + | + | - | - | - | - | - | - | - | - | - |
| <i>Pseudomonas fluorescens</i> | + | - | - | + | + | - | - | - | - | + | + | - |
| <i>Klebsiella pneumoniae</i> subsp Ozaenae, | - | - | | - | - | + | + | - | - | - | - | - |
| <i>Staphylocoque à coagulase négative</i> | + | + | + | - | - | - | - | - | - | - | - | - |

(+) = Presence; (-) = Absence; A = Inlet of filters (systemic water); B = Outlet of Filter / inlet to the RO device; C = outlet of the RO device / entry to the hemodialysis generator.

3.5. Distribution of bacterial genera

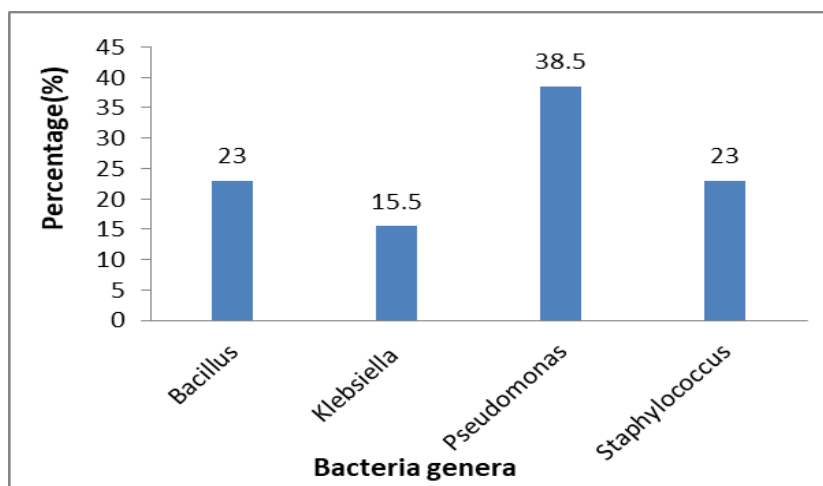


Figure 2 Isolated Bactéria as a function of genera

We observe from Figure 2 that *Pseudomonas* genus represented 38.5% of isolated bacteria, *Staphylococcus* 23%, *Bacillus* 23%, and *Klebsiella* 15.5%.

4. Discussion

Over the past two decades, there's been considerable progress in the understanding of microbial pathogenesis in hemodialysis patients, and the current focus is on the immunity of patients, bacterial virulence and the hemodialysis treatment process¹¹. It is now well established that the quality of water for hemodialysis depends on a complex chain of devices, procedures, and quality control implemented. Proper operation of the water treatment chains for hemodialysis needs preventive maintenance, periodic and regular replacement of worn or exhausted components by competent and trained personnel. Routine disinfection of reverse osmosis membranes and the water distribution system, including hemodialysis generators connected to the system should be subject to disinfection at least once a month or once a week. It is currently possible to use chemical and physical agents according to manufacturer's recommendations for a monthly disinfection of hemodialysis water treatment circuit.

Generally, in the hemodialysis center of Cameroon, disinfection of the water treatment circuit was carried out monthly with *TIUTOL KF*, an alkaline cleaning and disinfection concentrated solution composed with sodium hydroxide and hypochlorite (3.9 % of free chlorine). It is recommended to prevent biofilm and has bactericidal, fungicidal, tuberculocidal and virus inactivation properties.

Each week, patients on hemodialysis are exposed to 400 and 600 liters of water used for hemodialysis¹². High levels of bacteria may create a risk of bacteremia or endotoxemia to these patients because of the possibility of bacterial passage of endotoxins through the hemodialysis membrane¹³. A variety of microorganisms can multiply rapidly in HD water. If the level of bacterial contamination currently exceeds the acceptable limits (<100 CFU/mL), HD patients are exposed to septicemia or endotoxemia by Gram negative bacteria.

The high bacterial load (200 to 300 CFU/mL) detected in the first month of our study demonstrates that it was above international standards (>100 CFU/mL). These results are different from Montanari *et al*¹⁴ performed in a hemodialysis center in the city of Sao Paulo in Brazil, with a low load (2.5 to 3.0 CFU / mL) detected bacteria. Our results, however, are similar to those of Pisani *et al*³ performed in Campinas hospital in Brazil which reported a charge of 300 CFU / mL in water for HD. At points B and C of the water treatment circuit for hemodialysis, the bacterial loads are low (<100UFC/mL) after the samples from the second, third and fourth months of our study. This marks an efficiency in the disinfection system of the water treatment circuit for hemodialysis. These results are different from those obtained in the hemodialysis department of the University Teaching Hospital of Yaoundé where weaknesses were recorded in the water distribution circuit for HD¹⁵.

Most microorganisms isolated in water for HD were Gram-negative bacilli (54%), the Gram-positive cocci (23%) and the Gram positive bacilli (23%). These results are very similar to those of Rebecca¹; Silva *et al*⁴; Santos *et al*⁷ and Gueguim *et al*¹⁵. However, in the Santos *et al*⁷ study, about 90% of the bacteria isolated were Gram negative, with a clear predominance of the genus *Pseudomonas*, which was able to grow rapidly, even in sterile water, reaching high concentrations (> 100 000 CFU / mL) in less than 48 hours. The presence of glucose and bicarbonate in dialysis solutions, favours bacterial growth even faster and therefore a significant production of toxin causing frequent infections in hemodialysis, the leading cause of morbidity in these patients^{13,16}.

The most frequently isolated bacteria in the water for hemodialysis were of the genus *Pseudomonas* (38.5%), *Staphylococcus* (23%), *Bacillus* (23%) and *Klebsiella* (15.5%). These results are similar to those of Gueguim *et al*¹⁵ and Arvanitidou *et al*¹⁷ who reported a predominance of *Pseudomonas* (44%) and *Staphylococcus* (23%). Frequencies very different in isolation of 1.6% and 56% respectively, have both been described for the *Pseudomonas* genus by Pisani *et al*³ and Zunito *et al*¹⁸. The Arduino¹⁹ and Bambauer²⁰ studies have showed that the most commonly isolated bacteria in drinking water and water for hemodialysis were of the genus *Pseudomonas*. In our study, the frequency of the specie *Pseudomonas fluorescens* 38.5% (5/13) is of concern, given the well-known resistance of *Pseudomonas* to biocides and antibiotics. The genus *Pseudomonas* is often cited as a causal agent in sepsis reports and endotoxemia^{11, 19, 21}.

5. Conclusion

Given the weakened immune system of patients with chronic renal failure, we can conclude that the detection of a variety of bacteria in the hemodialysis water in this study indicates the need for regular and appropriate monitoring of

water for hemodialysis by the hemodialysis center of Douala General Hospital to ensure a better quality of life for patients undergoing this treatment

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest is associated with this work.

References

- [1] Rebecca Amato., Curtis, J., & Payne, G.M. (2013). Water treatment for hemodialysis: An update. *Nephrology Nursing Journal*, 40(5), 383-404, 465.
- [2] Lima, E.B., Gazineu, M.H., Paiva, S.C., Rêgo, R.C., Salgueiro, A. (2003). A. Implementation of the drinking water quality index (IQAC) in the urban area of the municipality of Rio Formoso, PE. *Food Hygiene*. 17, 88-94.
- [3] Pisani, B., Simões, M., Prandi, M.A.G., Rocha, M.M., Gonçalves, C.R., Vaz, T.M., Irino, K. (2000). Outbreak of *Pseudomonas aeruginosa* bacteremia in the hemodialysis unit of a hospital in Campinas, São Paulo, Brazil. *Rev. Inst. Adolfo Lutz*. 59 (1/2), 51-56.
- [4] Silva, A.M., Martins, C.T.B., Ferraboli, R., Jorgetti, V., Junior, R., Egidio, J. (1996). Review/Update in Dialysis: Water for Hemodialysis *J. Bras. Nef.*, 18(2), 180-8.
- [5] Riela, C.M., (2012). Principles of Nephrology and Hydroelectrolytic Disorders. 3rd ed. Rio de Janeiro, Guanabara Koogan, p. 607-608.
- [6] Ragon, A. (2004). "Water and Health in Healthcare Facilities".
- [7] Brunet, P. et Berland, Y. (2000). Water quality and complications of haemodialysis. *Nephrology Dialysis Transplantation*., 15, 578-580.
- [8] Santos, F., Santos, A.G., Biernat, J.C., Souza, M.E.L., Raubach, A., Aguirre, A., Antoniazzi, V.R., Scorsatto, K., Seibel, I., Demin, S.S., Hickmann, A.C., Oliveira, F.P., Borba, E.V., Simon, S., Santos, S.S. (2000). Endotoxin detection by the limulus amoebocyte lysate (LAL) test in hemodialysis units. *Rev. Virt. med.* 54: 115-119.
- [9] MANEDONG F. (2013). Renal failure: 11 patients die at the University Hospital - Because of the dysfunction that occurred at the hemodialysis center; Cameroon infos, September 2013. Consulted le 13-01-2015.
- [10] French Standard. (2008). Water treatment and distribution system for diluting concentrated solutions for hemodialysis – Design, operation, performance and safety requirements. S93-315: (2008)
- [11] Jaber, B. (2005). Bacterial infections in hemodialysis patients: pathogenesis and prevention. *Kidney Int.*, 67: 2508-2519.
- [12] Pontoriero, G., Pozzoni, P., Andrulli, S., Locatelli, F. (2003). The quality of dialysis water. *Nephrol. Dial. Transplant.*, 18 (suppl.7): 21-25.
- [13] Hoenich, A. et Ronco, C. (2007). Hemodialysis fluid: composition and clinical importance. *Blood Purif.*, 25: 62-68.
- [14] Montanari, B., Flávio, G.S., Miguel, J.O.C., Samuel, D.V., Regina, H. P., Clarice, Q.F.L, Karina, P. et Carlos H.G.M. (2009). *Revue Internationale. Médecine tropicale* . S. Paulo 51(1):37-43.
- [15] Cédric Gueguim, Nnanga Nga, François Kaze Folefack, Alain Ragon, Hortense Gonsu Kamga (2016). Microbiological Analysis of Hemodialysis Water at the University Teaching Hospital of Yaounde, Cameroon. *American Journal of Biomedical and Life Sciences*. Vol. 4, No. 6, 2016, pp. 81-86.

- [16] Oth, V.R. & Jarvis, W.R. (2000). Outbreaks of infection and/or pyrogenic reaction in dialysis patients. *Semin. Dial.*, 13: 92-96.
- [17] Arvanitidou, M., Vayona, A., Spanakis, N et Tsakris, A. (2003). Occurrence and antimicrobial resistance of Gram-negative bacteria isolated in haemodialysis water and dialysate of renal units: results of a Greek multicentre study. *Journal. appl. Microbiology.*, 95: 180-185.
- [18] Zunino, P. et Beltrán, L. (2002). Microbiological quality of hemodialysis water in a three-year multicenter study in Uruguay. *J. Nephrol.*, 15: 374-379.
- [19] Arduino, M.J., Bland, L.A., Agüero, S.M. (1991). Comparison of microbiologic assay methods for hemodialysis fluids. *Journal of clinical Microbiology.*, 29: 592-594.
- [20] Bambauer, R., Schauer, M., Jung, WK., Daum, V. et Vienken, J. (1994). Contamination of dialysis water and dialysate. A survey of 30 centers. *ASAIO Journal.*, 40: 1012-1016.
- [21] Oie, S., Kamiya, A., Yoneda, I. (2003). Microbial contamination of dialysate and its prevention in haemodialysis units. *J. Hosp. Infect.*, 54: 115-119