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Microbiological Analysis of Hemodialysis Water at the University Teaching Hospital of Yaounde, 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 University Teaching Hospital of Yaoundé 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 July and October 2015 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 83.3% (10/12) of non-compliance. Among the bacteria identified, nine (09) were Gram-negative bacilli including *Pasteurella haemolytica*, *Pseudomonas fluorescens, Pseudomonas paucimobilis, Aeromonas salmonicida* and *Klebsiella pneumoniae subsp ozaenae,* three (03) Gram-positive bacilli all *Bacillus sp* and six (06) Gram-positive cocci all of coagulase-negative staphylococci. The most frequently isolated bacterial genera were *Pseudomonas* (30.4%), *Staphylococcus* (26.1%), *Aeromonas* (13%), *Bacillus* (13%), *Klebsiella* (13%) and *Pasteurella* (4.3%). In this study, the high bacteriological contamination of the hemodialysis water with the detection of a variety of bacteria shows that the disinfection procedure of the distribution loop is not efficient and cannot prevent the development of a biofilm. A higher frequency of disinfection (almost every week), an increase of the concentration and time of contact of the chlorine disinfection product or the use of peracetic acid and a regular monitoring can contribute to improve the quality of the hemodialysis water at the CHUY to ensure a better quality of life for patients undergoing this treatment.

Keywords: Water, Hemodialysis, Microbiology, Contamination, Disinfection

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 lead to infectious, toxigenic and parasitic processes [1]. 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 needed [2]. 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 require 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 life [6].

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 Cameroon [9]. 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 University Teaching Hospital of Yaoundé 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 bottles [10].

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 22ºC) for 7 days [10]. The numbers 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 /July 2015	**Collection 2/ August 2015**	**Collection 3/ September 2015**	**Collection 4/ October 2015**
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).

From Table 1, the number of colonies is far above the norm (>100UFC / mL) for collections carried out in July, September and October for hemodialysis water. Our bacteriological results thus show the presence of a biofilm in the distribution loop because there are few differences in the number of isolated colonies between sampling points A, B and C.

3.2. Conformity After Culture

From Figure 1, 83.3% of samples were non-compliant with the number of colonies well above the norm (>100 CFU / mL), whereas only 16.7% of samples were compliant (<100CFU / mL).

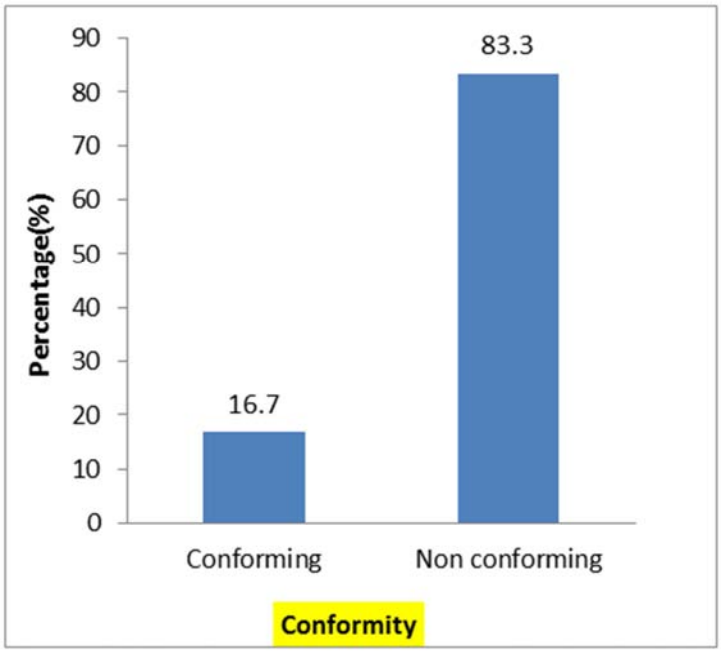

Figure 1. *Percentage of conformity after culture.*

3.3. Identification of Colonies After Culture

Gram staining was performed to determine the type of bacteria present in each sampling point. Table 2 below shows the results obtained.

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

Table 2. *Bacteria type as a function of each sampling point.*

Type of Bacteria	**Collection 1/ July 2015**			**Collection 2/ August 2015**			**Collection 3/ September 2015**			**Collection 4/ October 2015**			**Total/ Percentage**
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	
Gram - Bacilli	+	+	+	+	+	+	+	+	+	-	-	-	9 (50%)
Gram + Bacilli	-	-	-	-	-	-	-	-	-	+	+	+	3 (16,7%)
Gram + Cocci	-	-	-	+	+	+	-	-	+	-	+	+	6 (33,3%)

(+) = Presence (-) = Absence

3.4. Isolated Microorganism in Water for Hemodialysis

It is observed that the bacteria isolated vary depending on the sample. Table 3 below shows the results obtained.

Table 3. *Microorganisms species isolated in water for hemodialysis between July and October 2015.*

Isolated Bacteria	Collectio1 July 2015			Collection 2August 2015			Collection 3 September2015			Collection 4 October 2015		
	A	B	C	A	B	C	A	B	C	A	B	C
Aeromonas salmonicida	-	-	-	+	+	+	-	-	-	-	-	-
Bacillus sp	-	-	-	-	-	-	-	-	-	+	+	+
Klebsiella pneumoniae subsp Ozaenae,	+	+	+	-	-	-	-	-	-	-	-	-
Pasteurella haemolytica	-	-	-	-	-	-	+	-	-	-	-	-
Pseudomonas fluorescens	+	+	+	-	-	-	-	+	+	-	-	-
Pseudomonas paucimobilis	-	-	-	-	-	-	+	+	-	-	-	-
Coagulase négative Staphylococci	-	-	-	+	+	+	-	-	+	-	+	+

(+) = 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

From Figure 2, Pseudomonas genus represented 30.4% of isolated bacteria, Staphylococcus 26.1%, Aeromonas, Bacillus, Klebsiella and 13% and Pasteurella 4.3%.

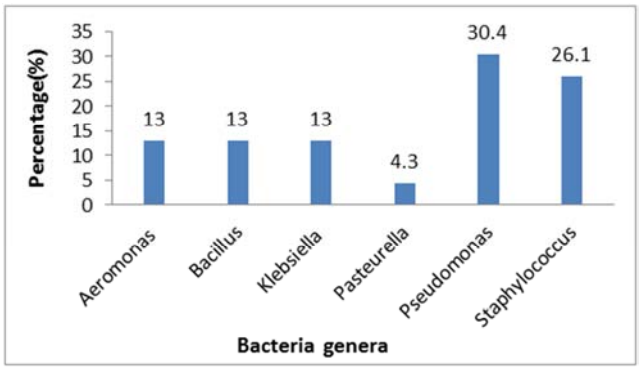

Figure 2. *Isolated Bactéria as a function of genera.*

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 process [11]. 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.

In the hemodialysis center of CHUY selected for our study, 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 hemodialysis [12]. High levels of bacteria may pose a risk of bacteremia or endotoxemia to thes patients because of the possibility of bacterial passage of endotoxins through the hemodialysis membrane [13].

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 350 CFU / mL) detected in our study shows that it was above the international standards (>100UFC / 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.

According to Ragon [6], the water distribution system for hemodialysis should be disinfected regularly (at least 1 time per month or 1 time per week) to avoid the development of a biofilm. Our results show the presence of a biofilm in the distribution circuit because there are few differences in the number of isolated colonies between sampling points A, B and C. This could reflect a weakness in the disinfection system of the distribution circuit of water for hemodialysis at CHUY.

Following the bad bacteriological results of the hemodialysis water, a new disinfection strategy is needed with the increase of the frequency and the use of a more powerfull disinfection product like peracetic acid in alternance with hypochlorite. The frequency of the disinfection process must moved from monthly to at least weekly.

Most microorganisms isolated in water for HD were Gram-negative bacilli (50%), the Gram-positive cocci (33.3%) and the Gram positive bacilli (16.7%). These results are very similar to those of Rebecca [1]; Silva *et al* [4];

Santos *et al* [7] and Reis *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 (>100000 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* (30.6%), *Staphylococcus* (26.1%), *Aeromonas* (13%), *Bacillus* (13%), *Klebsiella* (13%) and *Pasteurella* (4.3%). These results are similar to those of 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].

Arduino [19] and Bambauer [20] 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 species *Pseudomonas fluorescens* 21.7% (5/23) and *Pseudomonas paucimobilis* species 8.7% (2/23) 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 urgent need for regular and appropriate monitoring of water for hemodialysis by the hemodialysis center of CHUY to ensure a better quality of life for patients undergoing this treatment. There is an urgent need to modify the disinfection strategy by increasing both the frequency (almost every week), the chlorine concentration of the disinfection product and the time of contact to improve the quality of the hemodialysis water at the CHUY Center.

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