Arab Journal of Nephrology and Transplantation. 2014 Jan;7(1):27-31

AJNT

Original Article

Experience in Using Thermal Disinfection to Remove Viable Bacteria and Endotoxins in Centraly Distributed Reverse Osmosis Water

Ahmed Alayoud*[1], Mohamed Amine Hamzi[1], Abdelaziz Razkaoui[2] and Mohammed Benyahia[1]

1. Service of Nephrology, Hemodialysis and Kidney Transplantation, Military Hospital, Mohammed V. Rabat, Morocco.

2. Soludia Maghreb, Pharmaceutical laboratory, Sale, Morocco.

Abstract

Introduction: The water used for dilution of hemodialysis concentrates has to meet official quality recommendations regarding microbiology and chemical parameters. To avoid chemical use and to simplify treatments, hot water has been used to control microbial contamination of water distribution systems. In this study we evaluated the efficacy of heat disinfection in maintaining the quality of dialysis water generated by reverse osmosis (RO).

Methods: During the first part of the study, we consecutively used (1) continuous water circulation, (2) daily heat disinfection and (3) a combination of daily heat disinfection and weekly chemical disinfection while checking bacterial count and endotoxin level every 4-5 weeks. During the second part of the study, we continued using daily heat disinfection while checking bacterial count and endotoxin level on weekly basis.

Results: The endotoxin levels at all sampling points of the water treatment system were lower than 0.005/ ml throughout the study. The application of heat disinfection alone reduced bacterial levels but an escape phenomenon occurred. After an interval of 21 days, an exponential increase of bacterial count was noted and cultures from the RO unit revealed growth of Pseudomonas fluorescence. The addition of chemical disinfection was successful in eliminating micro-organisms. Throughout this study, micro-organisms and endotoxins were not detectable in dialysate fluid and substitution fluid in dialysis monitors.

Conclusion: The isolation of a thermo-sensitive organism from the RO unit after a period of relying on thermal disinfection suggests the existence of dead space in the RO unit that is not adequately exposed to heat but is accessible to chemical disinfection.

* Corresponding Author: E mail: a_alayoud@yahoo.fr

Keywords: Chemical disinfection; Dialysis Water; Microbiological Quality; Thermal disinfection.

The authors declared no conflict of interest

Introduction

The constant state of contamination in the water treatment systems has been associated with the chronic state of inflammation in dialysis patients [1]. Hence, the water used for dilution of hemodialysis concentrates has to meet official quality recommendations regarding microbiology and chemistry. Several recommendations and standards exist; and all of them set specific limits for bacterial counts and endotoxin levels [2-6]. Two different grades of water purity may be used for hemodialysis: pure water for standard hemodialysis and ultrapure water for hemo-dia-filtration [3]. According to the degree of desired water purity, the complexity and the cost of the water treatment system may differ significantly. It is therefore mandatory for each hemodialysis unit to establish protocols for the regular sanitization and disinfection of the water treatment system in order to prevent bacterial colonization and the formation of a biofilm [7,8]. The maintenance of water treatment systems requires frequent disinfection cycles of the complete chain that include chemical or heat disinfection, or a combination of both. To avoid problems resulting from chemical use and to simplify treatments, hot water has been used in dialysis units to control microbial contamination.

In our hospital, a new water treatment unit was installed in June 2010, including heat disinfection. In this unit, purified water is obtained from a purification system consisting of pre-treatment softener, activated carbon, downsizing microfilters, and a double reverse osmosis (RO) unit. The fluid distribution system is composed of the double RO unit, a heating unit (Hotfeed, Fresenius Medical Care Morocco), direct delivery loop, and dialysis stations (Figure-1). Since the installation of this unit, continuous water flow was maintained in the entire circuit and disinfection was done periodically using peracetic acid (Puristeril 340, Fresenius Medical Care Morocco). Microbiology and chemical monitoring of dialysis water was performed periodically, and no positive cultures were found in tap water (bacterial count was consistently "Zero" CFU/ml, endotoxin concentration was maintained at < 0.005 EU/ ml). After validating the integrity of this new water treatment unit for four months, we performed this study to investigate the efficacy of heat disinfection used alone in maintaining the quality of dialysis water.

Methods

The study was performed between October 2010 and July 2011 in the Military Hospital Dialysis Unit, Rabat, Morocco, and it consisted of two parts. During the first part of the study, our target was to satisfy the microbiological requirements for standard dialysis fluid in compliance with the recommendations of the European Pharmacopoeia (bacterial count <100 CFU/ ml and endotoxin content < 0.25 EU/ ml) [4], checking bacterial count and endotoxin content every 4-5 weeks. This part included three phases.

Phase I (one month): Water is kept in continuous circulation in the distribution loop without heat or chemical disinfection.

Phase II (two months): Continuous water circulation was maintained with the addition of thermal disinfection. Water from the RO unit is heated to 90° C before entering the distribution loop from which it is distributed to each of the dialysis stations allowing simultaneous hemodialysis monitor disinfection (Fresenius 5008 dialysis machine). This heat disinfection is performed every day for 340 minutes and is automatically controlled (Figure-1).

Phase III (five months): Continuous water circulation was maintained with a combination of daily thermal disinfection and weekly chemical disinfection.

In the second part of the study, our target was to satisfy the microbiological requirements for ultrapure dialysis fluid in compliance with the recommendations of The French Association for Normalization (bacterial count <100 CFU/litre and endotoxin content <0.25 EU/ ml) [5]. During this part, we continued using daily heat disinfection and checked the bacterial growth and endotoxin content on weekly basis for two months.

Samples for microbiological evaluation were taken at four different measurement points; A: output of RO device and heating unit (50 ml and 1000 ml for the first and second parts of the study respectively); B: dialysis fluid piping before returning into the heating unit (50 ml), C: dialysate fluid (100 ml) and D: substitution fluid (500 ml) in the dialysis monitor after double endotoxin retentive filters. Samples were sent to an independent bacteriological laboratory that is certified for microbiological analysis of dialysis water. To check bacterial level, we used the membrane filtration method (Micro Funnel 0.45µ, PALL Corporation USA). Sabouraud Dextrose Agar and Tryptone Soy Agar were used for the culture medium. Incubation temperature was 25°C for Sabouraud Agar and 35°C for Tryptone Soy Agar. Colonies were counted by visual observation after 7 days of incubation. To check endotoxin level, we used endotoxin measurement equipment (Limilus Amebocyte Lysat Chromo-LAL, CAPE CODE USA) utilizing the turbidimetric kinetic technique.

Results

During the first part of the study, microbiological monitoring results showed that we achieved the target bacterial count and the endotoxin level of standard dialysis water at all points of the water treatment system throughout the three phases. Endotoxin levels were maintained lower than 0.005/ ml and bacterial counts were consistently <100 CFU/ ml. It is noteworthy that the application of heat disinfection alone during phase II reduced bacterial levels to acceptable levels, but an escape phenomenon of microbiological growth was noted. Figure-2 shows that under this system of thermal disinfection alone, bacterial levels increased. The combination of heat disinfection with peracetic acid was effective in eliminating all micro-organisms.

In the second part of the study, the data showed an exponential bacterial growth after an interval of 21 days under heat disinfection alone (Figure-3). After four weeks of heat disinfection alone the bacterial count in RO water did not satisfy the requirements for ultrapure dialysis water although the targeted endotoxin level was fully satisfied. The endotoxin levels were consistently below 0.005 IU/ mL at all points. The addition of chemical disinfection was successful in removing this bacterial growth, achieving bacterial count <6 CFU/ Litre and endotoxin levels < 0.005 IU/ ml in all samples.

In the samples taken on 21st and 28th days from RO water during the second part of this study, Pseudomonas fluorescens was grown. Throughout this study, micro-organisms and endotoxins were not detectable in dialysate fluid (DF) and substitution fluid (SF) in dialysis monitors, even when the bacterial levels of RO water did not satisfy the requirements for ultrapure water. In all samples from DF and SF, endotoxin levels were lower than 0.005 EU/mL and bacterial levels were 0 CFU/500 ml.

Figure-1: Diagram explaining the design of the water treatment system at our unit and the direction of hot water flow during thermal disinfection; note dead space in the reverse osmosis (RO) water piping

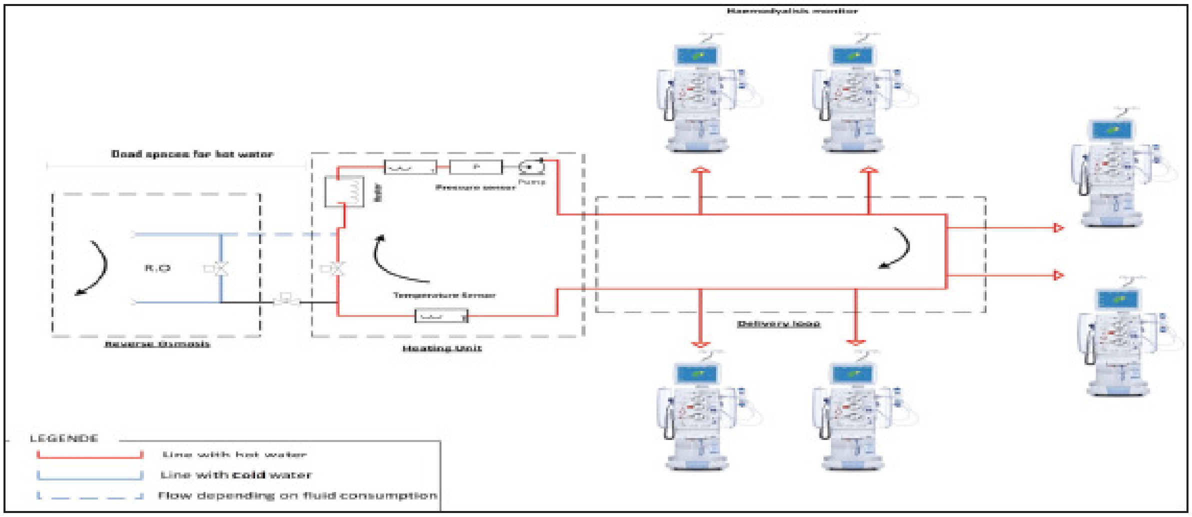


Figure-2: Levels of bacterial growth (CFU/ml) at several time points during the first part of the study; phase I: water is kept in continuous circulation without heat or chemical disinfection, phase II: daily thermal disinfection, phase III: combination of daily thermal disinfection and weekly chemical disinfection.

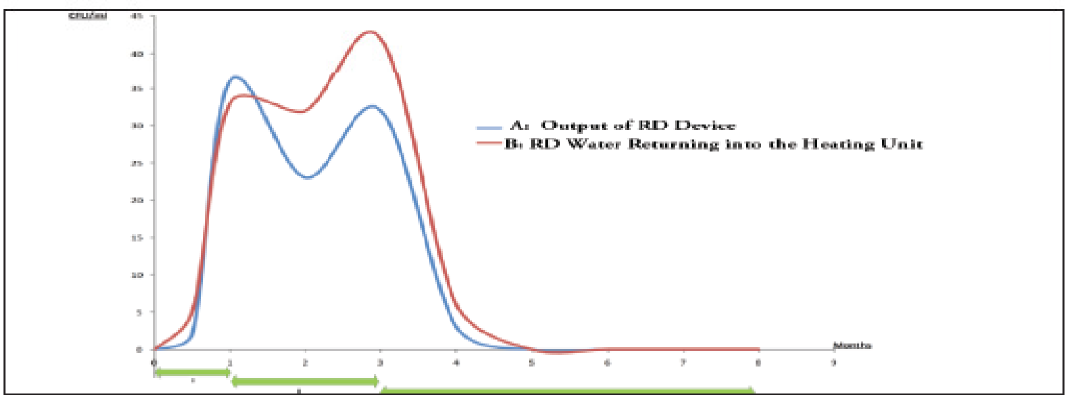
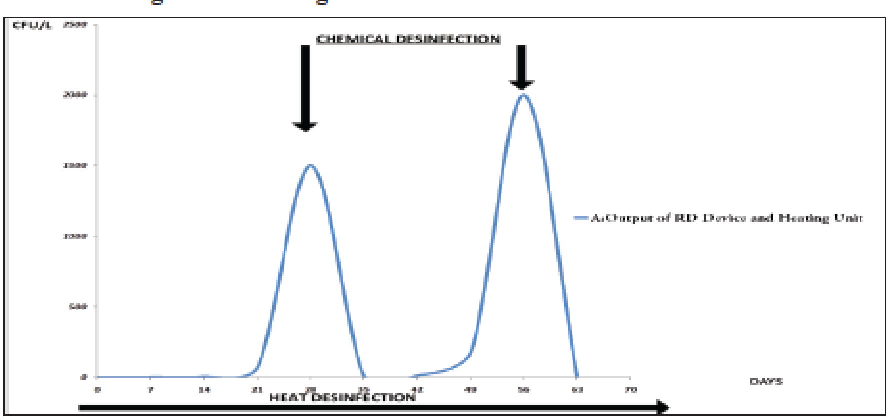


Figure-3: Levels of bacterial growth (CFU/litre) at several time points during the second part of the study. Bacterial levels increases after an interval of 21 days of relying on heat disinfection. The addition of chemical disinfection was successful in removing this bacterial growth.



Discussion

From the early days of dialysis it is known that water used for hemodialysis must be purified in order to prevent severe clinical side effects due to contamination of the water [7–11]. According to the degree of desired water purity, the complexity and the cost of the water treatment system may differ significantly [3]. A number of authorities and organizations have recently issued recommendations for microbiological quality of fluids in dialysis [12].

In order to have quality assurance in water systems, it is necessary to have a good disinfection strategy. This should determine the disinfection procedure and, more importantly, the frequency of disinfection [2, 13]. The criteria for choosing from several methods are based not only on general antibacterial activity but mainly on specific characteristics: contact time dependent activity, toxicity from residues, handling problems, biocompatibility, stability and material/device compatibility [14]. When using chemical disinfection, existing dialysis clinics and hospitals can utilize standard piping. However, it is possible that residual disinfectant may cause harm to the patients. Also, chemicals create a heavy burden for the environment if it is drained directly without any treatment [15]. In addition, the staff is exposed to the germicide solutions while repeatedly processing devices with them.

To avoid problems from chemicals use and to simplify treatments, hot water (>85 C) is used for disinfection of the distribution loop and monitors [14-16]. It is a convenient disinfection process that requires little rinse time. Hence, it could be used more often, thus not allowing biofilm to form. The temperature and contact time need to be established and validated by the manufacturer. Reaching the correct temperature for the right duration of time is necessary for successful disinfection [10]. Prescribed temperature degrees and contact time should also be periodically checked at the most distant place from the central heater [14]. In our water treatment system hot water is rinsed from distribution loop to the monitors, allowing simultaneous monitor disinfection.

In this study, we found that heat disinfection alone did not remove all micro-organisms, and bacterial growth of a thermo-sensitive organism (Pseudomonas fluorescens) was noted. The addition of chemical disinfection was necessary to control this bacterial level. Therefore, in our experience, the chemical disinfection method was superior to the thermal disinfection method.

It may be hypothesized that the incomplete effectiveness of hot water in our experience was due to biofilm formation. It is known that heat and peracetic disinfection will not remove microorganisms when a biofilm is established [10]. Considering the design of our distribution circuit, we suggest that there is a possibility of contamination in the RO system, particularly the osmotic membranes and internal piping system of reverse osmosis and connections between RO and the heating unit. This has already been described in some studies [2, 18]. In our experience, this dead space for hot water was accessible to chemical disinfection (Figure-1).

The solution to this problem would be to optimize the design of the water distribution circuit so that the heat energy can reach each point of the system, including RO membranes and connections. However, there is a possibility of heat causing deterioration in the RO system, and we need to consider the use of heat-resistant materials [14].

Conclusion

From our experience, one caveat of heat disinfection of water treatment systems could be the possible failure of adequate exposure of the entire RO unit. The addition of chemical disinfection to the thermal disinfection might be necessary to control for this shortcoming.

References

1. Smeets E, Kooman J, van der Sande F, Stobberingh E, Frederik P, Claessens P, Grave W, Schot A, Leunissen K. Prevention of biofilm formation in dialysis water treatment systems. Kidney Int. 2003 Apr;63(4):1574-6.

2. Nystrand R. Microbiology of water and fluids for hemodialysis. J Chin Med Assoc. 2008 May; 71(5):223-9.

3. European best practice guidelines for haemodialysis (part1) Section IV: Dialysis fluid purity. Nephrol Dial Transplant 2002;17(Suppl 7):45–62

4. Association Francaise de Normalisation. Fluides pour hémodialyse. AFNOR 2008: NF S93-315

5. Haemodialysis solutions, concentrated, water for diluting. European Pharmacopoeia 6 th ed. 2008; monograph 1167.

6. Quality of dialysis fluid for hemodialysis and related therapies. ANSI/AAMI/ISO 11663:2009 [cited] Available from: http://marketplace.aami.org

7. Vincent FC, Tibi AR, Darbord JC. A bacterial biofilm in a hemodialysis system. Assessment of disinfection and crossing of endotoxin. ASAIO Trans. 1989 Jul-Sep; 35(3):310-3.

8. Man NK, Degremont A, Darbord JC, Collet M, Vaillant P. Evidence of bacterial biofilm in tubing from hydraulic pathway of hemodialysis system. Artif Organs. 1998 Jul; 22(7):596-600.

9. Lonnemann G, Krautzig S, Koch KM. Quality of water and dialysate in haemodialysis. Nephrol Dial Transplant. 1996 Jun;11(6):946-9.

10. Brunet P, Berland Y. Water quality and complications of haemodialysis. Nephrol Dial Transplant. 2000 May;15(5):578-80.

11. Dunn J. Algae kills dialysis patients in Brazil. BMJ. 1996 May 11;312(7040):1183-4.

12. Nystrand R. Official recommendations for quality of fluids in dialysis -the need for standardisation. J Ren Care. 2009 Jun;35(2):74-81.

13. Canaud B, Martin K, Morena M, Bosc JY, Leray-Moragues H, Mahowashi M, Stec F, Hansel S. Monitoring the microbial purity of the treated water and dialysate. Saudi J Kidney Dis Transpl. 200Jul-Sep; 12(3): 325-6.

14. Cappelli G, Riccardi M, Perrone S, Bondi M, Ligabue G, Ligabue G, Albertazzi A. Water treatment and monitor disinfection. Hemodial Int. 2006 Jan; 10 Suppl 1:S13-8.

15. Nakahigashi H. How does the dialysis device take part in purification of dialysate? J JpnSoc Dial Ther 2007; 40:401–3.

16. Sakuma K, Uchiumi N, Sato S, Aida N, Ishimatsu T, Igoshi T, Kodama Y, Hotta H. Experience of using heat citric acid disinfection method in central dialysis fluid delivery system. J Artif Organs. 2010 Sep;13(3):145-50.

17. Lynch WH. Effect of temperature on Pseudomonas fluorescens chemotaxis. J Bacteriol. 1980 Jul;143(1): 338-42.

18. Liu H, Li G, Zhang X, Duan X, Yao Y. [A study on the endotoxin and bacterial contamination in reverse osmosis water and dialysis fluids]. Zhonghua Nei Ke Za Zhi. 1999 Dec;38(12):806-9.