Geget (2007) 7 : 61 - 71


Original Article

# Bacterial Contamination and Endotoxin Production in the Water Treatment System for Hemodialysis

**Ihab Z. El-Hakim, Nebal M. Darwish*, Nagwa A. El-Esnawy****

*Departments of Pediatrics, Microbiology*, Faculty of Medicine, Ain Shams University and Water Pollution Research Department**, Environment Research Division, National Research Center, Cairo, Egypt.*

## ABSTRACT

**Background:** Dead spaces and connections between units (segments) of fluid production and delivery in elder systems are a continuous source for bacterial growth, biofilm generation and endotoxin release. The role of bacterial contamination of dialysis water with respect to chronic inflammatory diseases associated with long-term hemodialysis (HD) therapy has been greatly underestimated. Pyrogenic substances of bacterial origin derived from contaminated dialysate penetrate intact dialyzer membranes with the consequence of the induction of an inflammatory response in the patient.

**Objectives:** The present study aimed to assess bacterial contamination and endotoxin production in the water treatment system for HD. It also aimed to evaluate the efficiency of decontamination measures applied along the water pathway in the system.

**Methods:** The study was conducted in the water treatment system of the Pediatric Dialysis Unit, Children's Hospital, Ain Shams University. Samples were examined for four consecutive weeks after changing the bacterial filter; each week three samples were collected. One sample was taken from tap water (sample A), another sample was taken from treated water just after the bacterial filter (sample B) and the third sample was taken from water in the returning pipe system not used by the hemodialysis machines (sample C). Quantitative methods were used for the total count of viable heterotrophic microorganisms (denoting bacterial contamination), total coliforms, fecal coliforms, enterococci, Pseudomonas spp. and the sulfite-reducing clostridia (denoting fecal contamination). The samples were assayed for endotoxin by the Limulus Amebocyte Lysate (LAL) kit employing a GEL-CLOT LAL. It is a qualitative non-kinetic assay.

**Results:** The results of the study showed that the methods of decontamination used in the water treatment system are efficient in decreasing the counts of all types of bacteria studied compared to tap water (sample A) with nearly equal efficiency for all types of bacteria. Also the passage of water in the pipe system of the HD unit to all dialysis machines did not affect its state concerning bacterial contamination as evidenced by the absence of rise in bacterial counts in sample C. Pseudomonas spp. did not show any growth on its specific medium althrough the study. It was also noted that the efficacy of decontamination methods used - particularly the bacterial filter – is affected by time as evidenced by the rise in bacterial counts in sample B over the 4 weeks of the study to become nearly totally inefficient after 4 weeks. Concerning endotoxin, it was positive althrough the study denoting complete inefficiency of the decontamination methods used in getting rid of endotoxin.

**Conclusions:** Tap water was heavily contaminated with heterotropic bacteria, fecal coliforms, enterococci, sulphite-reducing bacteria and total coliforms. The antibacterial measures adopted in the unit were not as efficient as expected. However, the method of decontamination used for the pipe system was efficient. Endotoxin was detected in all water samples. Recommendations: In order to improve the antibacterial measures used in the water treatment system of the HD unit under study, it is recommended to change the bacterial filter used more frequently or to use more recent bacterial filters. Regular bimonthly disinfection of the whole water treatment system (pre and post RO) utilizing the same disinfectants currently used that proved to be efficient in the post RO section is also recommended. Concerning endotoxin, it is expected that the better decontamination of the system might reduce the load of endotoxin, but the only specific solution - although financially difficult - is the use of polysulfone water filter that removes endotoxin from water at its entrance to the dialysis machine.

## INTRODUCTION

Since water is the main portion of the dialysate (the plasma-like salt bath that is responsible for cleansing the blood), it is considered a prescription or a drug and is prescribed by the physician and regulated by the US Food and Drug Administration (FDA) who put the Quality system regulation (QSR) guidelines. The purer the water, the more accurately the doctor's prescription will be delivered to the patient. Also, the more pure the water and thus the dialysate, the fewer clinical problems the patient will have over time[1].

Dead spaces and connections between units (segments) of fluid production and delivery in elder systems are a continuous source for bacterial growth, biofilm generation and endotoxin release[2].

Dialysis professionals always fight bacteria and biofilm in the system. Anytime there is downtime or inadequate disinfection, bacteria can grow. Once biofilm forms, it is almost impossible to remove even with disinfection measures[3].

Patients are exposed to about 120 liters of treated water during each dialysis treatment. All small molecular weight substances present in the water have direct access to the patient's blood stream as if they had been administered by IV injection. For this reason it is very important that the purity of the water used for dialysis be known and controlled. The Association for the Advancement of Medical Instrumentation (AAMI) has developed minimum standards for the purity of water used in hemodialysis[4].

The role of bacterial contamination of dialysis water with respect to chronic inflammatory diseases associated with long-term hemodialysis (HD) therapy has been greatly underestimated. Pyrogenic substances of bacterial origin derived from contaminated dialysate penetrate intact dialyzer membranes with the consequence of the induction of an inflammatory response in the patient. Reaching the patient's blood, bacteria-derived substances activate circulating mononuclear cells to produce proinflammatory cytokines such as interleukin-1 beta and tumor necrosis factor-alpha that mediate the acute phase response resulting in elevated levels of acute phase proteins (for example, C-reactive protein). The consequence is a state of microinflammation that may contribute to progressive inflammatory diseases in chronic renal failure such as beta2-microglobulin amyloidosis, protein catabolism, and atherosclerosis. The use of sterile dialysate reduces cytokine production and plasma levels of acute phase proteins, and may positively influence progressive inflammatory diseases in patients with end-stage renal failure[5].

## AIM OF THE WORK

The present study aimed to assess bacterial contamination and endotoxin production in the water treatment system for hemodialysis. It also aimed to evaluate the efficiency of decontamination measures applied along the water pathway in the system.

## MATERIALS AND METHODS

The study was conducted in the water treatment system of the Pediatric Dialysis Unit, Children's Hospital, Ain Shams

University. The components of the system and their specifications are illustrated in figure 1. Samples were examined for four consecutive weeks after changing the bacterial filter; each week three samples were collected. One sample was taken from tap water (sample A), another sample was taken from treated water just after the bacterial filter (sample B) and the third sample was taken from water in the returning pipe system not used by the hemodialysis machines (sample C).

Samples A were taken after 3 to 5 minutes of free flow through the tap and samples B and C from the faucets after application of a solution of hypochlorite (100 NaOCl/L) to faucets and the water was left to run for another 2 to 3 minutes. Gloves and long sleeves were worn when collecting the samples to prevent contamination from skin bacteria. All samples were collected into 500-ml glass containers that had been machine-washed, decontaminated with a 15% hydrochloric acid wash, followed by three ultra-pure water rinses and sterilized at 180°C for one hour. In samples A containers, sodium thiosulphate was added in a final concentration of 18 mg/L, to neutralize any residual chlorine and prevent its bactericidal action during sample transport.

Antimicrobial measures used in the system are the bacterial filter, the UV lamp in addnion to chlorine and citric acid used for washing the treated water tank monthly.

**Microbiological examination**

Quantitative methods were used for the total count of viable heterotrophic microorganisms (denoting bacterial contamination), total coliforms, fecal coliforms, enterococci, *Pseudomonas spp.* and the sulfite-reducing clostridia (denoting fecal contamination). The pour-plate method was used to estimate the number of live heterotrophic bacteria[6]. One ml, 0.5, 0.1 ml of each sample were processed on standard plate count agar (Difco) by the pour-plate technique and incubated for 48 ± 3 hours at 37°C. All plates were counted. The membrane filter technique was employed for total coliforms, fecal coliforms, fecal streptococci, *Pseudomonas spp.*, and sulfite-reducing clostridia: 100 ml of $10^{-1}$ diluted samples were filtered through membrane filters with 0.45 µm diameter pores. The membranes were then placed face up on m-Endo medium (Difco, 37°C, 24 hr) for total coliforms, on m-Fc agar (Difco, 44°C, 24 hr) for fecal coliforms, on Slanetz and Bartley medium (Difco, 37°C, 48 hr) for enterococci, on Cetrimide agar (Difco, 37°C, 48 hr) for Pseudomonas spp., and on membrane clostridial agar for sulfite-reducing clostridia (Difco, 37°C, 48 hr). All the agar media used contained trypton. The number of colonies for total coliforms, enterococci, sulphite-reducing bacteria is per 100 ml. The number of colonies for total heterotrophic bacteria is per 1 ml. The dilution plates that bear between 30 and 300 colonies were selected for counting. Bacterial count per milliliter of sample was calculated by multiplying the average number of colonies per plate by the reciprocal of the dilution used reported as colony forming unit/ml (CFU/ml).

**Endotoxin detection**

The samples were assayed for endotoxin by the Limulus Amebocyte Lysate (LAL) kit employing a GEL-CLOT

LAL reagent (Charles River Endosafe, USA). It is a qualitative non-kinetic assay. The test is based on the fact that serine protease zymogens found in ameobocyte lysate derived from American crab (Limulus polyphemus) are activated by bacterial endotoxin to initiate an enzymatic coagulation cascade that alters an abundant protein called coagulogen to produce a proteinaceous gel.

The presence of endotoxin was tested in sample A, B, and C in the four weeks. Of each test specimen 0.2 ml was added aseptically to assay tubes containing the LAL reagent, the contents of the tubes were mixed gently until they dissolved, then placed immediately in a 37°C incubator for 60 minutes. In water samples containing endotoxin a firm gel-clot is formed.

## RESULTS

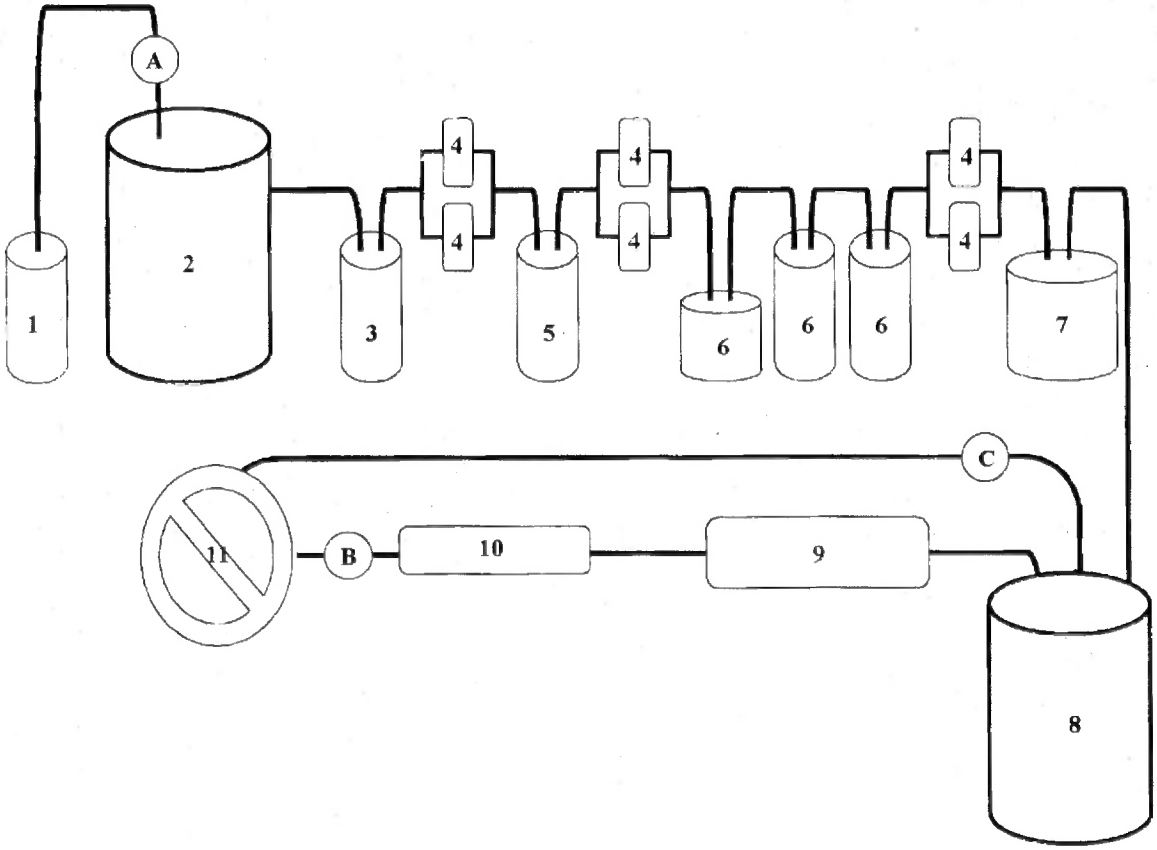
The results of the study showed that the methods of decontamination used in the water treatment system are efficient in decreasing the counts of all types of bacteria studied compared to tap water (sample A) with nearly equal efficiency for all types of bacteria. Also the passage of water in the pipe system of the HD unit to all dialysis machines did not affect its state concerning bacterial contamination as evidenced by the absence of rise in bacterial counts in sample C (Table 1 and Fig. 2). *Pseudomonas spp.* did not show any growth on its specific medium althrough the study.

It is also noted that the efficacy of decontamination methods used – particularly the bacterial filter – is affected by time as evidenced by the rise in bacterial counts in sample B over the 4 weeks of the study to become nearly totally inefficient after 4 weeks (Table 1 and Fig. 2).

Concerning endotoxin, it was positive althrough the study denoting complete inefficiency of the decontamination methods used in getting rid of endotoxin (Table 1).

**Table 1: Counts of various types of bacteria and endotoxin detection in the three studied water samples over the 4 weeks of the study.**

| Week | Sample | Heterotrophic bacteria (CFU/ml) | Total coliforms (CFU/ml) | Enterococci (CFU/ml) | Sulfite-reducing Clostridia (CFU/ml) | Fecal coliforms (CFU/ml) | Endo-toxin |
|---|---|---|---|---|---|---|---|
| 1 | A | > 3000 | > 3000 | 2820 | 2330 | 2660 | + ve |
| | B | 480 | 390 | 360 | 350 | 360 | + ve |
| | C | 320 | 340 | 330 | 310 | 320 | + ve |
| 2 | A | > 3000 | > 3000 | 2750 | 2610 | 2920 | + ve |
| | B | 760 | 1190 | 910 | 870 | 1150 | + ve |
| | C | 350 | 970 | 430 | 480 | 950 | + ve |
| 3 | A | > 3000 | > 3000 | 2670 | 2730 | > 3000 | + ve |
| | B | 1230 | 1720 | 1860 | 1910 | 1680 | + ve |
| | C | 960 | 1610 | 1720 | 1790 | 1600 | + ve |
| 4 | A | > 3000 | > 3000 | 2790 | 2760 | > 3000 | + ve |
| | B | 2450 | 2820 | 2320 | 2410 | 2730 | + ve |
| | C | 2100 | 2360 | 1970 | 1940 | 2280 | + ve |




| | |
|---|---|
| 1 | Sand filter (1) |
| 2 | Raw water tank |
| 3 | Sand filter (2) |
| 4 | Filter (pore size 10-25 micron) |
| 5 | Carbon filter (volume 26.93 Gals, with maximum pressure: 150 Psig 120°F Temp: 50°C with continuous follow rate: 4.7 GBM |
| 6 | Softener |
| 7 | RO (Universal AQUA TECHNOLOGIES INC. America's Inset Osmosis water purification system) 10-12 L/60 min (6 month) |
| 8 | Treated water tank |
| 9 | UV Lamp (Ideal Horizons) maximum operating Temp. 100°F Pressure: 125 PSI, maximum follow rate: 15 GBM maximum follow rate: 15 GBM Quartz otz0037 Lamp 12008 |
| 10 | Bacterial filter (Betafine-D Series, Aquapure, USA) |
| 11 | Hemodialysis unit |
| A, B and C | Sites of water sampling |

**Fig. 1: The components of the water purification unit of hemodialysis.**

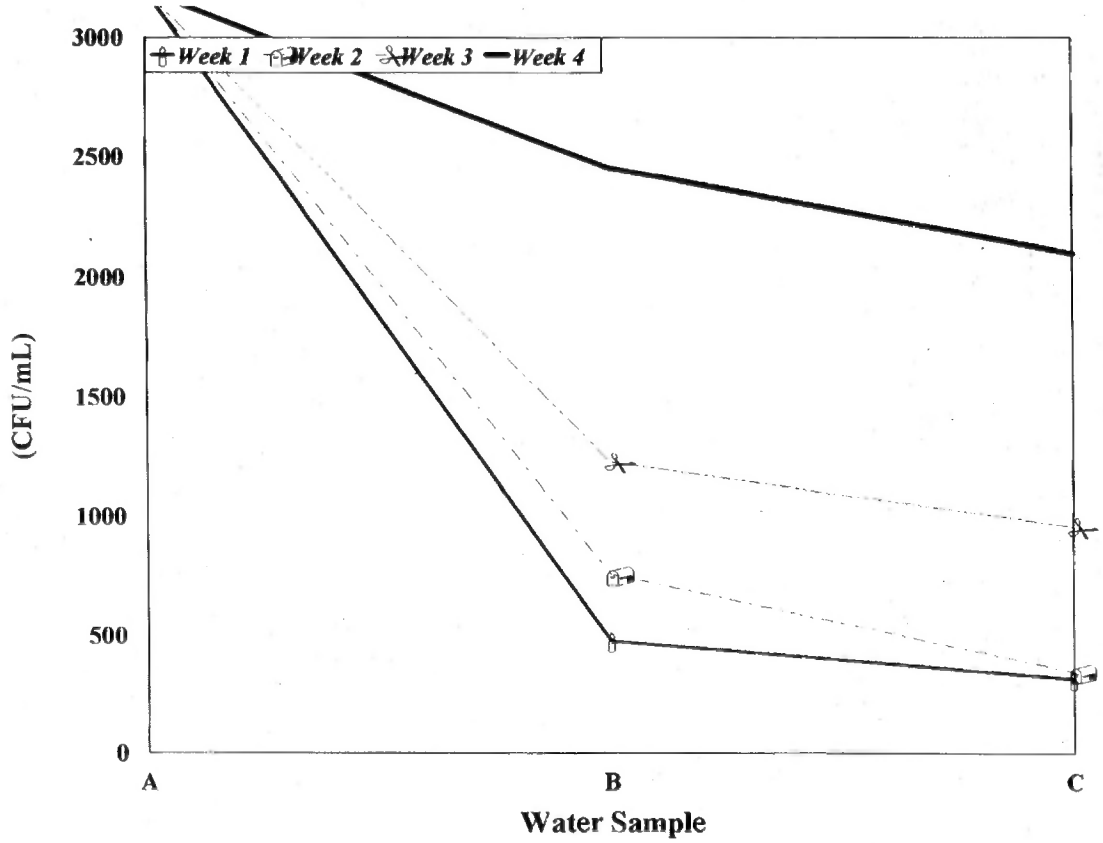



**Fig. 2: Changes in the counts of heterotrophic bacteria in the three studied water samples over the 4 weeks of the study.**

## DISCUSSION

The importance of bacteria and endotoxin-free, sterile dialysis fluid for long term, high quality HD treatment is obvious and very much demanded[2].

The quality of water used for HD cannot be dealt with, without at the same time reviewing the systems delivering it. In the water treatment system before reverse osmosis (RO), the incoming water determines the quality; after the RO, the maintenance in form of disinfection activities is decisive for the microbiological quality in the water system[7].

It must be noted that it is not only the number of microorganisms that is of importance but also what the microorganisms do in the fluid systems. Microbiological analysis is not normally able to tell the complete microbiological quality of the fluid systems, as the inner surfaces where the microbial growth takes place are not sent to any laboratory. Consequently, what is seen in samples is only what can come off the surface[7].

Dead spaces and connections between segments of fluid production and delivery in elder systems are a continuous source for bacterial growth, biofilm generation and endotoxin release[2].

The current methods, assessing the number of water bacteria, are insufficient; due to their large diversity, only a small fraction of them grow on used culture agars.

Bacterial cells are classified into three categories: the A cells represent cultivable cells; B cells are living though not cultivable whereas the C cells are dead cells. Endotoxin is only released from the C-type cells. It is our task to ensure that we keep the A-cell level low after the RO[8].

The bacterial colony counts of dialysis water and dialysates depends of which agars and culture conditions are used. The AAMI recommends to culture HD fluids on Tryptic soy agar (TSA) at 37°C for 48 hr. However, special nutrient-poor culture techniques, including low temperature (25 ± 2°C), and extended incubation time (> 48 hr) have yielded higher bacterial counts. Especially on Reasoner's 2A (R2A) media, several typical HD-associated water-borne bacteria appear to grow better, or sometimes even more selectively when using such culture conditions. So by using AAMI-approved culture methods the actual bacterial load of HD fluids may be underestimated or even missed. R2A produces significantly higher bacterial yields than TSA media for both (pre-treated) dialysis water and dialysates. Moreover, when the maximum limit of viable bacteria for all samples was considered as 100 CFU/ml (European criterion) or 200 CFU/ml (AAMI criterion), numerous TSA cultures would have been within bacteriological compliance (16 and 10% respectively), whereas the same samples cultured on R2A media showed colony counts higher than the upper permitted limits. In such cases TSA underestimates the actual bacterial contamination, and would falsely suggest a compliance with the microbiological regulations. In cases of R2A cultures, non-compliance would be missed in only a very low percentage (3 and 2% respectively)[9].

In the present study cultures were on media containing TSA as recommended by the AAMI, this may underestimate the actual bacterial count. All water samples studied revealed the presence of gram negative bacilli including total coliforms, enterococci, and sulphite-reducing bacteria. These types are known sources of endotoxin production. The number of bacteria in tap water (sample A) was very high throughout the study period denoting contamination of tap water with bacteria of fecal origin probably due to water pollution by sewage. This high contamination may affect the whole water treatment system and reduce the efficiency of antibacterial measures used (bacterial filter and UV).

In a study done in Spain, Bacterial counts < 200 CFU/ml were found in 100% of treated water samples obtained from 2 HD units[10]. A Similar study done in Alexandria, Egypt involving one private and one governmental HD units revealed 67% and 66.7% of bacteriologically acceptable treated water samples respectively[11].

In the present study the antibacterial measures used in the water treatment system were not as efficient as expected as evidenced by the high bacterial counts in sample B. This efficiency was clearly affected by the duration of use of the bacterial filter as the bacterial counts increased in the 4 samples over time. This might be due to the formation of biofilm on the bacterial filter over time that represents another source of bacterial shedding. Moreover, the study revealed that the pipe

system of the unit did not add to the bacterial load as evidenced by absence of change in the bacterial counts in samples C compared to samples B. This may prove the efficiency of monthly disinfection of the treated water tank and pipe system using chlorine and citric acid practiced in the HD unit under study.

In a study by Arvanitidou et al. (1999)[12] samples with endotoxin contamination derived from the mains water had significantly higher counts of fecal coliforms, enterococci and *Pseudomonas spp.* In another study by Zunino et al. (2002)[13] when heterotrophic bacteria counts were related to the different sampling points, the highest percentage of acceptable samples (< 200 CFU/mL) was obtained after UV or ozone bactericidal treatment (99%). This suggests that the use of bactericidal treatment devices after reverse osmosis units in HD water purification systems has a remarkably beneficial effect on water bacterial quality.

A variety of Gram-negative bacteria can persist in aqueous environments associated with HD system. These micro-organisms are capable not only for surviving, but also of multiplying rapidly in all types of waters, even those containing relatively small amounts of organic elements such as distilled, softened, deionised, or RO water[14].

Microbial contamination is characterized not only by the presence of bacteria, but also by high concentrations of biologically active by-products as endotoxin that apparently can stimulate blood monocytes to produce interleukin-1 (IL-1) that when circulating in high levels can result in pyrexia and possibly other as yet undefined symptoms and side effects[4].

In the present study, endotoxin was detected in all (100%) the water samples taken in the four weeks, denoting high contamination of water with endotoxin. Moreover, side effects that may be related to endotoxin were present in 40 out of the 82 (48.78%) patients treated with HD in the unit under study. In the study of Lamas et al. (2007)[10] acceptable endotoxin levels (< 0.25 UE/ml) were found in 100% and 70% of the water samples from the 2 HD units studied respectively. While in the study done in Alexandria, Egypt these figures were 100% and 57% in the private and governmental units respectively[11].

For several years now, attempts have been made to eliminate pyrogenic substances and ensure a sterile and endotoxin-free dialysis fluid. A recent dialysis fluid filter containing a membrane based on Polysulfone proved effective in reducing the bacterial contaminants in dialysis fluid, thus protecting patients from the potentially harmful acute and long-term life-threatening consequences of contaminated dialysis fluid[15].

Water for dialysis need not be completely sterile, because the dialyzer membrane is normally an effective barrier to both bacteria and endotoxin. However, the bacterial counts should be kept below 200 CFU/ml in the water by periodically disinfecting the water treatment system with appropriate disinfectants and by use of bacteriological filters[4].

Disinfection must be performed regularly and for all areas of the fluid system[7]. Gorke and Kittel (2002)[2] suggested system

disinfection to be carried bimonthly with peracetic acid 3.5% in > 0.1% solution at a mean temperature of > 15°C and for a minimum of 60 minutes. Also, the use of UV is quick, simple, relatively inexpensive and environment-friendly as it does not add any components to the water and creates no disinfecting by-products[1].

In conclusion, tap water was heavily contaminated with heterotropic bacteria, fecal coliforms, enterococci, sulphite-reducing bacteria and total coliforms. The antibacterial measures adopted in the unit were not as efficient as expected. However, the method of decontamination used for the pipe system was efficient. Endotoxin was detected in all water samples.

In order to improve the antibacterial measures used in the water treatment system of the HD unit under study, it is recommended to change the bacterial filter used more frequently (at least bimonthly) or to use more recent bacterial filters (as Flex N Nylon 66). Also the use of several bacterial filters and UV units in series might improve their performance by increasing contact time between water and UV light. Regular bimonthly disinfection of the whole water treatment system (pre and post RO) utilizing the same disinfectants currently used that proved to be efficient in the post RO section is also recommended. Concerning endotoxin, it is expected that the better decontamination of the system might reduce the load of endotoxin, but the only specific solution - although financially difficult - is the use of polysulfone water filter that removes endotoxin from water at its entrance to the dialysis machine. It is also recommended to study endotoxin at the level of the dialysis machine, in the dialysate concentrate and in the patient's sera for a more accurate correlation with the side effects occurring to the patients that might be related to endotoxin among other causes as well as determination of the efficiency of the dialyzer membrane in removal of such endotoxin.

**Acknowledgement**

The authors wish to thank the head of the Pediatric Dialysis Unit, Children's Hospital, Ain Shams University, as well as the medical, nursing and technical teams in the unit for their great cooperation without which this study would have not been completed.

## REFERENCES

1. **Arlington, V. (ed) (2001):** Water Treatment for Hemodialysis. In: Association for the Advancement of Medical Instrumentation (AAMI) Standards and Recommended Practices: 62.
2. **Gorke, A. and Kittel, J. (2002):** Routine disinfection of the total dialysis fluid system. Edtna Erca J; 28 (3): 130-3.
3. **Rockville, M. (1997):** Guidance for the Content of Premarket Notifications for Water Purification Components and Systems for Hemodialysis. In: FDA CDRH Branch, May 30.
4. **Baz, M.; Durand, C.; Ragon, A.; et al. (1991):** Using ultrapure water in hemodialysis delays carpal tunnel syndrome. Int. J. Artif. Organs; 14 (11): 681-5.
5. **Lonnemann, G. (2000):** The quality of dialysate: an integrated approach. Kidney Int. Suppl.; 76: S112-9.
6. **Eaton, A.; Clesceri, L. and Greenberg, A. (1995):** Standard methods for the examination of water and wastewater. In: Eaton, A.; Clesceri, L. and Greenberg, A. (eds). American Public Health Association: 53-74.
7. **Nystrand, R. (2001):** Dialysis fluid

contamination of pathways and life of microbes. Edtna Erca J.; 27 (3): 135-9.
8. **Trager, H. (2002):** The influence of bacteria in dialysis water on its endotoxin level. Edtna Erca J.; 28 (3): 121-4.
9. **van der Linde, K.; Lim, B.; Rondeel, J.; Antonissen, L. and de Jong, G. (1999):** Improved bacteriological surveillance of haemodialysis fluids: a comparison between Tryptic soy agar and Reasoner's 2A media. Nephrol. Dial. Transplant.; 14 (10): 2433-7.
10. **Lamas, J.; Alonso, M.; Sastre, F.; et al. (2007):** Dialysate bacteriological quality in a health district. Nefrologia; 27 (2): 191-5. (Abstract).
11. **El-Koraie, A.; Hazzah, W.; Abbass, A. and El-Shazly, S. (2007):** Bacteriological monitoring of dialysis fluid in 2 hemodialysis units in Alexandria, Egypt. Saudi Med. J.; 28 (8): 1234-8.
12. **Arvanitidou, M.; Spaia, S.; Askepidis, N.; et al. (1999):** Endotoxin concentration in treated water of all hemodialysis units in Greece and inquisition of influencing factors. J. Nephrol.; 12 (1): 32-7.
13. **Zunino, P.; Beltran, L.; Zunino, L.; et al. (2002):** Microbiological quality of hemodialysis water in a three-year multicenter study in Uruguay. J. Nephrol.; 15 (4): 374-9.
14. **Arvanitidou, M.; Spaia, S.; Katsinas, C.; et al. (1998):** Microbiological quality of water and dialysate in all haemodialysis centres of Greece. Nephrol. Dial. Transplant.; 13 (4): 949-54.
15. **Ikonomov, V.; Haase, G.; Stefanidis, I.; Melzer, H. and Mann, H. (2002):** Filtration fluid for hemodialysis treatment. Int. J. Artif. Organs; 25 (5): 379-85.