Bacteriological monitoring of dialysis fluid in 2 hemodialysis units in Alexandria, Egypt

Ahmed F. El-Koraie, MS, MD, Walaa A. Hazzah, MPH, Aleya A. Abbass, MPH, PhD, Sorya A. El-Shazly, MPH, PhD.

ABSTRACT

Objectives: To assess the bacteriological quality of dialysis fluid in 2 hemodialysis units in Alexandria, Egypt.

Methods: A total of 321 samples of hemodialysis fluids, 213 from unit A (a governmental unit), 108 from unit B (a private unit), both under the supervision of ministry of health, were collected from the water treatment system (WTS), treated water, concentrates, and final dialysate from the beginning of March to the end of August 2005. Samples were analyzed for enumeration of the total viable heterotrophic bacteria using the standard pour plate method, and for the determination of the total coliforms (TC) using the presence/absence method. Fifty samples were also examined for endotoxin detection by the Limulus Amoebocyte Lysate assay (LAL), employing the gel clot method.

Results: Percentages of acceptable samples of WTS were 67% from unit A and 66.7% from unit B, while the dialysate samples showed higher acceptability at unit B (86.1%) than unit A (51.7%). Eleven samples were detected as having TC. The LAL assay showed a range of 57-100% of samples exceeded 0.25 EU/ml. Analysis of these results and comparing them to other variables is further discussed.

Conclusion: The results demonstrate that hemodialysis centers need monitoring and preventive maintenance in order to ensure renal replacement therapy of good quality.

Saudi Med J 2007; Vol. 28 (8): 1234-1238

From the Departments of Internal Medicine and Nephrology (El-Koraie), Faculty of Medicine, and Microbiology (Hazzah, Abbass, El-Shazly), High Institute of Public Health, Alexandria University, Egypt.

Received 20th November 2006. Accepted 14th March 2007.

Address correspondence and reprint request to: Dr. Ahmed F. El-Koraie Department of Internal Medicine and Nephrology, Faculty of Medicine, Alexandria University, 39 Sekina bent, El-Hussein St., Kafr-Abdou, Roshdy, Alexandria, Egypt. Tel. +20 (3) 5453033. Fax. +20 (3) 4262995. E-mail: akoraie@hotmail.com

Dialysate is the product of a chain of processes including water purification, distribution of the purified water to individual dialysis machines, concentrate preparation, and formulation of dialysate from the purified water and concentrate. Concerns on dialysate purity have been raised with the extensive use of sodium bicarbonate, as a dialysate buffer favoring bacteriological proliferation, and the emerged dialysis-related pathology of end-stage renal disease (ESRD) patients.[1] Water treatment may have a dramatic effect on microbial contamination because of bacterial colonization of the different parts of the system such as carbon filters, softeners, or deionizers, or parts with low circulation such as tanks and taps. Formation of a biofilm that facilitates bacterial persistence at different points of the system, and protects bacteria from disinfection also increases the risk of contamination and high endotoxin levels in water.[2,3] The microbiological contamination, which is common in dialysis centers, results in by-products as bacterial endotoxin and its fragments that are capable of crossing both high- and low-flux dialysis membranes. Blood exposure to these substances activates leukocyte synthesis of pro-inflammatory cytokines, including interleukin-1 (IL-1) and tumor necrosis factor (TNF-alpha), as well as counter-inflammatory mediators such as interleukin-1 receptor antagonist (IL-1Ra). This may contribute to subclinical chronic inflammation, which has been incriminated in malnutrition, cardiovascular disease, immunodeficiency, and hypo responsiveness to erythropoietin therapy.[4] Thus, the present study aimed to assess the bacteriological quality of dialysis fluid in 2 hemodialysis (HD) units in Alexandria.

Methods. A total of 321 samples of HD fluids, distributed as 213 from unit A (a governmental unit), 108 from unit B (a private unit), both under the supervision of ministry of health, were collected during the period of the study from the beginning of March to the end of August 2005, at 2 HD units in Alexandria, Egypt (**Table 1**). All water samples were aseptically collected in 200 ml sterile wide mouthed plastic bottles. Four to five drops of sodium thiosulphate (100 gm/L)

were added only to bottles for sampling from the main pipe, primary storage tank, and distal to sand filtering system. Before collecting the water samples, any faucet fittings were removed. The faucets were cleaned by wiping with alcohol, left to dry, and then water was left to flow for one minute to flush any residuals in the connections and pipes. The final dialysate was allowed to flow for half a minute then 20 ml was aseptically collected and place into plastic containers. All the collected samples were transported on ice and delivered to the laboratory to be analyzed within 1-2 hours of collection. In case of expected delay, samples were stored at 4°C for a maximum of 18-24 hours. Samples from water treatment system (WTS) and concentrated dialysate were subjected to heterotrophic plate count (HPC) for enumeration of the total viable heterotrophic bacteria using the standard pour plate method, and the presence/absence (P/A) method for total coliforms (TC) detection. Samples of final dialysate were only examined for HPC.[5] Endotoxin detection was estimated in 50 samples collected from reverse osmosis (RO) water, treated water, and final dialysate. It was performed by limulus amoebocyte lysate (LAL) assay, employing the gel clot method (Pyrotell Single Test Vial of sensitivity 0.25 EU/ml was obtained from Associates of Cape Cod, Inc.).

Statistical analysis. Data were analyzed using SPSS version 13.0 as well as Epiinfo version 6. The probability value of <0.05 level was used as the cut off value for statistical significance, and the following statistical measures were used Chi square: (x^2), Z-test and testing agreement using Kappa test.

Results. As recommended by the European pharmacopoeia (EP),[6] water and dialysate samples were considered unacceptable if one or more of the following criteria were found; HPC ≥100 CFU/ml, endotoxin level ≥0.25 EU/ml, in addition to presence of coliforms. Out of 321 samples examined by HPC; 267 (83.2%) were acceptable. Regarding the samples examined by LAL, 44 (88%) were unacceptable (**Table 2**). Total coliforms were detected in 11 samples (4%) from unit A, distributed as; 5 samples from prepared bicarbonate, 2 from each of RO and second storage tank, and one from each of treated water and A-component. *Escherichia coli* (*E.coli*) was isolated from one sample of manually prepared bicarbonate. Percentages of acceptable samples of WTS were nearly similar in HD units A (67%) and B (66.7%). As regards filters in unit A, all samples of sand filter were acceptable, whereas 40% of carbon filters and 30% of softeners were acceptable, while the corresponding samples at unit B were inaccessible and excluded. For RO, second storage tanks and finally treated water from HD faucets 80%, 70%, and 50% of samples were acceptable in unit A, and 50%, 66.7%, and 66.7% in unit B. Regarding the dialysate, the percentage of acceptable concentrate samples results in unit A were 100% for acetate, 52.1% for bicarbonate, and 95.7% for A-component. In unit B, all concentrates samples were acceptable for both Bicart and A-component. The prepared bicarbonate acceptability results varied according to time of preparation, whereas 88.9% of prepared bicarbonate samples after 0-3 hours were acceptable, and only

Table 1 - Number of examined samples from different sampling points in both hemodialysis (HD) units.

Sampling points	Number of examined samples	
	Unit A	Unit B
A. Water treatment system (n=136)		
1. Tap water	10	6
2. First storage tank	10	6
3. Distal to sand filter	10	0
4. Distal to activated carbon filter	10	0
5. Distal to double softener	10	0
6. Distal to the reverse osmosis*	10	6
7. Second storage tank	10	6
8. HD room faucets*	30	12
B. Dialysate sampling		
1. Concentrated dialysate (n=89)		
Acetate solution	7	0
A-component (complementary solution A for bicarbonate based HD)	23	18
Manually prepared bicarbonate solution	23	0
Bicart capsules (dry powder cartridges)	0	18
2. Final dialysate (n=96)*	60	36
Total (n=321)	213	108

*Sampling points for Limulus Amoebocyte Lysate assay (n=50)

Table 2 - Results of the examined dialysis fluid samples from HD units according to the examined parameters, Alexandria, 2005.

Parameter	Number Of Examined Samples	Acceptable Samples		Unacceptable Samples	
		n	(%)	n	(%)
HPC	321	267	83.2	54	16.8
TC	225	214	95.1	11*	4.9
LAL	50	6	12	44	88

HPC = heterotrophic plate count, TC = total coliforms,
LAL = limulus amoebocyte lysate, *All TC recovered from unit A

28.6% were acceptable after 3-6 hours of preparation, (4-120 CFU/ml versus 70-2000 CFU/ml). In addition, it was noted that the bicarbonate samples compliance increased by giving instructions for proper handling of the prepared solution. The percentage of final dialysate samples acceptability was higher at unit B (86.1%) than unit A (51.7%). The relation between HPC of final dialysate and both treated water samples and concentrate samples at both HD units was found to be statistically significant ($p<0.05$), but no difference was found between the results and type of machines in use (**Table 3**). In unit B, 75% of the examined samples with LAL >0.25 EU/ml were unacceptable. The percentages of unacceptable samples according to different points were as follows: 83% from RO-water, 57% from finally treated water, and 82% from the final dialysate. All samples in unit A were (100%) unacceptable. The relation between endotoxin level in both treated water and final dialysate was found to be statistically significant. **Table 4** reveals the relation between LAL and HPC parameters among the examined 50 samples. Thirty samples were acceptable by HPC, and 4 of these were acceptable by LAL. It is also shown that 20 samples were unacceptable by HPC. Only 2 of these were acceptable by LAL while the remaining 18 were unacceptable by LAL. Also, it was found that out of 50 samples, 30 samples (60%) were acceptable when examined for HPC alone, while 20 samples (40%) were unacceptable. This percentage decreased to 12% acceptability when examined by LAL, and to 8% acceptability if evaluated by both HPC and LAL together, showing significant statistical difference.

Discussion. Bacterial contamination of treated water is a matter of serious concern in HD centers and improvement of microbiological quality is a permanent challenge for professionals at these units.[2] In the present study, HPC, TC, and LAL assay were used to assess the dialysis fluids quality. The results of the examined samples revealed that 83.2% and 95.1% of samples were acceptable in relation to HPC and TC parameters, and only 12% by LAL assay. This observed discrepancy between LAL assay and other parameters agrees with the reports of Klein et al,[7] Kulander et al,[8] and Bland et al,[9] who found no correlation between bacterial growth and endotoxin concentrations in water or dialysate samples. They assumed that low levels of bacterial growth might be associated with high endotoxin concentration, presumably because of bacterial adherence to and growth in dialysate tubing and release of endotoxin and its fragments into the dialysate and consequently to patients. In accordance with other workers, the present study showed that the lowest percentage of acceptable samples was at the activated carbon filter (40%), and the softener (30%). This decreased compliance of samples could be attributed to the fact that water going through them is already chlorine free. In addition, bacteria detaching from the biofilm developed on carbon were carried away by the flow and appear at the outlet of the filter.[10]

In our study, the overall compliance of treated water samples by HPC was nearly similar to those reported by Klein et al,[10] at 64.7%, and Laurence et al[11] at 68.2%. Zunino et al[2] reported variability of acceptable samples ranging from 32.8-100%, assuming that compliance could be increased by improving control programs on water bacterial quality. In addition, the type of culture medium and the conditions of incubation have considerable influence on the results and could be a possible factor for variation between different studies. The best result regarding bacterial count was reported in clinics using dry powder cartridges at each dialysis machine.[12] This agrees with our study results, where the Bicart used at unit B showed 100% acceptability, whereas in manually prepared bicarbonate solution in unit A acceptability dropped to 52.1%. The presence of indicator organisms is potentially dangerous and hence

Table 3 - The relation between HPC of final dialysate samples, and type of machine at HD unit A, Alexandria, 2005.

Machine Type	Number of Examined Samples	HPC (Dialysate)			
		Acceptable		Unacceptable	
		n	(%)	n	(%)
Fersenius 4008B	38	19	50	19	50
Gambro AK90	22	12	54.6	10	45.4
Total	60	31	51.7	29	48.3

HPC = heterotrophic plate count, HD = hemodialysis,
x^2:0.12 (P: 0.734212)

Table 4 - The relation between LAL, and HPC parameters among the 50 examined samples, Alexandria, 2005.

HPC	LAL		Total
	Acceptable (<0.25 EU/ml)	Unacceptable (>0.25 EU/ml)	
Acceptable	4	26	30
Unacceptable	2	18	20
Total	6	44	50

HPC = heterotrophic plate count, LAL = limulus amoebocyte lysate, EU = endotoxin unit
Observed agreement: 0.44 (Z: 0.96)

their absence denotes in general the safety of water. The TC test was used for monitoring the microbiological quality of water and dialysis concentrates in this study. Both TC and *E.coli* recovered from unit A, and probably indicates poor hygiene at the HD unit during the preparation of concentrates. However, neither TC nor *E.coli* was revealed from unit B. This again may be related to the fact that they use ready-made Bicart. However, Zunino et al[2] encountered TC in 0.5% of samples while Arvanitidou et al[13] reported much higher results, 12.3% for TC and 8.6% for *E.coli.*

Regarding the final dialysate, our study revealed a significant difference between its compliance in both units (51.7% and 86.1% acceptability at unit A and B). Many factors contributing to the dialysate formation should be considered; treated water and distribution system, concentrates, and the dialysis machines. In relation to the findings, there was a significant association between the final dialysate and each of treated water and concentrates in both HD units. In contrast, Baumbauer et al,[14] found no correlation between the level of contamination of dialysate and the water processing method or type of concentrate. Several reports have implicated dialysis machines in bacterial contamination of dialysate. Oie et al[15] suggested that the HD machines were the main source of contamination, where the tubing within the machine may be the site of biofilm development. However, this study demonstrated no significant difference between Gambro AK95 and Fersenius 4008B and the dialysate count. On the contrary, the acceptability of samples did not drop before and after the HD machine, which might be related to the fact that both machines are of a newer generation, which incorporates recent technology regarding continuous dialysate flow and the heating system and avoidance of closed circuits and loops.

Findings that moderate levels of bacterial products in dialysate can stimulate the production of cytokines across intact membranes (back-transport) have resulted in demands for improved microbiological quality of dialysate.[16,17] Pegues et al[18] suggested a potential transfer of pyrogenic substances from the dialysate during routine dialysis. Favero et al[19] showed that rates of pyrogenic reactions were directly related to levels of Gram-negative bacteria in the dialysate. As endotoxins are part of the bacterial cell wall, it is believed that the endotoxin level could be used to indicate the bacterial level. Several authors found no correlation between bacterial growth and endotoxin concentrations in water or dialysate samples. Possible explanations for that lack of correlation might be due to different bacterial strains having different endotoxin activity.[7-9,12] In addition, as LPS is released from debris of microorganisms, low CFU/ml could be associated with high endotoxin values.[20] In accordance with those workers, this study has shown poor agreement between HPC and LAL assay performed to assess the quality of treated water and dialysate. When results were further analyzed according to each parameter examined, it revealed that HPC showed 40% unacceptability, which increased to 88% and 92% by LAL and by both HPC and LAL. In accordance with our findings, Klein et al[7] found a relationship between endotoxin concentration in treated water and in dialysate, and did not find any correlation between bacterial counts and the endotoxin contamination levels.[10] Thus, Ledebo and Nystrand[12] recommended that endotoxin levels should always be measured in addition to bacterial count because they give a different and complementary picture of the microbiological quality of dialysis fluid.[12]

The findings of our study demonstrate that the measurement of endotoxin level (which is currently not routinely checked) should be integrated in the bacteriological monitoring of dialysate fluids. Readily prepared bicart capsules were proved to be much safer than the manually prepared bicarbonate powder, as measured by the bacteriological quality of the dialysate.

References

1. Canaud B, Bosc JY, Leray H, Stec F. Microbiologic purity of dialysate for online substitution fluid preparation. ***Nephrol Dial Transplant*** 2000; 15 Suppl 2: 21-30.
2. Zunino P, Beltran L, Zunino L, Mendez H, Percovich V, Rocca R, et al. Microbiological quality of hemodialysis water in a three-year multicenter study in Uruguay. ***J Nephrol*** 2002; 15: 374-379.
3. 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; 22 : 596-599.
4. Canaud B, Wizemann V, Pizzarelli F, Greenwood R, Schultze G, Weber C, et al. Cellular interleukin-1 receptor antagonist production in patients receiving on-line haemodiafiltration therapy. ***Nephrol Dial Transplant*** 2001; 16: 2181-2187.
5. Cleseri LS, Greenberg AE, Eaton AD, editors. Standard Methods for Examination of water and Wastewater. 20th ed. Washington, (DC): American Public Health Association (APHA), and Water Environment Federation (WEF); 1998. p. 9000-9221.
6. European Directorate for the Quality of Medicines, Council of Europe. Haemodialysis solutions, concentrated, water for diluting. In Monograph 1167. European Pharmacopoeia 4th ed. Strasbourg: Council of Europe; 2002. p. 2371-2372.
7. Klein E, Pass T, Harding GB, Wright R, Million C. Microbial and endotoxin contamination in water and dialysate in the central United States. ***Artif Organs*** 1990; 14: 85-94.
8. Kulander L, Nisbeth U, Danielsson BG, Eriksson O. Occurrence of endotoxin in dialysis fluid from 39 dialysis units. ***J Hosp Infect*** 1993; 24: 29-37.

9. Bland LA, Oliver JC, Arduino MJ, Oettinger CW, McAllister SK, Favero MS. Potency of endotoxin from bicarbonate dialysate compared with endotoxin from Escherichia coli and Shigella flexneri. ***J Am Soc Nephrol*** 1994; 5: 1634-1637.
10. Camper AK, LeChevallier MW, Broadaway SC, McFeters GA. Bacteria associated with granular activated carbon particles in drinking water. ***Appl Environ Microbiol*** 1986; 52: 434-438.
11. Laurence RA, Lapierre ST. Quality of hemodialysis water: A 7-year multicenter study. ***Am J Kidney Dis*** 1995; 25: 738-750.
12. Ledebo I, Nystrand R. Defining the microbiological quality of dialysis fluid. ***Artif Organs*** 1999; 23: 37-43.
13. Arvanitidou M, Spaia S, Katsinas C, Pangidis P, Constantinidis T, Constantinidis V, et al. Microbiological quality of water and dialysate in all haemodialysis centres of Greece. ***Nephrol Dial Transplant*** 1998; 13: 949-954.
14. Bambauer R, Schauer M, Jung WK, Daum V, Vienken J. Contamination of dialysis water and dialysate. A survey of 30 centers. ***ASAIO J*** 1994; 40: 1012-1016.
15. Oie S, Kamiya A, Yoneda I, Uchiyama K, Tsuchida M, Takai K, et al. Microbial contamination of dialysate and its prevention in haemodialysis units. ***J Hosp Infect*** 2003; 54: 115-119.
16. Nubé JM, Grooteman MPC. Impact of contaminated dialysate on long-term haemodialysis-related complications: is it that important? ***Nephrol Dial Transplant*** 2001; 16: 1986-1991.
17. Bommer J. Sterile filtration of dialysate: is it really of no use? ***Nephrol Dial Transplant*** 2001; 16: 1992-1994.
18. Pegues DA, Oettinger CW, Bland LA, Oliver JC, Arduino MJ, Aguero SM, et al. A prospective study of pyrogenic reactions in hemodialysis patients using bicarbonate dialysis fluids filtered to remove bacteria and endotoxin. ***J Am Soc Nephrol*** 1992; 3: 1002-1007.
19. Favero MS, Petersen NJ, Carson LA, Bond WW, Hindman SH. Gram negative water bacteria in hemodialysis systems. ***Health Lab Sci*** 1975; 12: 321-34.
20. Lonnemann G. Assessment of the quality of dialysate. ***Nephrol Dial Transplant*** 1998; 13 Suppl 5: 17-20.

Related topics

Tuzcu A, Bahceci M, Yilmaz ME, Turgut C, Kara IH. The determination of insulin sensitivity in hemodialysis and continuous ambulatory peritoneal dialysis in nondiabetic patients with end-stage renal disease. ***Saudi Med J*** 2005; 26: 786-791.

Han TM, Nankissor K, Pearce A, Assounga AG. Hemodialysis and ultrafiltration. A bridge to cardiac surgery. ***Saudi Med J*** 2004; 25: 1301-1303.

Al-Mueilo SH. Gastroduodenal lesions and Helicobacter pylori infection in hemodialysis patients. ***Saudi Med J*** 2004; 25: 1010-1014.