# Bacteriological Profile of Hemodialysis Fluid in Patients with Acute Kidney Injury (AKI) and Chronic Kidney Disease (CKD)

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**Abstract**

**Problem:** The association between severity of CKD (e.g., as measured by levels of estimated GFR) and risk of AKI has not been quantified until relatively recently. For several decades, many physicians believed that AKI was a self-limited process followed by complete recovery of kidney function to pre-AKI levels among survivors. **Approach:** The objective of this study is to assess the bacteriological quality of treated water and dialysate used in the HD unit of a tertiary care hospital. This is a cross-sectional and observational study where 50 samples were planned and possible to 48 only. The inclusion criteria are all AKD and CKD patients who were undergoing for hemodialysis and patients in antibiotic therapy were excluded. Treated water samples and dialysate samples were collected from each haemodialysis machine. The samples were further processed for bacteriological culturing and possible bacterial species were identified. **Findings:** Three out of 48 (6.25%) of treated water and seven out of 48 (14.6%) dialysate samples showed growth above acceptable limits of bacterial contamination. Bacterial colonies identified were *Staphylococcus aureus*, coagulase negative staphylococci (CONS), *Klebsiella* sp*, Pseudomonas aeruginosa, Escherichia coli* and *Enterobacter sp.* All the bacterial isolates were sensitive towards the battery of antibiotics used. No resistant strains identified in this study. **Conclusion:** This study had the observable data of maintenance of quality of dialysis water which is microorganism free for water treatment and distribution systems. Policy has to be adopted in order to regulate the quality of water used in HD. Adoption and strict implementation of standard disinfection protocols to minimize the pathogen exposure to patients.

**Keywords:** Hemodialysis fluid, AKI, CKD, Bacteriological profile

## Introduction

Acute kidney injury (AKI) (previously termed acute renal failure) is characterized by the rapid and sustained reduction of glomerular filtration rate resulting in the retention of nitrogenous (creatinine and urea) and non-nitrogenous metabolic waste products and dysregulation of body fluid volume status, electrolyte and acid-base homeostasis (Schiffl and Lang, 2013). Hospital acquired (HA) AKI may develop in a wide variety of clinical settings including ambulatory out-patients, general ward patients and in particular, critically ill-patients for whom AKI represents a common complication of both underlying illness and its treatment.

HA-AKI is common and its overall incidence is increasing in developed countries. This reflects increased acuity of underlying diseases, more aggressive radiologic, medical or surgical treatment of aged patients as well as increased detection of the renal disorder. HA-AKI is a heterogeneous syndrome that arises predominantly secondary to ischemia, nephrotoxins and bacterial sepsis, but rarely from genuine acute renal diseases. In the intensive care unit (ICU), AKI manifests itself in the majority of patients as part of multiple organ failure (Dennen *et al.,* 2010; Bellomo *et al.,* 2012).

AKI is a common medical problem among hospitalized patients and may be associated with multiple etiologies, occurring singly or in combination, including infectious diseases or conditions such as diarrheal

disease, HIV, malaria, glomerulonephritis and sepsis, toxins or herbal medications, autoimmune diseases, pregnancy-related conditions, trauma-related tubular injury, and iatrogenic causes including medications such as nonsteroidal anti-inflammatory drugs, hypovolaemia, and contrast induced nephropathy (Naicker *et al.,* 2008; Mahmoud *et al.,* 2014; Grace *et al.,* 2018).

In hemodialysis, huge amounts of water are used for diluting the concentrates to produce dialysis fluid. The water is produced on site by reverse osmosis units. The chemical and microbiological quality of the water is essential for dialysis patients. Reverse osmosis units produce water of acceptable chemical quality that can be kept throughout the water system. The microbiological water quality, on the other hand, does not depend on the reverse osmosis unit but on the maintenance of the whole water system. All over the world, dialysis units take water samples and send them to laboratories for cultivation and endotoxin tests. Depending on the method of microbiological analysis, the water may be judged to be very good even if in reality it is much worse and outside of standard recommendations. When standardizing the methods with adequate cultivation of water samples, the accuracy of the tests will be better, and as a result, dialysis units can use their resources for keeping the water systems in good shape, i.e. disinfect preventively and frequently and use less effort in collecting samples.

This will benefit patients, who will receive a high-quality dialysis fluid, thus eliminating the effects of microbiological impacts such as increased levels of inflammation markers (e.g. C-reactive protein) (Rolf, 2008). In the situation of performing hemodiafiltration by producing the substitution fluid on-line, it is even more important to have a sensitive method of microbiological verification to follow-up the hygienic quality. Microbial contamination of water can lead to biofilm formation in Haemodialysis (HD) system and release of endotoxins. Biofilm once formed is difficult to remove inspite of regular disinfection and is the constant source of endotoxins, peptidogycans and fragments of bacterial DNA that can cross the dialyser membrane and stimulate cytokine production and trigger elevation of acute phase reactants. These can give rise to acute intradialytic complications like fever, chills, hypotension, headache, nausea, cramps (Perez *et al.,* 2000).

Studies also suggest evidence of a possible relationship between water contamination and long-term morbidity, β2 amyloidosis, atherosclerosis (Montanari *et al.,* 2009; Lonnemann *et al.,* 2000). Microbial quality of dialysis fluids is still too often a neglected problem. It is critical to monitor the bacteriological quality of dialysis water. Good quality water can help improve patient's quality of life and could possibly increase their survival rates (Masakane *et al.,* 2009; Oumokhtar *et al.,* 2013). Immediate complications like fever, rigor in the patients can also be prevented.

The aim of this survey study is to assess the bacteriological quality of treated water and dialysate used in the HD unit of a tertiary care hospital.

**Materials and Methods**

This is a cross sectional and observational study. A total of 50 samples were planned but possible only to 48. The study period was for three 3 months and conducted after Institutional ethics committee approval (Ref. No. 798/TSRMMCH&RC/ME-1/2021 IEC NO. 033 dated 22.11.2021).

All AKD and CKD patients undergoing for hemodialysis were included and patients in antibiotic therapy were excluded. The treated water samples were collected immediately passed the water purification system (Reverse Osmosis) water tank and from Reverse Osmosis (RO) lines supplying the HD unit. Dialysate samples were taken from each haemodialysis machine where dialysate exits the dialyzer in the HD unit. The sample ports were disinfected with alcohol and allowed to dry before collecting the samples. At each point of collection, the valve was opened and water was allowed to flow for a minimum of 2 minutes at normal pressure and flow rate before the samples were drawn. Samples were collected using a clean catch technique to minimize potential contamination of the sample (Shiva et al., 2015).

The samples were vortexed and 0.1ml of treated water samples were pipetted and placed on the centre of the bacteriological culture plates. Dialysate samples were inoculated onto separate agar plates. The sample were spreaded with a cool alcohol flamed glass rod spreader onto the surface of the culture plates and the inoculated plates were incubated at $37^0$C for 24 to 48 hrs (Shiva et al., 2015).

For treated water, the colony count/ml was determined by multiplying the number of colonies seen by 10. The number of colonies seen was multiplied by 1000 to get the colony count per ml of dialysate sample. The acceptable limits of bacterial contamination for treated water and dialysate were taken as less than 200 CFU/ml and less than 2000 CFU/ml respectively in accordance with the Govt of India guidelines for Haemodialysis (Guidelines for Maintenance Hemodialysis in India, 2012; Gunjeet et al., 2018). Further the bacterial colonies were examined for the determination of genus and species using standard bacteriological procedures. The multidrug resistant strains were determined by Mueller Hinton agar drug diffusion assay.

Descriptive statistics including frequency, percentages, mean, median and standard deviation will be used to summarize and describe the data. Further it was presented as numerical values in the control group and as bar graphs in the patients. An alpha level of 0.05 was used for all significance tests.

**Results**

Three out of 48 (6.25%) of treated water and 7 out of 48 (14.6%) dialysate samples showed growth above acceptable limits of bacterial contamination (Figure 1 and 2).

***Figure 1: Analysis of treated water***

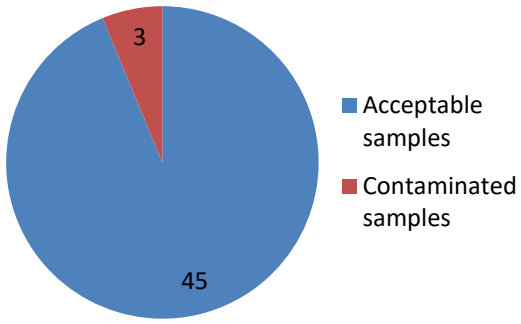
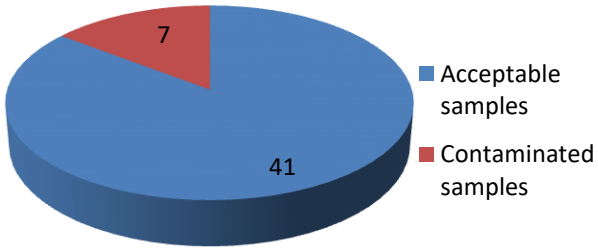



***Figure 2: Analysis of Dialysate sample***




In cases of samples showing unacceptable levels of bacterial contamination, disinfection of the water treatment system was repeated and follow-up cultures done until samples showed growth within acceptable limits. During follow-up procedures, no bacterial growth observed that indicated the disinfectant cleared the contaminants and provided the safe samples for patient management.

Bacterial colonies that appeared at 48 and 72 were isolated and identified. *Staphylococcus aureus*, coagulase negative staphylococci (CONS), *Klebsiella* sp, *Pseudomonas aeruginosa, Escherichia coli* and *Enterobacter* sp were the most frequent isolates. This study indicates that the media utilized, the time and temperature of incubation may result in a significant underestimation of the bacterial population of water and dialysis fluids, thus potentially placing the patient at a higher risk.

All the bacterial isolates were sensitive towards the battery of antibiotics used. No resistant strains identified in this study.

**Discussion**

The results of our study were found to be in the same range as other similar studies (Perez et al., 2000; Montanari et al., 2009). In a cross-sectional survey done across 51 chronic and acute dialysis centres in the central United States, 35.3% of the water samples and 19% of the dialysate samples did not satisfy the AAMI guidelines (Oumokhtar et al., 2013; Klein et al., 1998).

A study done in Germany observed that 17.8% of all water samples and 11.7% of all dialysate samples showed contamination higher than the accepted standard values (Bambauer et al., 1994). Study conducted by Asserraji et al., in Saudi Arabia found the incidence of unacceptable bacterial contamination of treated water to be 9.2% while dialysate samples did not show any contamination. They suggested that frequent disinfection of the water treatment plant is required to get better quality water for production of dialysis fluids (Asserraji et al., 2014).

In a similar study done by El-Koraie et al., out of 321 samples taken from different points in the water distribution system and the dialysate sampling system, 16.8% samples showed unacceptable growth. They concluded that HD centres need regular monitoring and maintenance to provide good quality haemodialysis (El-Koraie et al., 2007). Various studies have also suggested incorporation of endotoxin assays to monitor the quality of water used for production of dialysis fluids (Lonnemann, 2000; Lima et al., 2005).

In a survey done by the Japanese Society for Dialysis Therapy (JSDT) on bacteriological quality of dialysis fluid in all the dialysis facilities in Japan, 3.9% and 2.6% samples were found to be outside acceptable limits in 2006 and 2007 respectively. They found that the survival rate in dialysis patients in Japan was very high as compared to those of the other countries. The good quality of water and dialysate used in their dialysis facilities was suggested as one of the probable reasons for decreased rates of mortality in their patients (Masakane et al., 2009).

Different guidelines suggest different reference values as acceptable limits of contamination. The AAMI guidelines recommend <200 CFU/ml of treated water and <2000 CFU/ml of dialysate as permissible limits of contamination. The European Pharmacopoeia is more stringent, with growth ≥ 100 CFU/ml each of dialysate and treated water considered as unacceptable (Pontoriero et al., 2003; Hoenich et al., 2003).

This study highlights the importance of regular monitoring of quality of water used in HD units. Compliance could be increased further by frequent disinfection of the HD system. It is necessary to upgrade the disinfection protocols of the HD system. Further studies need to be done to identify the causes of contamination. Other options of disinfection of the water systems can be explored.

Prevention of infection is one of the few avenues available to reduce hospitalizations, control costs, and improve quality of life for these patients. Common pyogenic bacteria from the patient's endogenous flora are responsible for most infections in patients with end stage renal disease. The carriage rate of *S. aureus* in patients with end stage renal disease may approach 70%. Vascular access is the risk factor in more than 50% of the infections and *S. aureus* on the skin the most common pathogen. A previous episode of bacteremia is the most predictive risk factor for subsequent bacteremia, suggesting that the same patients have repeated infections and may be chronic carriers of staphylococcus. Mupirocin applied to the nares significantly reduces the carriage rate as well as subsequent rate of bacteremia. Unfortunately, clinical experience demonstrates that

universal use will ultimately lead to mupirocin resistance. Other strategies may have better results including limiting mupirocin prophylaxis to *S. aureus* carriers only.

The chances of infection of the vascular access are increased in patients with poor personal hygiene, malnutrition, and inadequate dialysis. The type of vascular access is a major risk factor for infection, with VC being at highest risk. Their use is to be discouraged without a plan for a safer VAD.

Twenty-five percent of the patients account for 50% of the total costs of caring for dialysis patients and more than 40% of deaths. Using vascular access data, past infection history, co-morbidity indexes, and physical activity scales we have developed an index to stratify patient risk for future infection. In an attempt to change the paradigm, we are focusing our resources to provide preventative home services in this frail and debilitated group of patients.

**Conclusion**

The quality of dialysis water depends on microbial contamination of untreated water, water purification techniques used and maintenance of water treatment and distribution systems. It is necessary to regularly monitor the quality of water used in HD units. Also, adoption and strict implementation of standard disinfection protocols of the water distribution and HD system is required to obtain good quality of water to minimize exposure of these immunodeficient patients to contaminated sources of water.

**References**

1. Asserraji, M., Maoujoud, A., Belarbi, M. and Elfarouki, R (2014). Monitoring the microbiological quality of dialysate and treated water. Saudi Journal of Kidney Diseases and Transplantation 25: 91-95.

2. Bambauer, R., Schauer, M., Jung, W.K., Daum, V. and Vienken, J (1994). Contamination of dialysis water and dialysate: A survey of 30 centers. ASAIO Journal 40: 1012-1016.
3. Dennen, P., Douglas, I.S. and Anderson, R (2010). Acute kidney injury in the intensive care unit: An update and primer for the intensivist. Critical Care Medicine 38: 261-275.

4. El-Koraie, A.F., Hazzah, W.A., Abbass, A.A. and El-Shazly, S.A (2007). Bacteriological monitoring of dialysis fluid in 2 hemodialysis units in Alexandria, Egypt. Saudi Medical Journal 28: 1234-1238.

5. Grace, I., Benedicte, N., Menelas, N., Vincent, D. and Onyema, O (2018). Clinical profile and outcome of patients with acute kidney injury requiring hemodialysis: two years' experience at tertiary hospital in Rwanda. Biomedical Research International 18: 1716420.

6. Guidelines for Maintenance Hemodialysis in India (2012). Ministry of Health & Family Welfare Govt. of India. Indian Journal of Nephrology 14: S1-46.

7. Gunjeet, K., Shankar, P., Raja, R., Pankaj, M., Krishnan, L.G. and Vivekanand, J (2018). Cost of hemodialysis in a public sector tertiary hospital of hospital. Clinical Kidney Journal 11: 726-733.

8. Hoenich, N.A. and Levin, R (2003). The implications of water quality in hemodialysis. Semin Dialysis 16: 492-497.

9. Klein, E., Pass, T., Harding, G.B., Wright, R. and Million, C (1990). Microbial and endotoxin contamination in water and dialysate in the central United States. Artificial Organs 14: 85-94.

10. Lima, J.R.O., Marques, S.G. and Gonçalves, A.G (2005). Microbiological analyses of water from hemodialysis services in Sao Luis, Maranhao, Brazil. Brazilian Journal of Microbiology 36: 103-108.

11. Lonnemann, G (2000). The quality of dialysate: An integrated approach. Kidney International 58: 112-119.

12. Mahmoud, L.B., Pariente, A. and Kammoun, K (2014). Risk factors for acute decompensation of chronic kidney disease in hospitalized patients in the nephrology department: A case-control study. Clinical Nephrology 81: 86-92.

13. Masakane, I., Takemoto, Y. and Nakai, S (2009). Bacteriological water quality in the central dialysis fluid delivery system from the survey of the Japanese Society for Dialysis Therapy. Blood Purification 27: 11-16.

14. Montanari, L.B., Sartori, F.G., Cardoso, M.J., Varo, S.D., Pires R.H., Leite, C.Q.F., Prince, K., Martins, C.H.G (2009). Microbiological contamination of a haemodialysis center water distribution system. Revista do Instituto de Medicina Tropical de Sao Paulo 51: 37-43.

15. Naicker, S., Aboud, O. and Gharbi, M.B (2008). Epidemiology of Acute Kidney Injury in Africa. Seminars in Nephrology 28: 348-353.

16. Oumokhtar, B., Lalami, A.E.O. and Mahmoud, M (2013). Prevent infection linked to the dialysis water in a hemodialysiscenter in Fez city (Morocco) The Pan African Medical Journal 16: 122-128.

17. Pontoriero, G., Pozzoni, P., Andrulli, S. and Locatelli, F (2003). The quality of dialysis water. Nephrology Dialysis Transplantation 18: 21-25.

18. Rolf, N (2008). Microbiology of water and fluids for hemodialysis. Journal of Chinese Medical Association 71: 223-229.

19. Perez, G.R. and Cinio, R.B (2000). Why and how to monitor bacterial contamination of dialysate? Nephrology Dialysis Transplantation 15: 760-765.

20. Schiffl, H. and Lang, S.M (2013). Current practice of conventional intermittent hemodialysis for acute kidney injury. Indian Journal of Nephrology 23: 395-402.

21. Shiva, V., Indumathi, V.A., Gurudev, K.C. and Shalini, A.N (2015). Bacteriological quality of treated water and dialysate in hemodialysis unit of a tertiary care hospital. Journal of Clinical and Diagnostic Research 9: 14-16.

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