

Bacterial contamination of hemodialysis water in three randomly selected centers in South Western Nigeria

OO Okunola, JO Olaitan¹

Department of Medicine, Nephrology Unit, Obafemi Awolowo University, Ile-Ife, ¹Department of Biological Sciences, College of Sciences, Engineering and Technology, Osun State University, Osogbo, Osun State, Nigeria

Abstract

Background: Hemodialysis (HD) is the most common method of renal replacement therapy for patients with either acute kidney injury in the failure stage or end stage kidney failure in Nigeria. The number of dialysis centers in Nigeria has risen exponentially from 10 centers two decades ago to more than 120 centers in 2015. The number of patients needing renal replacement therapy in the country in the form of HD has also risen close to a projected 2000/year. The outcome from HD in Nigeria is poor as a result of a myriad of interwoven factors such as complications of cardiovascular diseases and suboptimal dialysis dose primarily due to economic factors. These are often complicated by episodes of dialysis water related bacteremia, possibly as a result of the apparent lack of a standardized guideline or protocol for monitoring dialysis water treatment system which is the driving force of dialysis units.

Objectives: This is a multicenter laboratory-based study designed to determine the microbiological quality of samples of HD water and dialysate in randomly selected dialysis units in three major government teaching hospitals in Nigeria.

Methodology: Water samples were aseptically and serially collected from three HD units. The samples were taken from 6 points at each center coded A, B, and C over a 6-month period.

Results: The water system in the three dialysis centers were grossly contaminated with Gram-negative aerobic bacteria such as *Pseudomonas* species and *Moraxella* species at all the points in the three centers.

Conclusion: Conventionally, water treatment is a major determinant of morbidity and mortality in HD units, and the microbial quality is a major factor involved. There is evidence of bacterial contamination in the dialysis units sampled in this study. There is thus the compelling need for periodic microbiological monitoring of water after each treatment step. A uniform national guideline as part of an effective quality assurance protocol in infection surveillance is also advocated for dialysis units in Nigeria.

Key words: Hemodialysis, microbiological, Nigeria, water

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Address for correspondence:

Dr. OO Okunola,
Department of Medicine, Nephrology Unit, Obafemi Awolowo
University, Ile-Ife, Osun State, Nigeria.
E-mail: yok8t@yahoo.com

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Introduction

Dialysis is the process of removing toxins directly from the blood (hemodialysis [HD]) or indirectly via peritoneum (peritoneal dialysis) using diffusion across a semipermeable membrane.^[1] HD is one of the different forms of treatment for patients with uremic acute and end-stage kidney failure. As at the end of 2010, nearly one million people are receiving HD worldwide, 60.0% of who are treated in five countries (USA, Japan, Germany, Brazil, and Italy), and this constitutes only 12.0% of the world population.^[2] The total number of HD patients in Africa in 2007 was estimated at 67,700, this equates to an HD prevalence of 71.6 pmp, compared to a global prevalence of 223.0 pmp.^[3]

Dialysis practice (especially hemodialysis) is relatively evolving in Nigeria at a rapid rate. Chronic HD became available in Nigeria in 1981. However, the numbers of dialysis centers have risen exponentially from 10 centers a decade ago to a projected figure of 120 in 2015. The majority are in public hospitals (67.0%) and all are situated in major cities where <40.0% of the population reside.^[4]

The number of patients needing renal replacement therapy in the form of HD has also risen exponentially. Less than 10.0% of patients with chronic kidney failure are able to afford maintenance hemodialysis after 3 months of commencement due to socioeconomic factors.^[5]

Water treatment is the process whereby water goes through various levels of pretreatment, and a final purification process prior to its distribution through a hydraulic circuit. It is an important part of hemodialysis, and if this is not well processed, patients can develop septicemia or endotoxemia either directly or indirectly. Consequence of microbial contamination of dialysis water includes acute features such as headaches, nausea, cramps, and chronic manifestations such as the alteration of cardiovascular instability, worsening atherosclerosis, and malnutrition.^[6]

The pretreatment phase consist of passing water, obtained through a main source which may be ground or municipal water, through several tight filters basically to remove particulate matter. Further organic matters such as chlorine and chloramines are removed by activated carbon filters and then passed through charcoal water softeners which are a major site for microorganism growth.

The treatment phase consists of the reverse osmosis system which is described as the most cost-effective method for water purification from both organic and inorganic solutes, including organisms and endotoxins. The deionization system removes inorganic ions dissolved in the water by an ion-exchange process using cationic and anionic resins.

The final stage is the ultraviolet treatment using germicidal ultraviolet lamps. This kills all types of bacteria that have managed to pass through primary treatment devices leading to increased bacterial lipopolysaccharides and fragments, which are further removed by ultrafiltration.

Water is further delivered through loops and terminals for proportionating with dialysate concentrate and ultimately to different dialysis stations during treatment. Water for dialysis should, therefore, be ideally ultra-pure and free from any microbial contaminant.

There are several national and regional guidelines with respect to maximally acceptable limits of bacterial contamination of dialysis water. The American Association of Medical Instrumentation (AAMI) recommends the maximum acceptable level of viable bacteria count to be 200 colony forming units (CFU) per milliliter of water and endotoxin concentration of <2 IU/ml,^[7] while the European pharmacopoeia limit is set at 100 CFU/ml and endotoxin concentration of <0.25 IU/ml.^[8] Despite this, a number of centers, especially in Europe and South America still have levels of microbial contamination of both dialysis water and fluid in excess of the national standards.^[9,10]

There is generally a lack of standardized operational guidelines with regards to dialysis water quality in Nigeria. Even though frequency and access to dialysis are still suboptimal in our practice due to cost, our patients are still routinely exposed to a very significant amount of dialysis water.

There are limited data available on this hitherto neglected fundamental aspect in renal replacement therapy; hence, this study becomes imperative as contamination of dialysis fluids has major clinical consequences.^[11]

Methodology

Collection of samples

This is a cross-sectional study. It involved an assessment of bacterial contamination of dialysis water as a measure of determining its quality in three randomly selected government owned dialysis centers in three teaching hospitals in South Western Nigeria. The centers are approximately 43 km equidistant to each other and serve as referral hub for dialytic activities in their respective catchment areas.

The study period was from April 2011 to December 2012. A total of 36 samples were taken from 6 points at each dialysis units. Permission was sought and obtained from the head of the respective dialysis units involved, with the assurance that the result would be communicated directly to the dialysis units and the respective hospital authorities. The

centers were coded and subsequently identified as centers A, B, and C. The points were; (i) source/raw water (ii) water leaving the water reverse osmosis unit (iii) water leaving the storage tank (iv) water inlet into the machine (v) water outlet from machine (vi) dialysate water.

The samples were collected aseptically during active HD after 1 h by the same principal investigator and then transported in ice-packs maintained at 4°C to the coordinating microbiology reference laboratory located at the Department of Medical Microbiology of the Osun state University, Osogbo, Osun state, Nigeria within 6 h for analysis. This was repeated at two monthly intervals over the 6 months period.

Count and isolation of bacterial isolates

Analysis of total heterotrophic bacteria in the water samples was obtained on Tryptic Soy Agar (TSA) medium by pour plate method, 1 ml of water sample was introduced into 20 ml of TSA and poured into a conventional petri dish, allowed to set and incubated at 37°C for 24–48 h. Isolation of bacteria was by direct plating of the water samples (0.1 ml) on TSA by spread method under aseptic condition. The plates were incubated at 37°C between 24 h. Enumeration of CFU was carried out after 24 h. Identification and characterization of bacterial isolates were done on the basis of their cultural, morphological, and biochemical characteristics using Bergey's manual of determinative bacteriology standard reference.

Results

Results of center characteristics are summarized in Table 1 while analysis of water samples from the water distribution systems are summarized in Table 2. Gram-negative bacteria are the main contaminants of water and fluid. A total of 36 bacteria were isolated. *Pseudomonas* sp. was predominant in 55% followed by *Moraxella* sp. It was observed that both microbes were isolated from all the centers while *Bacillus* sp. was isolated from two centers. Contamination of dialysis fluid was in the range of $1.8\text{--}3.3 \times 10^2$ CFU/ml.

Centre C being the youngest of the centers, established <5 years ago understandably had the least number of colony isolated from the source up to the water inlet to the machine [Table 2].

Centre A, the oldest of the units had the largest amount of CFU, especially at points such as the source water and the inlet to the machine in addition to the dialysate [Table 2].

Centre B has grossly contaminated isolates at virtually all the points [Table 2].

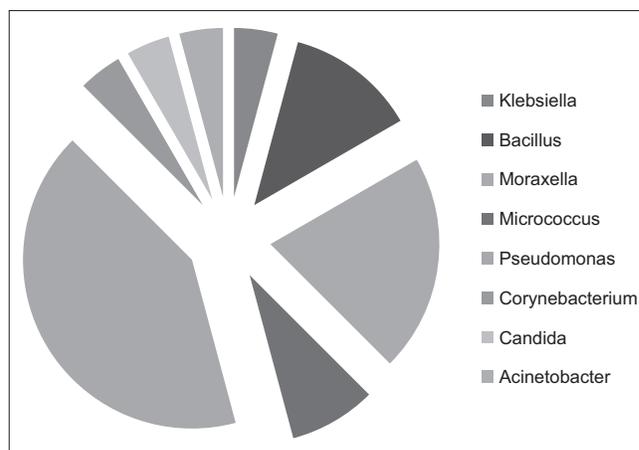


Figure 1: Percentage distribution of bacterial isolates from water samples

Table 1: Baseline data of dialysis centers and their water distribution systems

	Center A	Center B	Center C
Age of HD center (years)	24	22	10
Average number of dialysis sessions/month	76	60	48
Age of present water treatment system (years)	14	8	7
Total number of machines in operation over the preceding 3 months	16	6	2
Average capacity of treated water storage/m ³	2000	1000	500

HD=Hemodialysis

Table 2: Identification and quantification of the bacterial contaminants/center

	Center A	Center B	Center C	Mean cell count
Point 1 (source/raw water)	<i>Pseudomonas</i> , <i>Actinomyces</i> , <i>Micrococcus</i> (2.3×10^3)	<i>Pseudomonas</i> (3.3×10^2)	<i>Pseudomonas</i> (3.1×10^1)	(2.9×10^2) \pm 0.5
Point 2 (water from reverse osmosis)	<i>Moraxella</i> , <i>Micrococcus</i> (1.8×10^2)	<i>Bacillus</i> (1.14×10^2)	<i>Moraxella</i> , <i>Candida</i> (insignificant)	(1.0×10^2) \pm 0.9
Point 3 (water leaving storage tank)	<i>Pseudomonas</i> (1.0×10^2)	<i>Pseudomonas</i> (1.0×10^2)	<i>Pseudomonas</i> , <i>Moraxella</i> , <i>Klebsiella</i> (3.3×10^2)	(1.8×10^2) \pm 1.3
Point 4 (water inlet into dialysis machine)	<i>Pseudomonas</i> (2.0×10^2)	<i>Bacillus</i> (1×10^2)	<i>Pseudomonas</i> (1.2×10^2)	(1.4×10^2) \pm 0.5
Point 5 (water outlet from dialysis machine)	-	<i>Pseudomonas</i> (1.6×10^2)	<i>Pseudomonas</i> , <i>Candidiasis</i> (1.55×10^2)	(1.1×10^2) \pm 0.9
Point 6 (dialysate water)	<i>Bacillus</i> (2.0×10^2)	<i>Moraxella</i> (insignificant)	<i>Moraxella</i> (insignificant)	(0.7×10^2) \pm 1.2
Water contamination rate*** (%)	83	66	50	

*** Water contamination rate = $\frac{\text{Number of points infected / centre}}{\text{total number of dialysis points}}$

It should be noted, however, that the viable bacterial colony forming count was above the AAMI recommended levels.

Discussion

As a result of the increasing prevalence of chronic kidney failure and the compelling need for renal replacement therapy in acute kidney failure, there has been an increase in the demand for hemodialysis over the last three decades as more patients now utilize this modality. This is, however, constrained by infrastructural and technical factors among a host of other contending limiting factors.^[12]

There is also a pervading lack of maintenance culture in all the centers with frequent system and equipment dysfunctions as earlier reported by other workers.^[4] The incidence of bacterial contamination of hemodialysis water leading to septicemia in Nigeria was first reported in Lagos in 1996, and this was traced to the microbial content of the water treatment equipment.^[13] Since then there have been sporadic and often under-reported incidents of water-related infections in dialysis units. Such episodes of septicemia might have been misattributed to other causes such as viraemia or malaria infections.

It is also plausible that the episodes are under-reported as fewer people are on chronic hemodialysis due to the cost of a session of dialysis hence the total population at risk might not be adequate enough to make an objective assessment. This is unlike in developed countries where hemodialysis is subsidized under a social security nets incorporating health insurance schemes hence a substantial number of people in such climes have access to hemodialysis.^[14]

Gram-negative bacteria are the main contaminants of water in HD units as reported elsewhere and as seen in the results [Table 2].^[15] *Pseudomonas aeruginosa* was the commonly isolated organism in virtually all the points studied in the three centers [Figure 1], this is similar to findings virtually everywhere. Although in a similar study conducted in six centers in Lagos,^[16] *Escherichia coli* was the commonly isolated bacteria perhaps due to the fact that this is a densely populated town with virtually all the dialysis units located within living built up areas; thus, the risk of fecal contamination of the ground water used is high. All the dialysis centers in our study were not situated in densely populated areas and even though they all used deep ground water which is commonly used in Nigeria. Furthermore, *Pseudomonas* sp. is known to rapidly proliferate in dialysis fluids and this further poses a public health concern as it is often implicated as a primary cause of septicemia and endotoxemia through the production of exotoxin A.^[17]

Similar to what was obtained in a few centers in the United States,^[18,19] we also isolated *Moraxella* sp. from our samples. This is a Gram-negative organism which is often commensals on the mucosal surfaces of man and sometimes can cause opportunistic infections. Such infections is an additional potential cause of increased morbidity implication having been implicated as a cause of acute respiratory infections and blepharoconjunctivitis.^[20]

Buffers such as acetate or bicarbonate solutions are normally used in hemodialysis, however because of the side effects normally associated with acetate based buffers in terms of cardiovascular instability, most centers now prefer bicarbonate based buffering solutions. The drawback of this is that such solutions as used by all the centers in this study might have further aided bacterial growth and contamination as it constitutes an excellent growth medium for microflora.^[20]

It is also possible that in the tropics, seasonal changes might have further encouraged the growth of bacterial and other algae, this is termed *algal booms* and thus there is constant proliferation without concomitant analysis and maintenance of the water units, especially the water storage tanks, at the reservoir level and at the pretreatment levels.^[21]

Conclusion

Water quality is a major determinant of morbidity and mortality in HD patients conventionally. There is a gross bacterial contamination of dialysis water in three randomly selected dialysis units in South Western Nigeria. This pattern might be indicative of what is generally in some other centers in our environment.

There is a need for a monthly microbiological monitoring of HD water in Nigeria. Periodic cleaning and disinfection of the water treatment plants and distribution system are earnestly desired where are contamination to negate the possible adverse effects of using bacterial-contaminated water for hemodialysis. An acceptable national nephrology guideline on the standard of the maximal dialysis water microbial limit would also be appropriate.

Furthermore, correlation studies of the outcome of hemodialysis to contamination rate of water treatment plants and distribution systems would be informative to policy makers and care givers.

Limitation

Due to constraints with regards to procurement and logistics, we were unable to do a limulus ameocyte lysate test which is a qualitative test for detection of Gram-negative endotoxins. Furthermore, we did not screen for fungal, viral, many other bacterial, or mycobacterial organisms.

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Conflicts of interest

There are no conflicts of interest.

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