



RESEARCH ARTICLE

BACTERIAL CONTAMINATION OF DIALYSIS WATER AND DIALYSATE ATMUKALLA ARTIFICIAL KIDNEY CENTER IN MUKALLA CITY - HADHRAMAUT-YEMEN: RATE OF CONTAMINATION AND SENSITIVITY OF BACTERIAL ISOLATES TO ANTIBIOTICS

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Abstract



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Objective: Water treatment systems are a vital factor in dialysis therapy and precise control of hemodialysis water bacteriological quality is predominantly important in order to assurance a better quality of life of the hemodialysis patients. The purpose of this study was to detect the level of contamination in hemodialysis water and dialysate by bacteria in Mukalla Artificial Kidney Center and investigate the antimicrobial resistance patterns of isolated bacteria.

Methods: Forty eight samples of water and dialysate were assembled weekly over a period of 3 months from 4 points. Bacteriological analysis of samples was carried out then antimicrobial susceptibilities patterns of isolated bacteria were concluded by disk diffusion method.

Results: The mean of total count of bacteria for dialysis water and dialysate were higher than the recommended values (100 CFU/ ml). The isolated bacteria which colonized the hemodialysis systems were mostly Gram-negative bacilli as *Pseudomonas* sp., *Serratia* sp., *Citrobacter* sp. and *Enterobacter* sp. In general, most of the isolated bacteria were poorly responsive to antibiotics.

Conclusion: In conclusion: Dialysis water and dialysate not passed to meet the bacteriological provisions for hemodialysis. To reduce the hazard of contaminants for hemodialysis patients, a sufficient system for water treatment, disinfection of hemodialysis system, and bacteriological contamination monitoring of the water and dialysate are necessary.

Keywords: Bacterial contamination, dialysis water, dialysate, hemodialysis, Yemen.

INTRODUCTION

Hemodialysis patients endure from abnormalities of immune system as directly result of uremia and its metabolic consequences; cause them more vulnerable to infections. Such defects include impairment of the action of neutrophils, lymphocytes B and T and monocytes, causing defective antigen processing, antibody production and cell mediated immune response and thus to an increased incidence of microbial infections¹. These infections are the second leading cause of death among HD patients, with an attributed mortality rate of 14%^{2,3}. The morbidity and mortality of patients with ESRD are serious problems in Yemen as in the world⁴. In addition, hemodialysis is the most common method of renal replacement therapy for patients with either acute renal injury in the failure stage or end stage renal failure. Hemodialysis is the

process of removing toxins directly from the blood using diffusion across a semi permeable membrane⁵. Removing the harmful wastes and extra salt and fluids helps control the blood pressure and keep the proper balance of electrolytes⁶. Each patient using HD machine is exposed to large volume of water (400 L per week) used for production of dialysate, from which, if it is not properly treated, all the low molecular weight substances present in water as chemical, bacterial and toxic contaminants have direct access through the semi permeable membrane of dialyzer to HD patient's blood stream⁷⁻¹⁰. To prevent patients from risks of water contaminants there is a number of standards for quality of dialysis water and dialysate have been proposed¹¹. There are a number of regional and national guidelines with regard to maximally conventional limits of bacterial contamination of dialysis water. The American

Association of Medical instrumentation (AAMI) advises the highest acceptable levels of viable bacteria count to be 200 colony forming units (CFU) per milliliter of water and endotoxin concentrates of < 2 IU/ml, while European Pharmacopoeia (Ph. Eur.) maximum value is 100 CFU/ml and endotoxin concentrates of < 0.25 IU/ml^{12,13}.

However, no data are available regarding bacterial contamination in the Hadramout dialysis water distribution systems. Therefore, it is important to explore the possibility of contamination of dialysis water circulation systems in the dialysis center in Hadramout. In addition to that, this study was specially carried out to detect level of bacterial contamination and bacteriological quality of hemodialysis water and dialysate in Mukalla Artificial Kidney Center (MKC) in Mukallahcity, Hadhramout, Yemen.

METHODS

The samples were collected from MKC in Ibn Sena General Hospital in Hadhramout. The center consists of four rooms and having about 18 HD machines and performs approximately 1305 hemodialysis sessions monthly in three shifts a day. The water samples were collected weekly over a period of 3 months from four measurement points (sampling were repeated from the same points each month). The measurement points were:

1. Municipal water.
2. Return line of reverse osmosis loop.
3. Water prior to the machine.
4. Dialysate solution.

The samples had been collected in clean sterilized glass bottles of 250 ml capacity. These bottles were autoclaved before sampling at 121°C temperature for 15 minutes. At each point of collection the valve was disinfected by heat and using 70% isopropanol, then opened and water was allowed to flow for a minimum of 2 minutes at normal pressure and flow rate before the samples was drawn^{13,14,15}. Samples were then processed at Dar Alshifa Medical Specialized Center Laboratory.

Total enumeration of bacteria

The determination of total bacteria count in water samples was done by using pour plate method. Serial dilutions of water samples were made with peptone water and inoculated on to plate count agar. Plates were incubated at 37°C for 24 to 48 hours. The plates selected for counting were that producing 30-300 CFU/ml with some modulations⁵.

Test for total coliform bacteria

The total bacteria were determined by utilizing the most probable number (MPN) method. Aliquotsof 10, 1 ml and 0.1 ml of water samples and dialysate were collected from the four points mentioned earlier were added to tubes containing MacConkey broth. Test tubes were incubated at 37°C for 48 hours. After incubation, the production of acid and gas formation was considered positive. Number of the positive tubes was recorded and MPN was calculated according to MPN tables Positive tubes were selected for the

confirmed test procedures to detect the indicator bacteria of fecal origin *E. coli*. EMB media was being used¹⁶. The tubes that only showed turbidity were placed on MacConky agar, blood agar and nutrient agar to be tested for non-fermentivebacteria¹⁷.

Identification and characterization of isolating bacteria

The bacteria were isolated from developing colonies in the plate count agar as well as from MacConky broth were submitted to Gram stain and set of biochemical tests including the following: citrate test , urease test , kligler iron agar (KIA), oxidase test, catalease test, sulfide–indole – motility test (SIM) and coagulase test.

Antimicrobial resistance

Antibiotic sensitivities were done by using disk diffusion method on the Mueller-Hinton agar (Kirby-Bauer method)¹⁸. The antibiotic discs under study were: ceftazidime (CAZ 30 mcg), cefepime (CPM 30 mcg), ciprofloxacin (CIP 5 mcg), amikacin (AK 30 mcg), ceftriaxone, (CTR 30 mcg), piperacillin (PI 100 mcg) and trimethoprim (TR 5 mcg).

RESULTS AND DISCUSSION

The contamination of dialysis water and dialysate were above the (Ph. Eur.) recommended level: 100 CFU/ml. This indicates that there is a problem of biological contamination during water-treatment processes. In line with studies by Pisani *et al.*,¹⁹ and Heidarieh *et al.*,¹⁰ reported that the viable count always exceeded the recommended values.

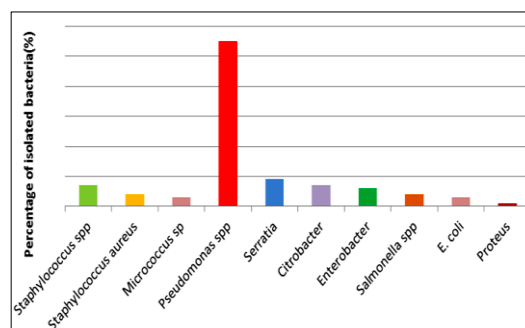


Figure 1: Isolated bacteria from hemodialysis center.

The maximum total count of bacteria was related to the back loop ($1.816 \times 10^3 \pm 2615.3$ CFU/ml) (Table 1). The minimum number of total bacteria was observed in the prior to machine ($1.78 \times 10^2 \pm 222.1$ CFU/ml). These results agreed with a similar study conducted by Oumokhtar *et al.*,⁷ that the maximum total bacterial count was related to back loop. These results have been found in current study suggest that dialysis system and tubing along the fluid pathways within dialysis supplies are the main source of contamination and biofilm development and result in the high levels of the bacterial contamination at different sampling points.

In present study, the maximum total count of coliforms was related to municipal water (3.41 ± 3.1 MPN/100 ml). The results refers to the presence of *Enterobacteriaceae* in water samples, and the contamination level of *Enterobacteriaceae* in

municipal water was more than it in dialysis water and dialysate and there was significant differences between them (p -value=0.030). Ninety-eight bacteria were isolated from all water samples in MKC. The water samples were contaminated by both gram-positive and gram-negative bacteria. Gram-negative bacteria (85.7%) were the main contaminants of water in MKC, while the gram-positive bacteria represented only (14.2%).

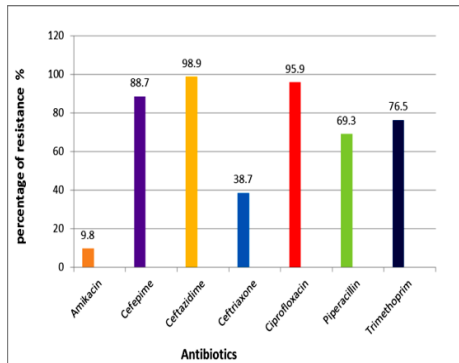


Figure 2: Antibiotic susceptibility of isolated bacteria in MKC.

This results agreed with Oumokhtar *et al.*,⁷ and Okunola and Olaitan,⁵ who reached that most isolated bacteria were gram-negative bacteria. The maximum number of isolated bacteria was for *Pseudomonas* sp. (55.1%), followed by *Serratia* sp. (9.18%), *Citrobacter* sp. (7.14%), non-coagulase *Staphylococcus* sp. (7.14%), *Enterobacter* sp. (6.12%), *Salmonella* sp. (4.08%), *S. aureus* (4.08%), *E. coli* (3.06%), *Micrococcus* sp. (3.06%) and *Proteus* sp. (1.02%) (Figure 2). The highest percentage of isolated bacteria was from the municipal water (30.6%), followed by prior to machine (24.4%), then dialysate solution 23.4% and the lowest percentage of isolated bacteria was from Back loop (21.4%) (Table 2). The most predominant isolated bacteria was *Pseudomonas* sp. (55.1%), this finding was in agreement with other studies conducted by Pisani *et al.*,¹⁹ and Lima *et al.*,²⁰ where the percentage of *Pseudomonas* sp. was the highest among the isolates. Also Arvanitidou *et al.*,²¹ Borges *et al.*,²² Montanari *et al.*,¹ Oumokhtar *et al.*,⁷ and Okunola and Olaitan,⁵ reported that *Pseudomonas* sp. was the most prevalent isolated bacteria as following: (27%), (32.5%), (44%), (52.8%) and (55%), respectively. This finding was attributed to that *Pseudomonas* sp. is recognized to rapidly grow in dialysis fluids and this consequence gives cause for anxiety, in view of the well-known resistance to antibiotics and biocides.^{2,23} Results of present study have showed that machine No. 5 was the most polluted among machines. The bacteria isolated from Machine No. 5 were as follows: *Pseudomonas* sp., *Staphylococcus* non-coagulase., *S. aureus*, *Micrococcus* sp. and *Enterobacter* sp. This device may be out of date or use more or more polluted. Result of present study suggests that there was a problem with the effectiveness of disinfectant used, also the biofilm development within the dialysis machine led to

contamination of the dialysate. The contamination level of the second month was the highest (52.9%), followed by the third month (33.8%) and the first month was (13.2%), with a statistically significant difference in the level of pollution between the three months (value of p =0.432). Also, there was a rapid increase in the level of bacteria numbers after the second periodic chemical disinfection of the water treatment system. This result agreed with study conducted by Oumokhtar *et al.*,⁷. The second chemical disinfection of the system has been done in the 2nd month. Finding of present study suggest that the municipal water contamination rate was high in the 2nd month also the biofilm have been installed in the water treatment system and hemodialysis machines despite the disinfection procedure routinely applied. Three membranes of the RO device were replaced in the third month and therefore it may be expected that this was the reason for decrease bacteria level to (33.8%) and thus the reduction of biofilm. Nazemi *et al.*, asserted that after each period of disinfection, there was observed increased contamination, which was due to the bacterial biofilms generated in the water pipes¹⁷. In addition, Nystrand stated that the occurrence of a biofilm on the pipes directs to a rapid re growth of bacteria after a few hours of disinfection of the water system²⁴. The 98 bacterial isolates showed variable resistance patterns. Antibiotic test results showed that a higher resistance was 98.9% against Ceftazidime (30 mcg). Ciprofloxacin (5 mg) and amikacin (30 mg) were the antibiotics that showed the lowest number of resistance isolates, 9.1% and 1.02%, respectively. In general, most of the isolated bacteria were poorly responsive to antibiotics. The most resistant bacteria were *Proteus* sp. and *E. coli*, they showed resistance to all antibiotics except Ciprofloxacin (Figure 3). The random use of antibiotics and transmission of resistant bacteria between patients were the main factors increasing antimicrobial resistance prevalence²⁵. This study reached to about 100% of *E. coli* were resistant to all antibiotics except Amikacin and Trimethoprim 66.6% while Ciprofloxacin 0% (100% of *E. coli* were sensitive to Ciprofloxacin). This finding was in line with the work of Romanus *et al.*, finding that 81% of *E. coli* were sensitive to Ciprofloxacin, 73% resistant to Trimethoprim²⁶. Also Arvanitidou *et al.*, showed that 0% of *E. coli* were resistant to Ciprofloxacin, while 100% were sensitive to Amikacin, Cefepime and Ceftazidime²¹. This finding was closed to a study by Omoya and Ajayi, finding that 100% of *Proteus* sp. showed resistance to Ceftriaxone while 0% showed resistance to Ciprofloxacin²⁷. Also Yah *et al.*, reported that *Proteus* sp. showed low resistance against Ciprofloxacin (6.1%)²⁸. *Pseudomonas* sp. showed the resistance to Amikacin (9.2%), Cefepime (83.3%), Ceftazidime (98.1%), Ceftriaxone (16.6%), Ciprofloxacin (1.8%), Piperacillin (46.2%) and Trimethoprim (70.3%). This finding was closed to what Romanus *et al.* reached, that *Pseudomonas* sp. were sensitive to Ciprofloxacin and Amikacin as 83%, 100% respectively²⁶. Borges *et al.*, also found that *Pseudomonas* sp. was sensitive to Amikacin and Ciprofloxacin as 64% and 77% respectively²².

Table 1: Mean values of total bacteria in treated water and dialysate.

Sampling points	Total count of bacteria CFU/ml			<i>p</i> value
	Back loop	Prior to machine	Dialysate	
Mean values ± SD	1.816×10 ³ ±2615.3	1.78×10 ² ±222.1	1.835×10 ² ±267.6	0.023

SD: standard deviation, *p* value: ≤ 0.05**Table 2: Bacteria isolated from the four points and their percentage at each point.**

Isolated bacteria	No. (%)				
	Municipal water	Back loop	Prior to machine	Municipal water	Back loop
<i>Pseudomonas</i> sp.	11 (11.2%)	14(14.2%)	16(16.3%)	13(13.2%)	54
<i>Proteus</i> sp.	0	1(1.02%)	0	0	1
<i>Salmonella</i> sp.	1(1.02%)	1(1.02%)	0	2 (2.04%)	4
Non-coagulase					
<i>Staphylococcus</i> sp.	3 (3.06%)	1(1.02%)	2(2.04%)	1(1.02%)	7
<i>S. aureus</i>	0	1(1.02%)	2(2.04%)	1(1.02%)	4
<i>Micrococcus</i> sp.	0	1(1.02%)	0	2(2.04%)	3
<i>Citrobacter</i> sp.	5 (5.10%)	0	2 (2.04%)	0	7
<i>Enterobacter</i> sp.	3(3.06%)	1(1.02%)	0	2(2.04%)	6
<i>E. coli</i>	3(3.06%)	0	0	0	3
<i>Serratia</i> sp.	4(4.08%)	1(1.02%)	2(2.04%)	2(2.04%)	9
Total	30	21	24	23	98
(%)	(30.6%)	(21.4%)	(24.4%)	(23.4%)	
<i>p</i> - value			0.96		

A study conducted by Khan *et al.*, reported that 80% of *Pseudomonas* sp. was resistant against Ceftazidime²⁹.

CONCLUSIONS

Hemodialysis water distribution systems in MKC were colonized with both gram negative and gram positive bacteria, which display multi-resistance to antibiotics. The CFU values for dialysis water and dialysate exceeded the limit of 100 CFU/ml. Therefore, an adequate water-treatment system, the efficient disinfection of haemodialysis equipment and dialyzers, and the microbiological monitoring of water and dialysate are key points in maintaining the quality of the renal replacement therapy service offered to patients with chronic renal disease.

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AUTHOR'S CONTRIBUTION

Al-Haik WM: laboratory works. **Al-Haddad AM:** revision and editing. **Bekeir SO:** writing, review and editing. **Al-Shamahy HA:** supervision, editing. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICTS OF INTEREST

There are no any conflicts of interest.

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