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Evaluation of growth of micro-organisms found in dialysis fluid: A microbiological study

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Abstract

Background: Dialysis is a type of renal replacement therapy wherein the artificial apparatus that eliminates extra water, solutes, & toxins supplements the kidneys' function of filtering the blood. Hence; the present study was conducted for evaluating growth of micro-organisms found in dialysis fluid.

Materials & methods: A total of 234 samples were enrolled. Sample fluid was collected after a fixed periodic interval of six days from Dialyzer machines used for the patients undergoing dialysis in the: National Institute of Medical Sciences and Research Jaipur, Rajasthan. Desirable Sample volumes were collected for both purified water and ultrapure dialysis fluid. All the results were recorded in Microsoft excel sheet and were subjected to statistical analysis using SPSS software.

Results: Out of 234 samples, 208 were dialysate sample while the remaining 26 were RO water samples. Out of 10 samples of dialysate group among which bacteria was identified, commonest bacteria to be identified was E. Coli found to be present in 70 percent of the samples. None of RO samples were positive for bacterial growth. Non-significant results were obtained while comparing the bacterial growth in between dialysate sample and RO samples. Biofilms production was present in 2 dialysate samples while it was absent in RO samples

Conclusion: With the demand for improved fluid quality in dialysis, it is important to establish well defined quality levels and the procedures necessary to reach them. The present standards for water for dialysis and dialysis fluid should be met before aiming for new targets

Key words: Dialysis, Microorganism, Biofilm

Introduction

Dialysis is a contraction of the Greek terms dia, which means "through," as well as lysis, which means "loosening or splitting." It is a type of renal replacement therapy wherein the artificial apparatus that eliminates extra water, solutes, & toxins supplements the kidneys' function of filtering the blood. Acute kidney injury (AKI), which causes a sudden deterioration of renal functioning, or chronic kidney disease (CKD), which causes a slow, steady loss of functioning, both require dialysis to maintain homeostasis.^{1, 2}

According to their prescription, subjects receiving hemodialysis thrice in seven days may be subjected to three hundred to six hundred litres of water. For patients receiving nocturnal therapies, the weekly dialysis fluid requirement rises to five hundred and eighty to eight hundred and sixty litres.^{1, 2}

A dialysis subject who receives treatment three times in seven days and uses one hundred and fifty litres of fluid every session is subjected to twenty-three thousand and four hundred litres of fluid annually. Bacteria can persist in harsh environments and create a biofilm, that assists them withstand biocides as well as other antimicrobial agents. In order to produce biofilms, bacteria adhere to surfaces as well as collect in a biopolymer matrix. Numerous prosthetic gadgets employed in nephrology, and the fluid routes of haemodialysis plants and monitors, have been found to contain biofilms, according to researches on the subject.³⁻⁵

Acinetobacter spp as well as *Pseudomonas aeruginosa* are among the microorganisms that are most frequently isolated, according to prior investigations. These two species are the most common opportunistic pathogens that can infect people receiving dialysis. Therefore, it is crucial to take into account the existence of them as biofilms in dialysis units as well as take steps to avoid their occurrence as biofilms. In order to increase the security of subjects as well as the standard of healthcare, it is crucial to assess infections as well as trends of antibiotic resistance among people receiving dialysis. Only a few papers from India have discussed the biofilms that develop in dialysis equipment and the techniques employed to control them.^{5, 6} Hence; the present study was conducted for evaluating growth of micro-organisms found in dialysis fluid.

Materials & methods

The present study was conducted for evaluating growth of micro-organisms found in dialysis fluid. A total of 234 samples were enrolled. Sample fluid was collected after a fixed periodic interval of six days from Dialyzer machines used for the patients undergoing dialysis in the: National Institute of Medical Sciences and Research Jaipur, Rajasthan. Desirable Sample volumes were collected for both purified water and ultrapure dialysis fluid. Tryptone glucose extract agar media was used for bacterial culture. Isolates of *P. aeruginosa* were cultured in BHI broth for 24 hours at 37°C. . All the results were recorded in Microsoft excel sheet and were subjected to statistical analysis using SPSS software.

Results

The present study was conducted in the department of microbiology of National Institute of Medical Sciences and Research Jaipur, Rajasthan with the aim of evaluating the microbiological purity of fluids used for hemodialysis. A total no. of 234 samples was collected from dialyzer machines. Out of 234 samples, 208 were dialysate sample while the remaining 26 were RO water samples. Out of 10 samples of dialysate group among which bacteria was identified, commonest bacteria to be identified was *E. Coli* found to be present in 70 percent of the samples. None of the RO sample were positive for bacterial growth. Non-significant results were obtained while comparing the bacterial growth in between dialysate sample and RO samples. Biofilms production was present in 2 dialysate samples while it was absent in RO samples.

Table 1: Distribution of samples

Samples	Number	Percentage
Dialysate sample	208	88.88
RO water sample	26	11.12
Total	234	100

Table 2: Identification of bacteria among dialysate sample

Bacteria	Number	Percentage
<i>Pseudomonas aeruginosa</i>	1	10
<i>E. Coli</i>	7	70
<i>Staphylococcus sp.</i>	2	20
Total	10	100

Table 3: Identification of bacteria among RO samples

Bacteria	Number	Percentage
Present	0	0
Absent	26	100

Graph 1: Comparison of bacterial growth in dialysate sample and RO sample

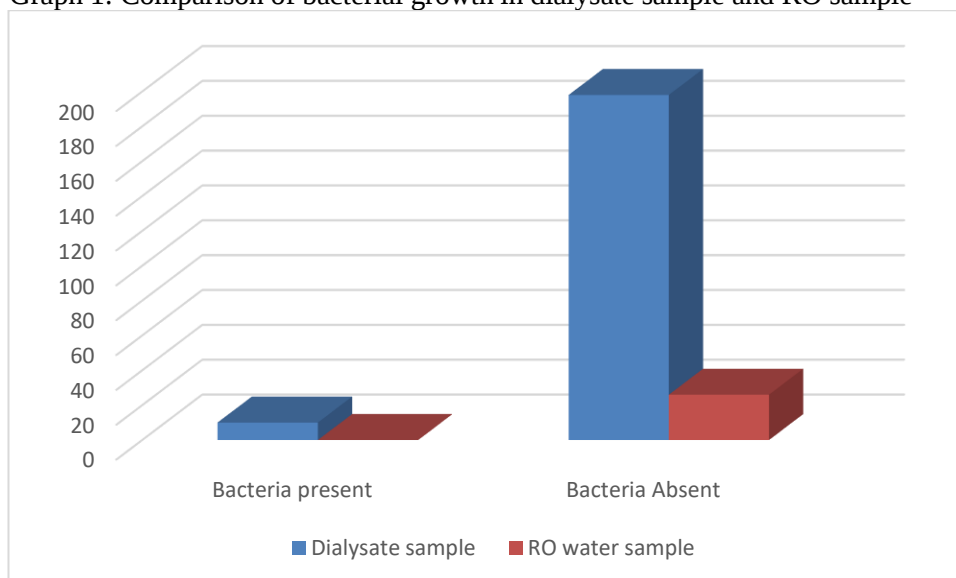


Table 4: Comparison of biofilm production

Biofilm production	Dialysate samples	RO samples
Number	2	0
Percentage	0.96	0

Discussion

The development of a biofilm is a very effective method which helps bacteria to survive in hostile conditions and to resist biocides and antimicrobial substances. Bacteria attach to surfaces and they aggregate in a biopolymer matrix to form biofilms. Studies on biofilms have shown their presence in many prosthetic devices which are used in nephrology as well as in the fluid pathways of haemodialysis plants and monitors. The biofilm formations in dialysis systems may be relevant, because they continuously release bacterial compounds and are resistant to disinfection. Once it is present, this community of bacteria increases the resistance to biocides due to slime production and, as a result, the chemical products for dialysis monitor disinfection and descaling

procedures do not result in an effective treatment. A germ- and endotoxin-free dialysate does not exclude the risks and hazards of bacteria and an endotoxin discharge from the biofilms, which may have developed on the fluid pathway tubing, may act as a reservoir for a continuous contamination. Efforts in the optimization of the cleaning and disinfection procedures which are used for haemodialysis systems should aim at detaching and neutralizing biofilms whenever necessary. The composition of the dialysis fluid is crucial in the normalization of the electrolyte composition of plasma water, homeostasis and the acid-base balance, and it should be individualized to the patient's requirements in the same way as the blood and dialysate flow rates are individualized, to ensure an optimal comfort and minimal complications which are associated with the procedure.⁷⁻⁹

The present study was conducted in the department of microbiology of National Institute of Medical Sciences and Research Jaipur, Rajasthan with the aim of evaluating the microbiological purity of fluids used for hemodialysis. A total no. of 234 samples was collected from dialyzer machines. Out of 234 samples, 208 were dialysate sample while the remaining 26 were RO water samples. Out of 10 samples of dialysate group among which bacteria was identified, commonest bacteria to be identified was *E. Coli* found to be present in 70 percent of the samples. None of the RO samples were positive for bacterial growth, *Pseudomonas* sp. was present. Non-significant results were obtained while comparing the bacterial growth in between dialysate sample and RO samples. Sornaranjani M et al 2022 determined the microbiological profile of peritoneal dialysis fluid in patients with acute and chronic renal failure and identify these organisms' susceptibility patterns. A total of 100 patients who were >18 years of age, acute and chronic renal failure patients who underwent Peritoneal Dialysis and patients on Continuous and Intermittent Peritoneal Dialysis were included. The study population included 100 patients who satisfied the inclusion criteria. 63% were males, and 37% were females. The patients had a mean age of 44.15 ± 13.89 . 28 samples were culture positive, out of which 13 (46.4%) were Gram-negative, 10 (35.7%) were Gram-positive and 5 (17.9%) were Fungal isolates. Among them, the majority were *Acinetobacterbaumannii* (20%) and *Candida non-albicans* (20%), followed by *Staphylococcus aureus* (16%), Coagulase-negative *Staphylococcus* (16%), *Klebsiellaoxytoca* (8%), *Klebsiellapneumoniae* (4%), *Enterococcus faecalis* (4%).¹⁰ Anversa, L et al in 2022 investigated the occurrence of fungi in dialysis water and dialysate, in addition to evaluating the susceptibility to antifungals and the biofilm production capacity of isolated microorganisms. The samples were collected in three hemodialysis units in Bauru (Brazil), every 15 days at post-reverse osmosis, reuse, and dialysate points. The fungi were isolated by spread plate on Sabouraud dextrose agar. Filamentous fungi were phenotypically identified and yeasts were subjected to molecular evaluation of the ITS region. Susceptibility test to antifungals was carried out by the broth microdilution method and biofilm production capacity was evaluated in microtiter plates using crystal violet staining. Fungi were isolated in 52/216 (24.1%) samples, with an average count of 16.3 (10-40) CFU/mL. Overall, 61 microorganisms were identified, with 54 (88.5%) filamentous fungi and 7 (11.5%) yeasts. The main genera included were *Penicillium*, *Cladosporium*, *Scedosporium*, *Rhinoctadiella*, *Fusarium*, and *Emmonsia*. Most isolates showed high values of minimum inhibitory concentration for 5-flucytosine and fluconazole and 35/45 (77.8%) isolates were classified as strong producers of biofilm.¹¹

In the present study, Biofilms production was present in 2 dialysate samples while it was absent in RO samples. Chaoui L et al 2022 assessed the degree of contamination of HD water by bacteria at the HD center of Mohammedia, Morocco, in addition to evaluating the antimicrobial resistance of isolated bacteria. Fifty four water samples were taken, the appropriate cultures were used to isolate the pathogenic bacteria, which were identified biochemically and molecularly by 16S RNA sequencing. The isolated bacteria that colonized the HD systems were mostly Gram-negative bacilli, such as *Stenotrophomonasmaltophilia*, *Pseudomonas* spp., and *Burkholderiacepacia*. Results of

the antibiotics test showed remarkable resistance levels. Among *Pseudomonas* spp. and *S. maltophilia*, 10 strains were classified as multidrug-resistant (MDR), and 4 as extensively drug-resistant (XDR). The diversity of bacterial strains isolated in the water used for HD treatments, and their worrying resistance levels pose a significant risk to patients.¹²

Conclusion

With the demand for improved fluid quality in dialysis, it is important to establish well defined quality levels and the procedures necessary to reach them. The present standards for water for dialysis and dialysis fluid should be met before aiming for new targets. The quality levels should be achieved in sequence because high levels are based on conditions required to reach a lower level. It may be realistic to aim for a stepwise improvement of the dialysis fluid quality from its present status to ultrapure fluid.

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