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Original Article

Comparison of Reasoner's 2A Agar and Muller Hinton Agar Media for Microbiological Monitoring of Dialysis Water

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Abstract

Background and Aim: Microbiological culture of dialysis water is a routine safety measure. In, Khorramabad laboratories perform these cultures on Muller Hinton Agar (MHA) at 35–378C for 48 h, not on the Reasoner's 2A agar (R2A agar) at 17–238ºC for 7 days recommended by international standards, the objective of the present study was the comparison of the efficiency of R2A and MHA media in the counting of heterotrophic bacteria in the samples of water collected in dialysis centers from 2 hospitals in Khorramabad, from September to November 2019.

Methods: A total of 165 samples of treated water in dialysis centers were collected aseptically and then transported in ice-packs to the Department of Medical Microbiology of the Lorestan University of Medical Sciences and the pour plate technique was carried out for the enumerating of heterotrophic bacteria. Finally, bacterial colonies were counted after incubation at 34±2ºC for 48 hours on MHA and 25ºC for 1 week on R2A.

Results: Results showed heterotrophic bacterial counts in R2A were greater than those in MHA in 89% of the samples, so enumeration of heterotrophic bacteria should be carried out in R2A agar associated with longer incubation times, because of the greater sensitivity. The proportion of water samples yielding colony counts ≥200 CFU/mL by R2A -7d was significantly different from the proportion by MHA-48h ($p<0.001$).

Conclusion: The results proposed using R2A agar combined with relative low culture temperature (20-25°C), and an extended incubation time (7-10 days) is more efficient. However, as the spectrum of bacterial contamination is not similar for dialysis centers and countries, many studies using different media and culture parameters are required to confirm this.

Keywords: Dialysis Water; Heterotrophic Bacteria; Pour Plate; Reasoner's 2A Agar; R2A agar.

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Introduction

Patients receiving hemodialysis are exposed to great content, of dialysis fluid approximately 120 liters in a single dialysis treatment (1). The presence of a nonselective semipermeable membrane, which operates as a barrier between blood and dialysis fluid, provides a direct path for the transformation of contaminants into the blood flow (2). A series of purification procedures such as deionization, carbon filtration, and reverse osmosis (RO) is commonly used to remove chemical pollutants from water used in hemodialysis. These processes are also an effective barrier against microbiological contaminants (3). Also, the tubing system (hydraulic circuit) of the dialysis machines could promote bacterial growth and biofilm formation. Biofilm acts as a source of bacterial fragments such as DNA and endotoxin which are delivered into the water and potentially able to permeate dialysis membranes(4). To prevent patients from hazards of water contaminants several standards for the quality of dialysis water and fluid have been suggested (2). The association for the advancement of medical instrumentation (AAMI) standards has represented

the most perfect standards for the chemical and microbial quality of dialysis water (5).

The intact membrane of the dialysis machine should prevent the contamination of the blood with bacteria from the dialysis fluid. However, infections may still occur when membrane integrity is compromised, when microbial contamination of the water is high or when contamination occurs in the utilization of the dialysis machines (6-8).

Classical approaches for the enumeration of microorganisms in water include plate counts, membrane filtration, and the most probable number technique (9). As occurs in any microbiological technique, results of microorganism enumeration are influenced by culture media used, as well as by the incubation conditions (10, 11).

There are two basic culture media used in microbiological analyses: complex media with high nutritional content such as Trypticase Soy Agar (TSA) and Muller Hinton Agar (MHA). These media are shown for the isolation and enumeration of heterotrophic bacteria isolated from animals and humans. There are also simple media with few nutrients, such as Reasoner's 2A Agar (R2A agar), used in the detection of oligotrophic bacteria, slow-growing, and in the enumeration of heterotrophic bacteria conformed to aquatic environments and that require the low concentration of nutrients (10, 12, 13). Techniques that use simple culture media, conformed with longer incubation periods (5 to 7 days) at lower temperatures (20 to 28°C) are more sensitive in determining microbial contamination of water for human utilization and of water for dialysis (14-16). However, the use of more complex culture media may improve the improvement of microorganisms when longer incubation times and lower temperatures are used. The last editions of the United States Pharmacopeia (12) and the European Pharmacopoeia (17) recommend the use of R2A medium for the enumeration of heterotrophic bacteria in treated water, incubated between 20 and 25°C for 4 to 7 days, or between 30 and 35°C from 3 to 4 days.

Considering the importance and the need to evaluate the microbiological quality of water used in dialysis centers, the objective of the present study was to compare the efficiency of R2A and MHA media in the enumeration of heterotrophic bacteria in treated water collected from dialysis centers.

Methods

A total of 165 samples of treated water were collected in the dialysis center of two hospitals in Khorramabad city, the west of Iran. The study period was from September to November 2019.

Samples were collected aseptically after allowing the water to flow for two or three minutes, according to the recommendations of the American Public Health Association (APHA) (14). 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 Lorestan University of Medical Sciences and the interval between collection of the samples and the beginning of the analyses was no more than six hours. This was repeated at one monthly interval over the three months.

The pour plate technique was carried out according to the recommendations of official compendia for the enumerating of heterotrophic bacteria (12). Aliquots of 1 mL of the samples were transferred in quadruplicates to the center of sterile Petri dishes, and 20 mL of R2A, molten and cooled to 45ºC, were placed in one of the dishes; 20 mL of MHA, also molten and cooled to 45ºC, were poured in the one other plate. Plates were homogenized and, after the medium was solidified, they were incubated in an inverted position at respectively. Finally, bacterial colonies were counted after incubation 34 ± 2ºC for 48 hours on MHA and 25ºC for 1 week on R2A.

Statistical analysis

The following comparisons were made of the proportions of samples yielding bacterial colony counts≥200 CFU/mL, MHA-48h versus R2A-1W; we used chi-squared and the T-test to determine a significant difference between the means of two microbiological culture media. Statistical analyses were carried out using the

software SPSS 19 for Windows with a 95% confidence interval.

Results

Means of the heterotrophic bacteria/mL counts obtained with each of the culture media were calculated for the 165 samples (Figure 1).

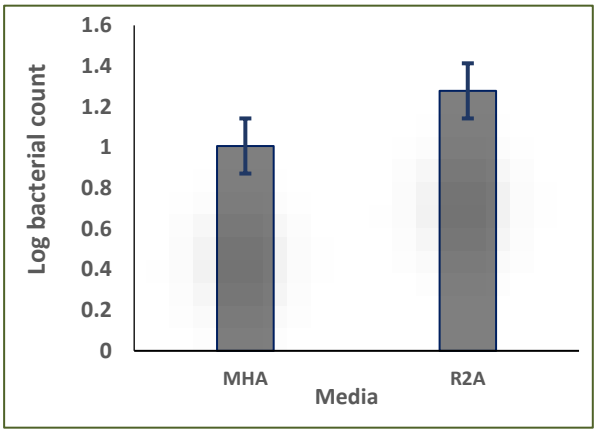

Figure 1. The average number of bacteria counted in the two different culture media

In the MHA, the number of positive samples was 43(26.2%) and in the R2A, the number of positive samples was 16(9.8%), which according to the chi-square test, this difference was statistically significant (p< 0.001) (Figure 2).

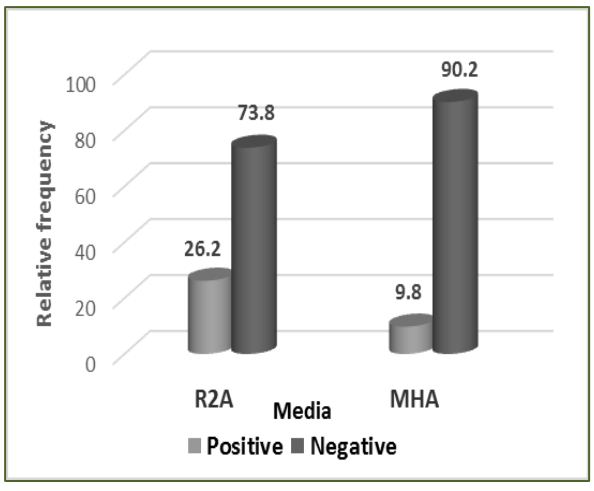

Figure 2. Relative frequency distribution of bacterial count results in the studied samples by type of culture medium.

In the samples of Shohaday Ashayer Hospital, the number of positive samples was 38 (23.2%) and in the samples of Shahid Rahimi hospital, the number of positive samples was 5 (3%), which according to the chi-square test, this difference was statistically significant (p< 0.001) (Figure 3).

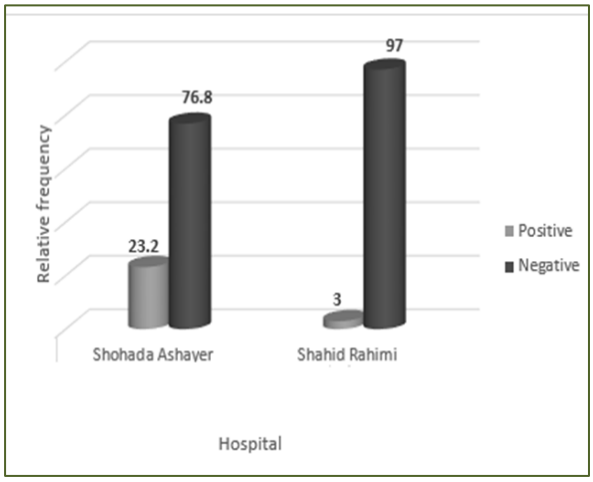

Figure 3. Relative frequency of bacterial count distribution test results in the studied samples by hospital type.

Discussion

As a result of the increasing prevalence of chronic kidney failure and the persuade need for renal replacement therapy in acute kidney failure, there has been an increase in the request 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 (18). 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 (19).

Over the last decade, several studies aimed at evaluating the quality of dialysis water, especially microbial quality, have been performed in developed countries (3, 5).

Microorganisms that grow in extreme environments show better results in laboratory culture when they are incubated in conditions that simulate these environments. Because of this, bacteria associated with water for dialysis grow better in low nutrient culture media, such as R2A, when incubated for more than 48 hours and at temperatures around 25ºC (20).

There are several national and regional guidelines 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 (21) while the European pharmacopeia limit is set at 100 CFU/mL and endotoxin concentration of <0.25 IU/mL (22).

Besides comparing the efficiency in the enumeration, this study also evaluated the impact of the culture media in the evaluation of the quality of the samples, considering the maximum threshold of 200 CFU/mL determined by AAMI recommends. In the 165 samples, bacterial counts of over 200 CFU/mL were obtained in 26.2% of them using R2A, and in 9.8% of them using MHA. These data suggest that MHA media underestimated bacterial contamination of the samples and may erroneously indicate that these samples complied with the microbiological standards determined by the official regulations. R2A, a low-nutrient culture medium, showed better results than MHA in the evaluation of bacterial contamination in water for dialysis when incubated at around 25ºC for 1 week (23, 24).

Also, the results of this study showed that the number of positive samples was 38 (23.2%) in the samples of Shohaday Ashayer Hospital, and the number of positive samples was 5 (3%) in the samples of Shahid Rahimi hospital. Similar to other studies, the degree of contamination of dialysis centers has different results depending on the method of disinfection (25, 26). Due to the high rate of contamination in the Shohaday Ashayer Hospital is necessary to perform regular disinfection for the water treatment units in this dialysis center. Enumeration of heterotrophic bacteria in dialysis water should be carried out in R2A associated with longer incubation times, to minimize the risks to the patient under dialysis, because of the greater sensitivity of this culture medium.

Conclusion

Water quality is a major determinant of morbidity and mortality in patients with hemodialysis conventionally. Therefore, dialysis water must be monitored routinely and constant and vigorous control of the hemodialysis water treatment system is essential to improve outcome. We recommend using R2A agars combined with a low culture temperature, and an extended incubation time.

Conflict of Interest

The authors declared that they have no conflict of interest.

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Ethics

The Ethics Committee of Lorestan University of Medical Sciences (Lorestan, Iran) approved the project.

Ethics Committee Cod: IR.LUMS.REC.1397.110

URL:

https://ethics.research.ac.ir/EthicsProposalView.php?id=32526).

Authors' Contributions

Somayeh Delfani and Babak Hadian participated in study design and data collection and evaluation.

Iman Pouladi and Setareh Soroush contributed to all experimental.

Khatereh Anbari contributed to data and statistical analysis, and interpretation of data.

Faranak Rezaei drafted the manuscript, supervised the study, and contributed to the study design and edition of the manuscript.

All authors performed editing and approving the final version of this paper for submission. All authors read and approved the final manuscript**.**

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