

Occurrence of fungi in dialysis water system and dialysis solution in dialysis centers in Ahvaz, Iran.

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ABSTRACT

Objectives: The aims of the present study were to examine the water and dialysate for determining the diversity, occurrence, and distribution of fungi in hemodialysis centers of Ahvaz Jundishapur University of Medical Sciences.

Methodology: One hundred and sixty eight water samples from dialysis machines, and dialysis solution were collected. The samples were cultured on Sabouraud's dextrose agar and incubated at room temperature.

Results: Our study demonstrated that various fungi were present in the water system in haemodialysis centers. Totally, eight genera of filamentous fungi and three genera of yeasts and yeast-like were identified. The most common filamentous fungi were *Fusarium*, *Penicillium* and *Aspergillus*, whereas, *Candida tropicalis* was the most frequently isolated yeast.

Conclusion: The water supply for hemodialysis units could be contaminated with several fungal species that occur during piping into kidney machine. In addition, contamination with fungi due to contact with plastic materials in kidney machine pipes is more considerable. For minimizing the exposure of patients suffering from chronic renal insufficiency to contaminated water sources, water distribution systems should also be monitored for mycological contamination. In addition dialysate solutions should also be kept for preventing fungal contaminations.

KEY WORDS: Hemodialysis, Water, Fungal contamination, Dialysis solution.

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INTRODUCTION

Water usually is used in dialysis centers largely during a day. Reverse osmosis units produce water which is acceptable in chemical quality. However, the microbiological quality of water is an important aspect for safe and effective hemodialysis. Each patient is exposed to 18000-36000 liter of water during dialysis per year.¹ High levels of microbial contamination can be found in dialysis equipment and affect patients. Several saprophytic fungi are natural inhabitants of soil and water; they are found as rare pathogens in a normal host. Hemodialysis patients are susceptible to such pathogens because of their immune system is significantly decreased and infections are frequent cause of death in patients.²

Several investigators believe that hospital water can be a potential source of fungal pathogens.³⁻⁷

Fungi can grow more rapidly in the treated water as well as culture media and prevent their viability for several months.³ Reverse osmosis parts, carbon filters, dead spaces, tanks and taps are ideal place for microorganisms growth. Tanks and tubing are suitable sites for formation of biofilms.^{2,3,8} Usually water is distributed in PVC pipes throughout the entire water system. As a result during such procedure, water transports microbial material to dialysis machines. In addition, dialysate fluids are used for dialysis which still remains non-sterile and could be contaminated by several fungi.³ Routine microbial checks are usually performed at hemodialysis centers however, microbial analysis is not included. Several investigators believe the microbial growth in dialysis fluids results in the presence of endotoxins.^{9,10} Several saprophytic fungi have been isolated from water in hemodialysis centers, including *Trichoderma*, *Cladosporium*, *Aspergillus*, *Fusarium*, *Penicillium*, *Verticillium*, *Chrysosporium*, *Acremonium*, and *Candida parapsilosis*.^{2,3,7}

The aims of the present study were to examine the water and dialysate for determining the diversity, occurrence, and distribution of fungi in hemodialysis centers of Ahvaz Jundishapur University of Medical Sciences.

METHODOLOGY

The present study was conducted during one month (2009) in two hemodialysis centers in Imam Khomeini and Golestan hospitals of Ahvaz Jundishapur University of Medical Sciences. One hundred and fifty samples of water were collected during dialysis treatment, at four points in the water system. These points included tap receiving water, treated water in storage tank, kidney machines (inlet lines to dialysis machines and outlet port of the dialysis machines). In

Table-I: The frequency of positive samples.

Sample type	Samples	Contaminated	
	No.	No.	%
Outlet water	53	36	67.9
Inlet water	53	4	7.6
Feed water	5	0	0
Storage water	4	0	0
Dialysate	53	6	11.3
Total	168	46	27.4

addition, dialysate solutions for each machine were sampled (53 samples) (Table-I). Samples (at least 10 ml) were collected in the sterile containers and then sent directly to the medical mycology laboratory at Jundishapur University of Medical Sciences. 25µl of each sample was cultured on Sabouraud's dextrose agar, SDA (Merck, Germany), and incubated at ambient temperature for one week aerobically.

All fungal isolates were counted and each isolate subcultured on fresh SDA for the isolation of a pure, single colony for identification. Macroscopic and microscopic morphology (slide culture technique) were used for the identification of filamentous fungi. In addition, germ tube formation, microscopic morphology on Cornmeal agar (Difco, UK) plate with Tween 80, and morphology on CHROMagar Candida (CHROMagar Candida Company, Paris, France) were applied for identification of yeasts and yeast likes.

RESULTS

A total of 168 samples, from the two haemodialysis centers (Imam and Golestan centers) in Ahvaz, Iran were collected during summer 2009. Imam and Golestan haemodialysis centers were equipped with 20 and 10 artificial kidney machines respectively and they had been in use for several years. Both haemodialysis centers use water from a public supply originating from river water. One hundred and fifty water samples collected from different points water distribution system yielded 40 fungal isolates.

Table-II: Fungal species found in samples.

Organisms	No	%
Yeasts	24	41.4
<i>Fusarium</i>	14	24.1
<i>Penicillium</i>	8	13.8
<i>Rhizopus</i>	3	5.2
<i>A. Terreus</i>	2	3.4
<i>Paecilomyces</i>	2	3.4
<i>Alternaria</i>	1	1.7
<i>Acremonium</i>	1	1.7
<i>A. flavus</i>	1	1.7
<i>A. niger</i>	1	1.7
<i>Cladosporium</i>	1.	1.7
Total	58	100

In addition 6 of 53 (11.3%) dialysate samples were positive for fungal isolates (Table-I).

None of the 46 samples (feed and storage water) produced any fungal growth. Filamentous fungi and yeasts (yeast likes) were isolated from 24(41.4%) and 34(58.6%) of samples respectively. Multiple fungal growths were detected in 28.3% of samples (12, 26.1% two organisms, one, 2.2% three organisms) and 33, 71.7% was yielded one organism. Among the filamentous fungal isolates, *Fusarium* (14, 24.1%) was the most frequent species followed by *Penicillium* (8, 13.8%) (Table-II). In the present study 24 yeasts and yeast likes were also identified. *C. tropicalis* was the most common yeast (7, 29.2%) followed by *C. cruzei* (5, 20.8%), *Saccharomyces* species (3, 12.5%), *Trichosporon* species (3, 12.5%), *C. glabrata* (1, 4.2%), *C. guilliermondii* (1, 4.2%), and *Candida* species (4, 16.6%).

Details of the levels of fungal contamination in samples are shown in Table-III. The highest contamination level (>100000 cfu/ml) was observed in 4.3% of samples, whereas 37% of samples had the range of 1-99 cfu/ml (Table-III).

DISCUSSION

Water system in a dialysis center is large. There are several square meters of surface of reverse osmosis membranes, distribution piping, storage tanks and several meters of inlet lines. In addition outlets lines from each kidney machine should be calculated. All these equipments must be kept in good condition, with no microorganism growing. As a result the monitoring of microorganisms in water system used for hemodialysis is extremely important. Several reports have showed that there are risks associated with dialysis water contamination.^{11,12} Patients suffering from chronic renal insufficiency are more susceptible to fungal diseases.

In the present study only 4 of 53 samples collected at the inlet water were contaminated with fungi and presented low fungal counts (1-99 cfu/ml). It is demonstrated that water treated by reverse osmosis is free from fungal elements and it is in agreement with the results of other researchers.² Outlet water from kidney machine usually consist of solutions of salts, glucose during dialysis procedure.² In addition, fluid from dialysis machine is enriched by addition of nitrogen and carbon-containing waste compounds from the patient's blood that provide suitable medium for rapid growth of fungi.² The high levels of fungal contamination in such samples indicate that these fungi could grow well in outlet water. In our study, fungi were recovered from 67.9% of outlet water samples of the

Table-III: Yeast and mould counts in various samples.

Organisms	Contaminated	
	No	%
1-99	17	37.0%
100-999	11	23.9%
1000-9999	11	23.9%
10000-99999	5	10.9%
>100000	2	4.3%
Total	46	100%

haemodialysis units. This percentage is lower than that reported by Arvanitidou et al⁸ (76.2%) and Pires Gonsalves et al² (90%) who reported from haemodialysis units in Greece and Brazil, respectively.

Hemodialysis patients are usually exposed to a lot of water during hemodialysis process. High concentrations of fungi in water can pose risks of fungemia or endotoxemia. In addition the presence of high concentration of fungal elements in outlet water is important for contamination in kidney machine. Our study showed that the fungi most frequently associated with outlet water (followed by dialysis solution and inlet water) were *Fusarium* and *Candida*. Previous studies have shown that the predominant fungi isolated from dialysis water were *Fusarium*², *Cladosporium*,^{2,3} *Aspergillus* species and *Penicillium*³ and *Trichoderma*.⁷ The results suggest that the water supply for hemodialysis units could be contaminated with fungal species during piping into kidney machine. For minimizing the exposure of patients suffering from chronic renal insufficiency to contaminated water sources, water distribution systems should also be monitored for mycological contamination. In addition dialysate solutions were also kept for preventing fungal contaminations.

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