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Survey of fungal flora in the old and new water distribution systems in Aradan, Iran

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Original Article

Abstract

One of the great concerns of potable water consumers regarding its quality is the pollution of potable water with pathogenic microorganisms. Fungi in drinking water are responsible for changing the taste and odor of water. Health problems are possible, originating from mycotoxins, animal pathogens and allergies. Water samples were collected between December 2014 and June 2015 from different sites of municipal water supply system of Aradan, Iran. In this descriptive study, a volume of 100 ml of tap drinking water samples (n = 60) were collected in sterile bottles. All water samples passed through sterile 0.45 micrometer filters. The filters were placed directly on Sabouraud dextrose agar and incubated at 20°C for 4-7 days. Routine mycological techniques were applied to identify the grown fungi. Three dominant fungi genera in water were Cladosporium, Aspergillus and Penicillium, the concentrations [Colony forming unit (CFU)/100 ml] of which were higher in old and new water distribution system. Among Aspergillus species, A. flavus had the highest frequency; from 555 fungal colonies, 11 different fungal genera were identified. Various fungi were present in the tap water. Present study showed that average concentration of fungi increased in old water distribution.

KEYWORDS: Drinking Water, Fungi, Distribution Age, Aradan

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Introduction

One of the great concerns of potable water consumers regarding its quality is the pollution of potable water with pathogenic microorganisms. Microorganisms like different kinds of bacteria, viruses and parasites are known potable water pollutants which can lead to the epidemics caused by water in several ways.[1] Many potable water services rely exclusively on microbial monitoring like all coliforms and E. coli in order to get insured of potable water.[2] In recent years, viruses and parasites have been accepted more or less as qualitative indicatives of potable water.[3] Fungi are an ever-present and various collection of eukaryotic organisms; fungi are microorganisms with high dispersion in the nature[4] and can be found in almost all parts of the environment and grow largely in the water.[5] They were rarely considered as pathogenic microorganisms of water.[1] There have been recently more studies about fungi in waters and the fungi and their metabolites have been considered as dangerous potable water pollutants, therefore it is very important to study fungi in potable water.[5] Fungal diseases are among the most common infections in human. The presence of fungi in

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potable water is associated with generation of smell and taste in water.[6] Filamentous fungi in water may oxidize distribution system pipes[4] and lead to sanitary problems caused by mycotoxins and allergies.[5,6] The presence of hypothetically pathogenic types, such as A. fumigatus in tap water has led to speculations whether hospital water distributions may serve as a transmission route for fungal infections. Some studies have centralized on analyzing hospital water systems with respect to the presence of fungi.[7-12]

The exceptions are some precautionary recommendations related to high-risk patients in hospitals, as previously mentioned. Mycotoxins are poisonous metabolites generated by some groups of fungi which are often related to foodstuffs and the mold smell in Paris potable water distribution systems has been attributed to fungi. In addition, they generate their own battery of compound with characteristic odors and tastes.[6] The potential public health impact is deteriorating water quality in distribution system piping networks with time. According to these, presence of fungi in potable water can damage its quality and will threaten human health; therefore, this study aimed to survey and compare the amount of fungal flora in potable water distribution system in Aradan, Iran, in two new and old distribution system.

Materials and Methods

In this descriptive-sectional study, 60 samples containing 200 ml potable water were collected from pipelines of 6 parts of Aradan during two seasons of winter and spring in 2015 (each season 30 samples). Sampling was performed in different places of six spots (60 times); beginning, middle and end of the drinking water distribution network of the city three of which were from old distribution networks and the other three were from new distribution networks. Before sampling, discharging valve was disinfected, and then the valve was left open. Then 200 ml water samples were gathered in polystyrene sterile bottles along with some drops of one percent sodium thiosulfate solution (120 mg/l sodium thiosulfate) to neutralize remaining chlorine of water.[2-4] In each time of sampling, pH and free residual chlorine were measured in situ. To evaluate pH, a pH meter kit and reagent phenol red was used, and to evaluate the free residual chlorine, chlorine photometer kit and N,N-Diethyl-p-phenylenediamine (DPD) reagents were used.

In order to remove the fungi, 100 ml of water was filtered with sterile 0.4 micrometer filters. The filters were placed directly in Sabouraud dextrose agar environment under full sterile conditions, then plates containing filters were incubated in 27°C for 4 days. During this time the fungi growth in plates was controlled every day and the number of developed colonies was recorded and reported according to colony forming unit (CFU) per 100 ml water.[2-4]

Fungi breed and species (if required) were identified through standard methods of mycology based on slide-cultivation and by apparent structure and developed colony microscopy. Statistical analysis was done by SPSS software (version 17.0, SPSS, Inc., Chicago, IL, USA). Analysis of variance (ANOVA) with Tukey's post-hoc test for pairwise comparisons were used to compare means in different stations. The one sample Kolmogorov–Smirnov test was employed to evaluate the normality of the data. Kruskal–Wallis test was used for the nonparametric equivalence of one-way analysis of variance if the normality assumption was redundant. The Mann–Whitney U test was employed for assessing significant difference of the bacterial concentrations in two seasons, old and new distribution networks. P-values less than 0.05 were considered significant.

Results and Discussion

Fungi density and diversity by season:

Abundance and density of fungi in winter and spring were high in Cladosporium spp, Penicillium spp and Aspergillus spp. Aspergilluses found in this study were

Aspergillus niger, Aspergeillus flavus and Aspergillus fumigatus. The most abundant and dense of all was Aspergillus flavus. Fungi were more diverse in spring than in winter (Figure 1).

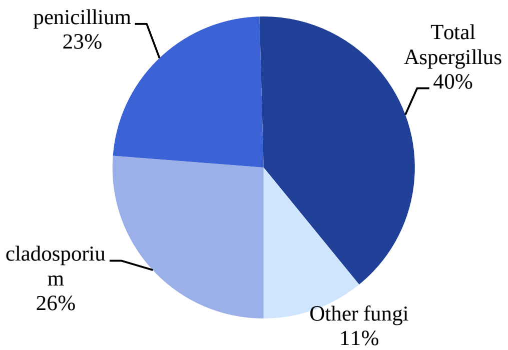

Figure 1. Percentage of fungi isolated in winter seasons sampling water distribution

It should be noted that we could identify other fungi in spring such as: Fusarium spp, Alternaria spp, Monilia spp, Mucor spp, Rhizopus spp, Drechselera spp, Mycelium and yeasts (Figure 2).

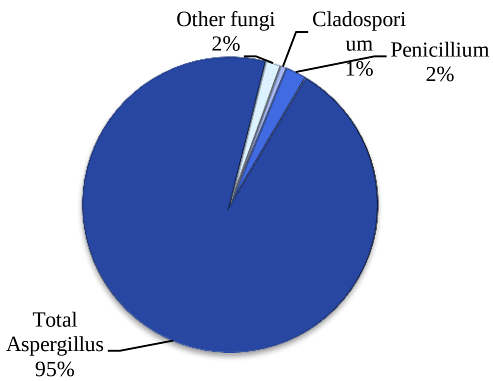

Figure 2. Percentage of fungi isolated in spring seasons sampling water distribution

According to table 1, the amount and diversity of bladed fungi such as Aspergillus spp were higher in spring than winter (P_v = 0.031). The average density of colorful fungi like Cladosporium spp, Alternaria spp and bladed fungi like Penicillium spp were higher in winter than in spring and there was a significant difference in their density (P = 0.003 and P = 0.014). There was generally a significant difference between the amount and diversity of fungi in winter and spring and their quantity was higher in spring (14.79 CFU/100 ml) than in winter (10.28 CFU/100 ml) (P = 0.040).

The total fungi concentrations in stations at different distribution networks are shown in figure 3. Among all the stations, the highest concentration (16.44 CFU/100 ml) was observed at the end of the old water distribution network, while the lowest concentration (9.88 CFU/100 ml) was observed at the end of the new distribution network. The reason could be increase in biofilm concentration in parts of the system that were in end points or blinded area of the network.

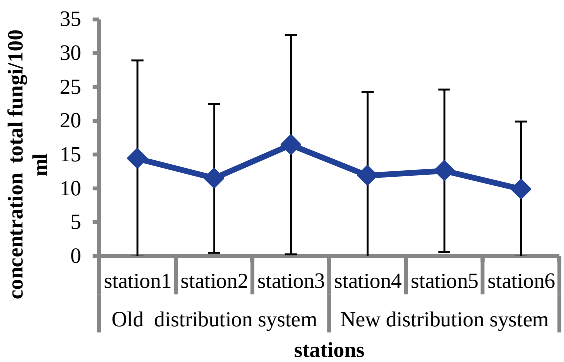

Figure 3. The average and standard deviation concentration of fungi (CFU/100 ml), in water of two distribution networks
CFU: Colony forming unit

Table 1. Average concentration of fungi (number /100ml) water distribution, measured in two seasons

Season	Fungi				
	Cladosporium spp	Penicillium spp	Total Aspergillus spp	Other fungi species	Total fungi
Winter	2.7 ± 7.14	2.40 ± 4.6	4.07 ± 7.64	1.12 ± 3.10	10.28 ± 14.01
Spring	0.1 ± 0.21	0.035 ± 0.9	14.40 ± 18.21	0.25 ± 0.86	14.79 ± 16.84
Differences between concentration of fungi	P = 0.003	P = 0.014	P = 0.031	P > 0.050	P = 0.040

Of all the samples collected during both winter and spring, 98.9% had fungi which showed that the water was polluted by fungal spores. In the old distribution system, we observed fungal development in all collected samples. According to previous studies conducted in Iran, the amount of positive samples in Sari was 93.3% of all measured samples. Some general results obtained from previous studies are: the recovery of fungi varied between 7.5%–89% positive samples, and that the levels of fungi in the samples varied significantly in several studies. Fungi have been recovered from all types of water, from raw water to treated water, and from heavily contaminated water to distilled or ultra-pure water. Fungi have also been conveyed from bottled drinking water.[13-15]

Prevalent fungi in this study were Cladosporium spp, Aspergillus spp and Penicillium spp. which was similar to other studies conducted in Iran and other countries.[3,4,16,17] Other fungi identified in this study were Fusarium, Alternaria, Monilia, Mucor, Rhizopus, Drechselera, Mycelium and yeasts. Some of these fungi were separated from samples in similar studies.[18-19] The average of all fungi in spring was higher than the average in winter. The prevalent fungi in both seasons were almost the same; however, their amount in spring was higher than winter. In winter Cladosporiums spp and Penicilliums spp were higher than spring. In a similar study, the amount of Acremonium was higher in winter.[6] Acremonium spp is one of the colorful fungi which is in the same family with Cladosporium spp. But in spring, the amount of Aspergillius was abundant. One reason for the development of this type of fungi is increase in environmental temperature which is the favorable condition for Aspergillius. There was also a significant difference between the amount and diversity of fungi in spring in comparison with winter perhaps due to the favorable weather and that the plants start to grow. Since the water source for city distribution system is surface water (river), the temperature of this source is influenced by the environment and its temperature. This may increase the amount and diversity of fungi. Also, according to a similar study, the amount of fungi in surface waters was higher in spring and autumn than other seasons.[6] The amount of fungi increases with the increase in distribution system age, perhaps because of high growth of biofilm in distribution system or water leakage from the system.

Density and diversity of fungi based on water distribution system age:

According to figures 4 and 5, in old and new distribution systems, the highest isolated fungi were Cladosporium spp (18% and 14%), Aspergilluses spp (65% and 69%), Penicillium spp (13% and 13%) and other fungi (4% and 4%).

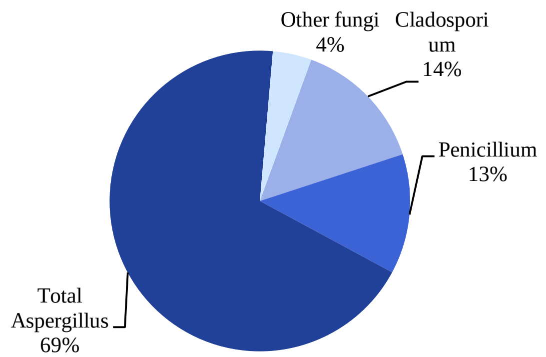

Figure 4. Percentage of fungi isolated in old water distribution system

The amount and diversity of Cladosporium spp, Penicillium spp and all Aspergilluses spp were higher in the old distribution system, and there was a significant difference in the density of these fungi in the old distribution system compared to the new one (P = 0.04 and P = 0.042, P = 0.038) (Table 2).

Since water is an influencing factor on the community health, measuring the amount of different kinds of microorganisms in water has an important role in identifying the origin and the reason of its pollutions. The studies indicated that different factors influenced the amount of fungi in water, such as the type of water source, chlorine and season.[2,6]

Table 2. Average concentration of fungi (number/100ml), measured in old and new water distribution System

Distribution	Fungi				
	Cladosporium spp	Penicillium spp	Total Aspergillus spp	Other fungi species	Total fungi
New water distribution system	1.9 ± 5.60	1.70 ± 2.78	9.03 ± 17.90	0.55 ± 1.87	11.46 ± 15.20
Old water distribution system	2.7 ± 1.84	2.03 ± 3.25	10.06 ± 18.24	0.66 ± 2.58	14.12 ± 22.10

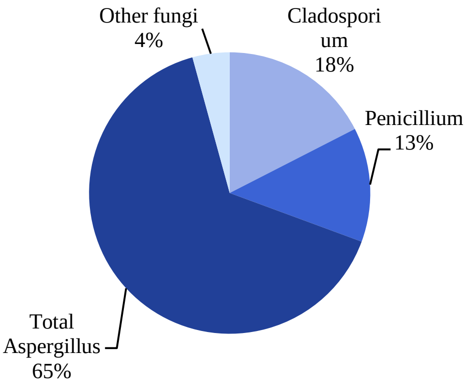

Figure 5. Percentage of fungi isolated in new water distribution system

Fungi have the capacity to grow on the substrate, forming part of microbial biofilms on tube surfaces, remains, or sediments. They are likely to become established where there are cracks, pitting or dead ends.[20-21] Several studies have detected fungi in biofilms on water and wastewater pipe surfaces.[22-24] Some authors have suggested that the rate of water contamination depends on the origin of the water, arguing that surface water is more contaminated than underground water.[25]

Members of the hyphomycetes were the only fungi identified in this study. While this fungal class is always the most represented in similar reports,[26] the genera distribution is inconsistent. In this study, Aspergillus spp were the most dominant fungi recovered from the water system, accounting for 65% and 69% of all those isolated from old and new distribution respectively.

Therefore, other parameters need to be taken into account to explain these results. For example, the presence of biofilms in the tubes could be a source of water contamination.[6,27]

Conclusion

Fungi contamination occur in a wide range in water distribution systems, many of them produce potential toxin, tastes and odor. They have the ability to cause disease or allergy in humans, act as contaminators in food and beverage industry, or decrease esthetical quality of water regarding smell and taste. Therefore, it is essential to keep the amount of fungi reaching the consumers under surveillance. In this study, the amount of fungi increased with the increase in supply system age, perhaps because of high growth of biofilm in distribution system or water leakage in the system.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

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References

1. Hageskal G, Lima N, Skaar I. The study of fungi in drinking water. Mycol Res 2009; 113(2): 165-72.
2. Pereira VJ, Basilio MC, Fernandes D, Domingues M, Paiva JM, Benoliel MJ, et al. Occurrence of filamentous fungi and yeasts in three different drinking water sources. Water Res 2009; 43(15): 3813-9.
3. Hageskal G, Gaustad P, Heier BT, Skaar I. Occurrence of moulds in drinking water. J Appl Microbiol 2007; 102(3): 774-80.
4. Mayahi S, Mosavi B, Hedayati MT, Movahedi M, Shokohi T. Mycoflora assessment in drinking tap water (Sari, Iran). J Gorgan Uni Med Sci 2011; 13(4): 114-9. [In Persian].
5. Al-gabr HM, Zheng T, Yu X. Occurrence and quantification of fungi and detection of mycotoxigenic fungi in drinking water in Xiamen City, China. Sci Total Environ 2014; 466-467: 1103-11.
6. Goncalves AB, Paterson RR, Lima N. Survey and significance of filamentous fungi from tap water.

Int J Hyg Environ Health 2006; 209(3): 257-64.
7. Anaissie EJ, Costa SF. Nosocomial aspergillosis is waterborne. Clin Infect Dis 2001; 33(9): 1546-8.
8. Anaissie EJ, Stratton SL, Dignani MC, Lee CK, Summerbell RC, Rex JH, et al. Pathogenic molds (including Aspergillus species) in hospital water distribution systems: a 3-year prospective study and clinical implications for patients with hematologic malignancies. Blood 2003; 101(7): 2542-6.
9. Hapcioglu B, Yegenoglu Y, Erturan Z, Issever H. Heterotrophic bacteria and filamentous fungi isolated from a hospital water distribution system. Indoor Built Environ 2005; 14(6): 487-93.
10. Pires-Goncalves RH, Sartori FG, Montanari LB, Zaia JE, Melhem MS, Mendes-Giannini MJ, et al. Occurrence of fungi in water used at a haemodialysis centre. Lett Appl Microbiol 2008; 46(5): 542-7.
11. Kanzler D, Buzina W, Paulitsch A, Haas D, Platzer S, Marth E, et al. Occurrence and hygienic relevance of fungi in drinking water. Mycoses 2008; 51(2): 165-9.
12. Panagopoulou P, Filioti J, Petrikkos G, Giakouppi P, Anatoliotaki M, Farmaki E, et al. Environmental surveillance of filamentous fungi in three tertiary care hospitals in Greece. J Hosp Infect 2002; 52(3): 185-91.
13. Cabral D, Fernandez P. Fungal spoilage of bottled mineral water. Int J Food Microbiol 2002; 72(1-2): 73-6.
14. Fujikawa H, Wauke T, Kusunoki J, Noguchi Y, Takahashi Y, Ohta K, et al. Contamination of microbial foreign bodies in bottled mineral water in Tokyo, Japan. J Appl Microbiol 1997; 82(3): 287-91.
15. Ribeiro A, Machado AP, Kozakiewicz Z, Ryan M, Luke B, Buddie AG, et al. Fungi in bottled water: a case study of a production plant. Rev Iberoam Micol 2006; 23(3): 139-44.
16. Hageskal G, Knutsen AK, Gaustad P, de Hoog GS, Skaar I. Diversity and significance of mold species in Norwegian drinking water. Appl Environ Microbiol 2006; 72(12): 7586-93.
17. Hageskal G, Vralstad T, Knutsen AK, Skaar I. Exploring the species diversity of Trichoderma in Norwegian drinking water systems by DNA barcoding. Mol Ecol Resour 2008; 8(6): 1178-88.
18. Arvanitidou M, Kanellou K, Constantinides TC, Katsouyannopoulos V. The occurrence of fungi in hospital and community potable waters. Lett Appl Microbiol 1999; 29(2): 81-4.
19. Nagy LA, Olson BH. The occurrence of filamentous fungi in drinking water distribution systems. Can J Microbiol 1982; 28(6): 667-71.
20. Mara D, Horan NJ. Handbook of water and wastewater microbiology. London, UK: Academic Press; 2003.
21. Paterson RR, Lima N. Fungal contamination of drinking water. In: Lehr J, Keeley J, Lehr J, Kingery TB, Editors. Water encyclopedia. New York, NY: John Wiley & Sons; 2005.
22. Doggett MS. Characterization of fungal biofilms within a municipal water distribution system. Appl Environ Microbiol 2000; 66(3): 1249-51.
23. Paterson RR, Goncalves AB, Lima N. Mycological examination and biofilm formation in drinking water. Proceedings of the 8th International Mycological Congress; 21-25 August 2006; Cairns, Australia; 2006. p. 129.
24. Skaar I, Qstensvik O. Moulds in water from Oset and Skullerud waterworks (in Norwegian) [Report]. Oslo, Norway: Oslo Water and Sewerage Works; 2005.
25. Warris A, Voss A, Abrahamsen TG, Verweij PE. Contamination of hospital water with Aspergillus fumigatus and other molds. Clin Infect Dis 2002; 34(8): 1159-60.
26. Hayette MP, Christiaens G, Mutsers J, Barbier C, Huynen P, Melin P, et al. Filamentous fungi recovered from the water distribution system of a Belgian university hospital. Med Mycol 2010; 48(7): 969-74.
27. Ortolano GA, McAlister MB, Angelbeck JA, Schaffer J, Russell RL, Maynard E, et al. Hospital water point-of-use filtration: a complementary strategy to reduce the risk of nosocomial infection. Am J Infect Control 2005; 33(5 Suppl 1): S1-19.