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Fungi Occurrence Assessment in Drinking Water Distribution Systems and Its Relationship with Fecal Indicator Bacteria

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A R T I C L E I N F O

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ABSTRACT

Introduction: As recommended by World Health Organization, consumption of the fungal contaminated water does not cause to serious infection, but may lead to healthy or aesthetic problems. The aim of this research was to assess the occurrence of fungi in water and its relationship with fecal indicator bacteria.

***Materials and Methods*:** 110 water samples were collected from different location of water distribution systems in Aliabad-e Katul City, North of Iran during April to November 2018. Enumeration of coliforms bacteria and fungi were performed by multiple tube fermentation and membrane filtration method, respectively; as described in Standard Methods.

Results: Our results showed that fungi and total coliform were detected in 31 (28.4%) and 26 (23.9%) samples, respectively. Among samples of fungi positive, 22 (20.2%), 7 (6.4%), and 5 (4.6%) water samples were positive for *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp., respectively. However, none faecal coliform and *E. coli* were observed in all examined samples, proposing the absence of faecal pollution in water. The mean and SD residual chlorine and pH were 0.55 ± 0.23 (mg/l) and 7.30 ± 0.30, respectively. The statistical analysis showed a remarkable difference between the prevalence of total coliforms and fungal species ($P < 0.001$).

Conclusion: Presence of potential opportunistic pathogens fungi in potable water can be considered as a health risk, especially for immuno-suppressed individuals. Therefore, cleaning the processes such as biofilm removal and addition of the free chlorine concentration can be effective to decrease fungi contamination and total coliform from water distribution system.

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Introduction

Water is necessary to human life and an acceptable supply must be available to all people. As recommended by the World Health Organization (WHO), consumption of drinking water should not pose any important threat for human health particularly older persons, pregnant women, young children and people with immune-system deficiencies during their lifetime [1]. Diseases associated with contamination of the drinking water have a major impact on the human health burden [2]. Infants and debilitated individuals, those who live under unsanitary and unhealthy situations are at the greatest risk of water borne diseases [3].

According to the WHO report, two million children die annually due to consumption of contaminated drinking water [4]. Bacteria, protozoa, and viruses have known as the most important cause of waterborne diseases due to microbial contamination of drinking water. Nevertheless, other microorganism such as helminths worm and fungi must be considered because they also can diseases but at a lower level than viruses and bacteria [5]. Fungi, as a varied group of microorganisms belonged to the Eumycota kingdom [6]. In respect to microbial ecology, fungi are saprophytes that obtain the main section of their metabolic carbon from degrading organic matter. Some species of fungi acting as parasites causing of disease such as skin and nail infections, mucosal infections, respiratory infection, etc. [7]. Over recent years, an increasing concern has raised associated with the occurrence of fungi in water distribution systems and its potential pathogeny [8 - 11]. For instance, in a study conducted by Hageskal, Trichoderma viride detected in Norwegian drinking water as the most dominant species[12]. This type of fungi have known as cause of asthma disease in children living in water-damaged homes [13]. In another study, Warris et al. also reported that Aspergillus *fumigatus* was detected from 49 percent of the examined taps water in Oslo University Hospital, Norway [14]. *Aspergillus fumigatus* is one of the most important fungal pathogen causing infections in patients in hospitals, and its infection rates are increasing [15].

Fungi infections occasionally related to annul host immunity due to the viral disease. They are principally prevalent among the individuals with immunodeficiency virus, hematological disturbance and hormonal disorders, organ transplantations, antibiotic consumers, and more medical users [16]. Nonetheless, according to WHO recommendation, the using of fungal contaminated drinking water has not lead to serious infections [8]. Possible reasons may be lack of knowledge about the health problems of fungal in water, different cultivation procedures, and heterogeneous mechanisms of fungal pathogenicity, small number of article about fungal contamination of tap water and occurrence of diseases in humans [17]. A number of research showed that fungi growing in Drinking Water Distribution Systems (DWDS) can cause aesthetic problems such as taste and odour or may lead to health problems due to biofilms formation inside the taps and in tap water by releasing a large quantity of products, known as the secondary metabolites [18]. Some of the secondary metabolites are toxic for animals and threat may present a risk for human health in higher concentrations or under prolonged time of exposure [7]. For this reason, secondary metabolites, fungal cell wall components, and the fungal load may contribute to the emergence of allergies and other opportunistic and systemic infections [9].

In recent years, although fungi have been repeatedly reported as causative agents of the respiratory, mucosal, cutaneous, and subcutaneous infections, they remain largely overlooked in regulations of the water quality. Consequently, evaluation of the opportunistic and pathogenic fungi in drinking water can be helpful for microbiological drinking water safety [7]. For this reason, the present study was designed to evaluate occurrence of the fungi in drinking water as well as to survey their relationship with the indicator of Total Coliform (TC) bacteria.

Materials and Methods

Water samples

In this cross sectional study, 110 drinking water samples were prepared from different stations of DWDSs in Aliabad Katoul City located in North of Iran (figure 1) from April to November 2018. Samples were gathered in sterile glass bottles, transmitted to environmental microbiology laboratory by a water coleman, and were analyzed instantly after coming to the lab.

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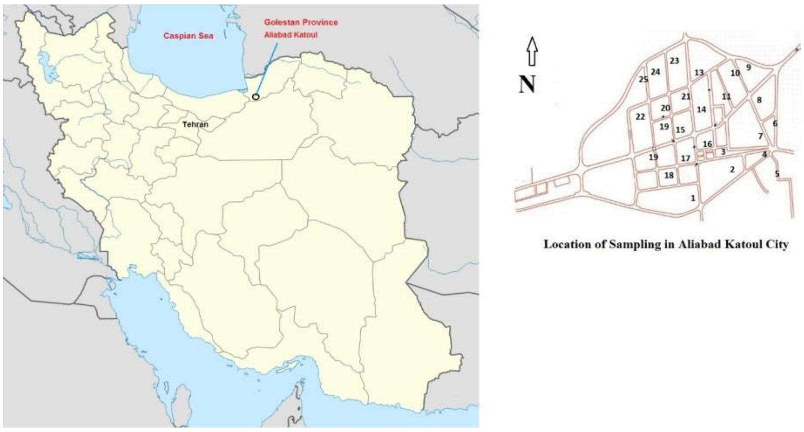

Figure 1: Location of the research in Iran (Aliabad Katoul city, north of Iran)

Microbial enumeration

Identification of the total and fecal coliform (FC) bacteria were performed by multiple-tube procedures according to explained in the Standard Methods for Examination of Water and Wastewater [19]. TC was examined based on the fermentation of lactose by acid and gas production after 48 h of incubation at 35°C. For sample with positive of lactose broth, simultaneous inoculation into brilliant green lactose bile broth and EC broth were also further carried out to confirm TCs and FCs, respectively. Results were reported as the most probable number (MPN) of identified TCs and FCs per 100 ml of water. For detecting fungi spp., 100 ml of samples were concentrated by membrane filters with 0.45 mm pores size. The membranes were located on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (50 mg/ml), incubated at 24 ± 0.5 °C and observed daily within the week. Sub-culturing of the colony on SDA in the same medium, were performed to prepare a pure colony for identification tests. Each isolate was verified by macroscopic and microscopic morphology (slide culture techniques using SDA). Additionally, residual free chlorine concentration and pH were measured by a Palin kit in the sampling site [19].

Statistical analysis

IBM SPSS Statistics 20.0 was used for analyses of data. A Chi-Square test was performed to show that there is association between the presence or absence of fungi with the presence or absence of indicator bacteria.

Ethical issues

This study was approved by the research ethics committee of Golestan University of Medical Sciences, Iran with Ethics Code of IR. GOUMS. REC. 1395.71.

Results

The results of this investigation revealed that fungi and TC were detected in 28.4% and 23.9% of the samples, respectively. Among samples of fungi positive, 22 (20.2%), 7 (6.4%), and 5 (4.6%) water samples were positive for *Aspergillus*, *Rhizopus*, and *Penicillium* species, respectively. TCs were detected within the concentration range of 1 - 43 MPN/100 ml. Nonetheless, none *faecal coliform* and *E. coli* were found among samples positive for TC. The mean and SD residual chlorine and pH were 0.55 ± 0.23 (mg/l) and 7.30 ± 0.30, respectively. The pH values (6.8 to 7.8) and free chlorine concentration (0.1 to 1) were within the range of drinking water legal requirements. Analytical results of the measured parameters are summarized in Table 1.

Table 1: Analytical results of the measured parameters from 25 DWDSs

No. of station	No. of samples	Free chlorine concentration Mean ± SD	pH Mean ± SD	Number of water samples positive for *Total coliform*	*Aspergillus* sp.	*Rhizopus* sp.	*Penicillium* sp.
1	4	0.55 ± 0.17	7.25 ± 0.30	1	1	0	0
2	5	0.54 ± 0.15	7.04 ± 0.33	1	0	0	0
3	4	0.45 ± 0.06	7.05 ± 0.25	1	1	0	0
4	4	0.83 ± 0.24	7.05 ± 0.25	0	0	0	0
5	4	0.65 ± 0.17	7.40 ± 0.43	0	0	0	0
6	4	0.58 ± 0.15	7.25 ± 0.44	0	0	0	0
7	5	0.42 ± 0.11	7.44 ± 0.41	0	1	0	0
8	5	0.40 ± 0.10	7.20 ± 0.28	2	1	1	0
9	4	0.55 ± 0.17	7.10 ± 0.35	1	0	1	0
10	4	0.83 ± 0.24	7.25 ± 0.30	0	0	0	0
11	4	0.58 ± 0.17	7.15 ± 0.30	0	0	0	1
12	4	0.40 ± 0.12	7.20 ± 0.49	3	2	1	0
13	5	0.44 ± 0.26	7.56 ± 0.17	0	1	1	0
14	4	0.53 ± 0.1	7.60 ± 0.23	0	0	0	1
15	5	0.38 ± 0.08	7.44 ± 0.41	1	3	0	0
16	4	0.95 ± 0.10	7.10 ± 0.20	0	0	0	0
17	4	0.88 ± 0.25	7.35 ± 0.38	0	0	0	0
18	4	0.90 ± 0.12	7.10 ± 0.20	0	0	0	0
19	5	0.62 ± 0.28	7.52 ± 0.18	1	0	0	0
20	4	0.58 ± 0.30	7.40 ± 0.43	3	0	1	0
21	5	0.40 ± 0.07	7.12 ± 0.36	2	3	0	0
22	5	0.40 ± 0.01	7.24 ± 0.43	1	3	0	0
23	5	0.38 ± 0.08	7.48 ± 0.33	4	3	0	1
24	4	0.45 ± 0.06	7.50 ± 0.2	2	0	0	0
25	4	0.33 ± 0.50	7.50 ± 0.2	3	3	0	1
Total	109	0.55 ± 0.23	7.30 ± 0.34	26	22	5	4

The correlation analyses indicated that chlorine concentration had a significant effect on the TC population in water ($p = 0.016$); whereas, pH did not show any significant effect on the TC of water ($p = 0.867$). Furthermore, the statistical analysis showed a remarkable difference between the prevalence of TCs and fungal species with the sampling stations ($P < 0.001$). However, in 5 samples, where no TC was detected, two cases of *Aspergillus flavus*, two cases of *penicillin*, and one *Rhizopus* were observed.

Discussion

Microbiological quality of the drinking water in public DWDSs plays a significant role in protecting public human health [1]. Our finding showed that microbial quality of the DWDSs was safe in accordance with the FC and *E. coli* as common faecal indicators of bacteria recommended by WHO [20]. For the most part, tracking of indicator bacteria provides a high level of water safety because they appear in big amount in the feces of human and animals. Nevertheless, lack of the FC recovery from a sample, it does not mean to say that other microorganisms doesn't present in DWDSs. In this regard, presence of the TCs in a distribution system can be caused by the bacterial regrowth within the distribution system biofilm [1]. It also indicates that DWDS may be vulnerable to contamination by more harmful microorganisms. Under this circumstance, a high level of disinfectant, an effective contact time, a thorough flushing of the system, as well as a re-analyze to approve that water is without TC are necessary [4]. According to WHO recommendations, when more than 10 samples analyzed from distribution systems in a given time, no successive samples from the same location or not over than 10 percent of samples should show the presence of TC bacteria. Therefore, analysis for TCs should be carried out in all drinking water distribution systems.

Our findings showed that fungi were present in DWDS, but the presence of *Aspergillus*, *Penicillium*, and *Rhizopus*, as potential pathogenicity species is indicative of a greater risk that may lead to health problems. For instance, an outbreak of human's skin irritations was reported by Aslund in a study were performed in Rabacka city, Sweden [8]. The aerosolized of fungi through water passes installations such as showers into the air flow confirm the hypothesis of fungal aerosol can cause serious health hazard to hospitalized individual [22]. This finding is similar to the results reported by Mayahi et al., who found *Aspergillus*, *Penicillium*, and *Rhizopus* species from a WDS [22]. In general, the detection rate of fungi was in a wide range of 7.5 to 89 % of the positive samples depending on temperature of water, water retaining time, type of disinfectants and its concentration in the water, existence of the assimilable organic carbon and biodegradable organic carbon in water, availability of inorganic nutrient, properties of the pipe material, and the sediments load of pipes [7].

Our results also demonstrated that concentration of free residual chlorine and pH values were within the acceptable levels for drinking water. As a result, presence of TCs or fungi may be associated with biofilm growth within a water distribution system. Similar results reported by Hageskal at al. Who isolated fungus species from biofilms of drinking water systems [8]. It should be noted that it is very difficult to eliminate from colonized biofilm matrices by water treatment processes when fungi formed in biofilms of water systems. Therefore, improving the quality of water must be done before the water reaches to the consumers in a municipal water distribution system. Additional, adequate process control measures and operator training also needed for ensuring the safety of drinking water.

Conclusions

Presence of the opportunistic pathogens fungi in water can be considered as a potential danger for human health especially among children, elderlies, and immuno-suppressed individuals, particularly under prolonged time especially for immuno-suppressed persons. Increase of the disinfectant dosage, flush of the water mains, checking for the existence of cross-connections and loss of pressure can decrease both fungi contamination and TC growth in WDS.

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Conflict of interests

The authors confirm that there are no conflict of interest regarding the publication of this article.

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