Fungal contaminants in man-made water systems connected to municipal water

Duygu Göksay Kadaifciler and Rasime Demirel

ABSTRACT

Water-related fungi are known to cause taste and odor problems, as well as negative health effects, and can lead to water-pipeline clogging. There is no legal regulation on the occurrence of fungi in water environments. However, much research has been performed, but further studies are needed. The main objectives of this study were to evaluate the fungal load and the presence of mycotoxigenic fungi in man-made water systems (for homes, hospitals, and shopping centers) connected to municipal water in Istanbul, Turkey. The mean fungal concentrations found in the different water samples were 98 colony-forming units (CFU)/100 mL in shopping centers, 51 CFU/100 mL in hospitals, and 23 CFU/100 mL in homes. The dominant fungal species were identified as *Aureobasidium pullulans* and *Fusarium oxysporum*. Aflatoxigenic *Aspergillus flavus* and ochratoxigenic *Aspergillus westerdijkiae* were only detected in the hospital water samples. *Alternaria alternata*, *Aspergillus clavatus*, *Aspergillus fumigatus*, and *Cladosporium cladosporioides* were also detected in the samples. The study reveals that the municipal water supplies, available for different purposes, could thus contain mycotoxigenic fungi. It was concluded that current disinfection procedures may be insufficient, and the presence of the above-mentioned fungi is important for people with suppressed immune systems.

Key words | aflatoxin, DNA sequencing, fungi, municipal water, ochratoxin, thin-layer chromatography

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INTRODUCTION

Microbial contamination in man-made water systems has recently emerged as a growing problem (Sautour *et al.* 2012; Al-gabr *et al.* 2014). Municipal water passes through kilometers of pipelines and, most of the time, is stored for periods before use. Microorganisms are introduced into the water during supply and are transferred by water flow, during which they can adhere to the inner surfaces of pipes and produce biofilm layers, which accumulate over time. Microorganisms associated with biofilms, when intermittently separated from the biofilm matrix, can create new biofilm layers elsewhere in pipelines. Therefore, these biofilm fragments and microorganisms can spread throughout water distribution systems; this condition affects the hygienic quality of the water. Water-related fungi are related to taste or odor problems, contamination of food, corrosion of water supply pipelines, and various health problems (Anaissie *et al.* 2001; Hageskal *et al.* 2009).

Doggett (2000) was the first to report the presence of fungi in municipal water distribution-system biofilms; *Aspergillus* and *Penicillium* were the most common biofilm genera found. Consequent studies show that allergic and opportunistic pathogen members of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Trichoderma*, and *Cladosporium* have spread to homes, dental clinics, and hospitals via water distribution systems (Anaissie *et al.* 2001; Hapcioglu *et al.* 2005; Hageskal *et al.* 2006, 2009; Sautour *et al.* 2012; Göksay Kadaifciler *et al.* 2013).

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Direct contact of contaminated water with damaged human tissue or inhalation of bioaerosols can cause skin irritations and a variety of diseases. It has been reported that showering and sink washing spreads fungi present in hospital water systems into the air as bioaerosols; they remain in the air for a long time and cause opportunistic infections such as fusariosis (Anaissie *et al.* 2001). In recent years, studies in hospitals have focused on *Aspergillus fumigatus* and its effect on patients with suppressed immune system diseases, such as diabetes, cancer, and AIDS (Chazalet *et al.* 1998; Pfaller & Diekema 2004).

It is known that members of the genera *Aspergillus*, *Penicillium*, and *Fusarium* are important mycotoxin producers. *Aspergillus flavus*, which is known to produce aflatoxins (B2 and G2), has been isolated from a cold-water storage tank (Paterson *et al.* 1997). Al-gabr *et al.* (2014) also detected aflatoxins, fumonisin, and trichothecenes in drinking water systems. Furthermore, an *in vitro* study by Russell & Paterson (2007) demonstrated the production of zearalenone by *Fusarium* spp. in drinking water. Nevertheless, previous studies indicated that the production of mycotoxin in water is low; the concentration of mycotoxin may increase because of long-term storage of water in reservoirs. Furthermore, Hageskal *et al.* (2009) suggested that small amounts of mycotoxins in the human body, a result of long-term consumption of contaminated water, might lead to health problems. The production and importance of mycotoxin in water environments are still poorly known.

There are currently no limiting criteria for the presence of total fungi, mycotoxigenic fungi, and mycotoxins among standards for drinking and municipal water supplies. However, previous studies have determined that mycotoxins have negative health effects on higher organisms, and the presence of biomass effects drinking-water quality. The objectives of this study were: (i) to evaluate the culturable total fungi and mycotoxigenic fungi content of drinking water and/or municipal water sources distributed via municipal supply systems; and (ii) to determine the relationship between culturable fungal concentrations in water samples and physical-chemical parameters such as temperature, pH, and free chlorine.

METHODS

Water sampling and fungal isolation

Water samples (500 mL each) were collected from 86 different man-made water systems, including 49 homes, 13 shopping centers, and 24 hospitals, directly connected to municipal water supplies in the city of Istanbul, Turkey. These 86 water systems were the only buildings for which we were able to obtain permission for sampling. Although the water samples from the hospitals and shopping centers were sampled from faucets, the fact that the municipal water was first collected in a container and then supplied to the faucet was provided by management. A chlorine neutralizer, 0.05 mL of 10% sodium thiosulfate solution, was added to 500 mL sterile, sample bottles (Nagy & Olson 1982). Water samples were concentrated by filtration through 0.45 μm pore-sized nitrocellulose (Millipore, USA) filters. These filters were placed on Sabouraud Dextrose Agar (Oxoid, UK) plates containing streptomycine antibiotic (SDA), in triplicate, and incubated at 25 °C for 7–10 days (Al-gabr *et al.* 2014). After incubation, the fungal colonies were counted and a colony-forming unit (CFU) per 100 milliliter (CFU/100 mL) was calculated. The colonies were subcultured on Potato Dextrose Agar (PDA) (Oxoid, UK) slants and stored at 4 °C. The sample water temperature, pH, and free chlorine values were also measured.

Thin-layer chromatography

Fungal isolates were cultured on yeast-extract sucrose agar and incubated at 25 °C for 7 days. Agar plugs containing mycelium were cut out of the colony center and margined to the edge, close to other colonies, using a 6 mm diameter cork borer. The plugs were transferred to sterile screw-cap tubes and 1 mL of methanol was added. Extractions were performed ultrasonically for 15 min with sonication. Extracts of 20 μL were spotted on thin-layer chromatography (TLC) plates (20 × 20 cm) with silica gel 60 without non-fluorescence. After the spots were air dried, the TLC plates were placed in an eluent tank filled with toluene, ethyl acetate, and formic acid (90%) (5:4:1 vol/vol/vol);

elution was performed for 15–30 min (Frisvad & Ve Filtenborg 1983; Samson *et al.* 2010). After elution, the plates were air dried in a fume hood and then examined in visible light, 366 nm and 312 nm, with comparison to ochratoxin A (OTA) and aflatoxins (AFs) (B1, B2, G1, and G2). A CAMAG HPTLC was used for detection (Frisvad & Ve Filtenborg 1983; Samson *et al.* 2010). Fungal extracts belonging to the same species were shown to be identical or similar to secondary metabolite profiles. Therefore, the fungal isolates which displayed different metabolite profiles were selected for molecular identification.

Morphological identification of fungi

Fungal isolates were inoculated into Malt Extract Agar (Oxoid, UK) and PDA and then identified to genus level according to generally accepted standards (Barnett & Hunter 1999).

Molecular identification of fungi

Fungal isolates were inoculated into Malt Extract Agar and incubated at 25 °C for 7 days. Genomic DNA was extracted from the pure cultures using microbial DNA isolation kits (MO BIO Laboratories, Inc., USA). Standard gene regions, which are internal transcribed spacer (ITS) regions of rDNA genes, were used for molecular characterization. These regions were amplified using the primer pairs V9G; TTACGTCCCTGCCCTTTGTA (forward) and LS266; GCATTCCCAAACAACTCGACTC (reverse) (Samson *et al.* 2010), and polymerase chain reaction (PCR) reactions were carried out in 25 μL of final reaction volumes. Each tube contained 1 μL of genomic DNA; 2.5 μM of forward and reverse primer; 2.5 μL of 10 × Taq buffer + KCl–MgCl2 (Bioline, UK); 2.5 μL of 25 mM MgCl (Fermentas, CA, USA); 2 μL of 2.5 mM dNTPmix; 0.25 μL of 5 U/μL Taq DNA polymerase (Bioline, UK), and 11.75 μL of sterile deionized water. DNA amplification was performed in a thermocycler with an initial denaturation step for 5 min at 95 °C, followed by 35 cycles of denaturation for 45 sec at 95 °C, annealing for 30 sec at 56 °C, and extension for 2 min at 72 °C. A final extension at 72 °C was performed for 6 min (Samson *et al.* 2010). To confirm the amplification of solely the ITS, 5 μL of PCR product together with a marker (GeneRuler™ 50 bp DNA Ladder, Fermentas, CA, USA) was resolved by gel electrophoresis on 1% agarose gel containing 5 μg/mL of GelRed™ in 1 × TAE buffer. The gel samples were photographed via a gel documentation system (M02 4611; Uvitec). PCR products were cleaned up using EXOSAP-IT (Amersham Pharmacia Biotech, Little Chalfont, UK) and used for sequencing. The ITS region was sequenced using ITS1; TCCGTAGGTGAACCTGCGG (forward) and ITS4; TCCTCCGCTTATTGATATGC (reverse), sequencing reactions were performed with a CEQ™ DTCS Quick Start Kit (Beckman Coulter, CA, USA), and sequenced using a CEQ™ 8000 Genetic Analysis System. The sequences were allocated to GenBank accession numbers and compared with those deposited in the NCBI GenBank database. Fungal author names and fungal names were standardized according to the Index Fungorum website (2016).

Statistical analyses

Statistical analyses were carried out using the Spearman's correlation coefficient test (IBM SPSS, Version 21, USA). The test was used to examine the relationship of fungal concentrations with selected parameters such as temperature, free chlorine, and pH. Significant differences were considered at $p < 0.05$.

RESULTS

Fungal concentrations in man-made water systems

The minimum-maximum range of fungal concentrations in homes, hospitals, and shopping centers were determined as 0–289, 1–800, and 0–300 CFU/100 mL, respectively (Table 1).

The highest isolation frequency of fungi was recorded for water samples collected from hospitals (100%), followed by shopping centers (84.6%) and homes (79.5%).

Identification of fungal flora

A total of 228 fungal isolates were sub-cultured. A comparison of the metabolite profiles of the 228 isolates determined 82 different profiles, and these isolates were selected for

Table 1 | Concentrations of fungi in shopping center, home, and hospital water samples

	Shopping center ($n = 13$)			Home ($n = 49$)			Hospital ($n = 24$)		
Range of fungal counts (CFU/100 mL)	0	1–50	51–300	0	2–100	101–289	0	1–100	800
n	2	4	7	11	34	4	–	23	1
Mean of fungal counts	98			23			51		

CFU/100 mL = colony-forming units per 100 mL; n = number of samples.

molecular identification. A total of 34 species and 16 genera were identified (Table 2).

The lowest number of fungal species was isolated from the shopping centers (eight species), while the highest number of fungal species was isolated from the homes (32 species). The genera *Penicillium* (10 species) and *Aspergillus* (eight species) had higher species diversity than the other genera. The following genera were represented by only one species each: *Alternaria*, *Botryosphaeria*, *Byssochlamys*, *Coprinopsis*, *Gibberella*, *Paecilomyces*, and *Periconia*. However, the most prevalent fungal species were *Aureobasidium pullulans* (808 CFU/100 mL) and *Fusarium oxysporum* (809.9 CFU/100 mL). *A. pullulans* was isolated from homes, hospitals, and shopping centers, while *F. oxysporum* was isolated from homes and shopping centers. In addition, several other fungi were identified such as *Alternaria alternata*, *Aspergillus clavatus*, *Aspergillus fumigatus*, *Cladosporium cladosporioides*, and *Exophilia* sp.

Mycotoxigenic fungi in water samples

All fungal isolates were compared to OTA and AF (B1, B2, G1, and G2) mycotoxin standards; it was determined that AF and OTA were able to produce 11 and one isolate(s), respectively. *Aspergillus flavus* and *Aspergillus westerdijkiae*, which were diagnosed as toxigenic fungi, were isolated from the hospital water samples.

Measurements of chemical-physical parameters

The minimum-maximum range of the water chemical parameters, free chlorine and pH, were determined as 0–3 ppm and 6.74–7.7 ppm, respectively. Free chlorine was identified in 7.6% of the shopping centers, 12.5% of the hospitals, and 46.9% of the homes. The mean temperature of the water samples was 23 °C.

According to the correlation coefficient test, there was a positive correlation between fungal concentrations in homes and temperature ($p = 0.00$, $r = 0.517$), while there was a negative correlation between fungal concentrations in homes and free chlorine ($p = 0.039$, $r = -0.306$). The results of the analyses indicated that there was a positive correlation between fungal concentrations in the shopping centers and temperature ($p = 0.045$, $r = 0.564$). A negative correlation between fungal concentrations in the hospitals and temperature ($p = 0.021$, $r = -0.467$) and pH ($p = 0.041$, $r = -0.420$) was determined.

DISCUSSION

Fungi generally produce slowly progressing chronic infections. Nonetheless, people with suppressed immune systems might experience fatal, acute infections (Iatta *et al.* 2009). In particular, the air we breathe contains *Aspergillus, Penicillium, Cladosporium, Alternaria*, and *Fusarium*, which may cause aspergillosis, allergic rhinitis, anaphylactic pneumonia, chronic bronchitis, and asthma. The frequency of cases of these infections has been increasing (Singh 2005). It has been reported in many articles that fungi, which are predominantly present in the soil and air, can adapt to live in man-made water systems. Recent studies have determined that *Aspergillus* and *Fusarium* spp. which are infectious agents in patients, originated from hospital water (Anaissie *et al.* 2001; Ao *et al.* 2014). Our observation that fungi are regularly present in water systems corroborates the findings of others, but their significance for hygiene and public health seems to be less evident.

Table 2 | Total number of fungi in the man-made water systems

Fungi	Accession number	Total number (CFU/100 mL)	Sample source
Alternaria alternata (Fr.) Keissl. 1912	KX610142, KX610143, KX610159, KX610165	19.6	Hm, S
Aspergillus awamori Nakaz. 1907	KX610171	10	Hm, H
Aspergillus clavatus Desm. 1834	KX610123	2	Hm
Aspergillus calidoustus Varga, Houbraken & Samson 2008	KX610170	1	Hm
Aspergillus pseudoglaucus Blochwitz 1929	KX610153	19	Hm, H
Aspergillus westerdijkiae[a] Frisvad & Samson 2004	KX610128, KX610164, KX610169	75	H, Hm, S
Aspergillus flavus[a] Link 1809	KX610125, KX610172	13	H
Aspergillus fumigatus Fresen. 1863	KX610137	21	Hm, H
Aspergillus versicolor (Vuill.) Tirab. 1908	KX610119, KX610121, KX610161, KX610173,	6	Hm, H
Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud 1918	KX610176	808	Hm, H, S
Aureobasidium sp. Viala & G. Boyer 1891	KX610115	2	Hm, S
Botryosphaeria dothidea (Moug.) Ces. & De Not. 1863	KX610122	4	Hm, H
Byssochlamys spectabilis (Udagawa & Shoji Suzuki) Houbraken & Samson 2008	KX610124	1	Hm
Chaetomium globosum Kunze 1817	KX610114, KX610158	2	Hm, H
Chaetomium sp. Kunze 1817	KX610120	1	Hm
Cladosporium cladosporioides (Fresen.) G.A. de Vries 1952	KX610155, KX610162	80	H, Hm, S
Cladosporium cucumerinum Ellis & Arthur 1889	KX610156	3	Hm
Coprinopsis cinerea (Schaeff.) Redhead, Vilgalys & Moncalvo 2001	KX610168	1	Hm
Exophiala sp. J.W. Carmich. 1966	KX610129	1	H
Fusarium chlamydosporum Wollenw. & Reinking 1925	KX610163	9	Hm
Fusarium oxysporum Schltdl. 1824	KX610126, KX610140, KX610141	809.9	Hm, S
Fusarium solani (Mart.) Sacc. 1881	KX610127, KX610175	60	S
Gibberella intricans Wollenw. 1930	KX610166	1	Hm
Paecilomyces divaricatus (Thom) Samson, Houbraken & Frisvad 2009	KX610144	1	Hm
Penicillium adametzioides S. Abe ex G. Sm. 1963	KX610135	1	H
Penicillium commune Thom 1910	KX610132, KX610146, KX610149	11	Hm, H
Penicillium chrysogenum Thom 1910	KX610118, KX610133, KX610138, KX610150, KX610152	11	Hm, H
Penicillium citrinum Thom 1910	KX610136, KX610174	2	Hm, H
Penicillium dierckxii Biourge 1923	KX610131	30	H
Penicillium dipodomyicola (Frisvad, Filt. & Wicklow) Frisvad 2000	KX610117, KX610130, KX610134	278	H, Hm, S
Penicillium griseofulvum Dierckx 1901	KX610113	1	H
Penicillium polonicum K.M. Zaleski 1927	KX610148, KX610151, KX610157	35	Hm, H
Penicillium rubens Biourge 1923	KX610139, KX610147	5	H

(*continued*)

Table 2 | continued

Fungi	Accession number	Total number (CFU/100 mL)	Sample source
Penicillium spinulosum Thom 1910	KX610167	2	Hm
Periconia byssoides Pers. 1801	KX610154	18	Hm, H
Pseudozyma sp. Bandoni 1985	KX610116	1	Hm
Talaromyces minioluteus (Dierckx) Samson, N. Yilmaz, Frisvad & Seifert 2011	KX610145	1	Hm
Talaromyces pinophilus – (Hedgc.) Samson, N. Yilmaz, Frisvad & Seifert 2011	KX610160	1	Hm
Non-identified fungi		155	Hm, H
Yeast		358	S

Hm, home; H, hospital; S, shopping center.
[a]Mycotoxin producer; CFU/100 mL = colony forming unit per 100 millilitres.

According to several authors, there are no international, acceptable guidelines and isolation procedures for evaluating drinking-water or municipal-water quality for the presence of fungi. Therefore, the culture mediums and isolation methods that researchers choose might affect the number of fungi found in their investigations. In the vast number of previous studies, the membrane filtration method was used, but the isolation media method was varied. The current study, along with many others that have tested domestic and hospital waters, used Sabouraud Dextrose Agar (Anaissie *et al.* 2001; Hapcioglu *et al.* 2005; Al-gabr *et al.* 2014). In the current study, the mean fungal concentrations of the different water source samples were 23 CFU/100 mL in homes, 51 CFU/100 mL in hospitals, and 98 CFU/100 mL in shopping centers; previous studies corroborate these findings. However, a previous survey concerning the quantification of fungi in drinking water from a public network, houses, tanks, and lakes reported that 97.91% of the water sample fungal concentrations were below 100 CFU/100 mL (Al-gabr *et al.* 2014). Several researchers have determined fungal concentrations of 1–300 CFU/100 mL in homes, hospitals, and municipal water systems (Hapcioglu *et al.* 2005; Hageskal *et al.* 2006).

The current study highlights that water from shopping centers and hospitals contains more fungi than domestic waters. It is considered that the absence of fungi in some home-water samples may cause the average of household water samples to be lower than the other water systems (Table 1). This result may be explained in terms of biofilm formation or chlorine.

Biofilm formation

It is known that municipal water is an oligotrophic environment for microbial growth. Therefore, microorganisms are able to proliferate in the favorable microhabitat provided by biofilms on pipe surfaces. Domestic water is used immediately, but other sampled systems use storage containers and the water is subsequently used. It is possible that fungi attach to a biofilm layer formed on the surface of the container during storage and their number increases with time. The biofilm acts as a fungal reservoir, and fungi might be transferred at intermittent intervals to the water (Göksay Kadaifciler *et al.* 2013). As opportunistic pathogens, if fungi accumulate in this biofilm, there may be a potential health risk for people with immune suppression and children.

Chlorine

Being the most preferred disinfectant in water supply systems, this chemical agent can be effective on planktonic microorganisms, but it cannot penetrate biofilms (Kim *et al.* 2002). Another property of chlorine is that it is volatile; even though municipal water is chlorinated, it is considered that the level of free chlorine decreases in the water as it flows through pipelines over distance. In fact, many water samples kept in containers do not contain free chlorine, but domestic water samples contain 46.9% chlorine. In conclusion, in the current study, home water samples showed a

negative correlation between the amount of chlorine and the number of fungi, which was also found by Al-gabr *et al.* (2014). However, there was no relationship between free chlorine and the water samples of other buildings. It is thought that the non-detection of chlorine in the hospital and shopping-center water samples may have affected the results of the statistical test. Despite this, there were also a high number of fungi in the home water samples with high chlorine levels. It is suggested that disinfection may not be effective when municipal water is stored in containers for a long time.

Studies carried out in Turkey on the presence of microfungi in hospitals and dental clinics connected to municipal water supply systems have focused on identification based on morphological characteristics (Hapcıoglu *et al.* 2005; Göksay Kadaifciler *et al.* 2013). The current study is the first to collect water samples from different sources under heavy use in Istanbul and to conduct a mycotoxin search and ITS-PCR identification. Because fungi might be responsible for opportunist infections, the screening process in aquatic environments was made in terms of fungal bio-varieties. Therefore, both classical morphological and molecular techniques were used for the determination of the mycobiota in the water distribution systems (Al-gabr *et al.* 2014). It has been reported that the different PCR techniques used by ITS region primers are very sensitive for the identification of environmental fungal isolates (Al-gabr *et al.* 2014).

As opportunist mycotic infections have increased in recent years, studies of the identification of *Fusarium* spp. and toxigenic fungi in aquatic systems have also increased (Sautour *et al.* 2012; Al-gabr *et al.* 2014). *Fusarium* is present in aquatic environments and may infect human lungs via aerosolization or skin lesions. It has been reported that *Fusarium solani* and *Fusarium oxysporum* are the most common causes of keratitis and fusariosis (Nucci & Anaissie 2007). In the current study, *Fusarium oxysporum*, *Fusarium solani*, and *Fusarium chlamydosporum* were also found in homes and shopping centers. This result suggests the possibility that people might be exposed to *Fusarium* during their daily activities, such as showering and wearing contact lenses. Therefore, the presence of *Fusarium* may be a potential health risk. Aflatoxin and ochratoxin are known to have carcinogenic, teratogenic, and immunotoxic effects. While direct skin contact and inhalation of these toxins are thought to be of minor importance for public health, ingestion is the most significant way they invade the human organism. However, Hope & Hope (2012) state that inhalation of ochratoxin is associated with human illness. In the current study, hospital water temperatures ranged between 16 °C and 28 °C, which is suitable for the production of mycotoxins. *Aspergillus westerdijkiae* and *Aspergillus flavus* isolates, that have toxigenic properties, were isolated from the hospital water samples. The detection of these species could be life threatening for immunocompromised patients. The significance of toxigenic fungi in water is not totally understood; however, these organisms may have negative effects on health.

Aureobasidium pullulans and *Fusarium oxysporum* were detected in high concentrations in the current study's water samples. *A. pullulans* is widely distributed in different environments; although it is known to be saprophytic, it has also been reported to be a causative agent of cutaneous infection and pneumonia (Hawkes *et al.* 2005). *F. oxysporum* is a common soil inhabitant; however, it has been isolated from water systems (Hageskal *et al.* 2006; Sautour *et al.* 2012). The concentration of these fungi, in comparison with the concentrations of other fungal species isolated from water samples, is quite high (Table 2). This result could be explained by the biofilm in these systems protecting these fungi from the harmful effects of disinfection and provide a suitable environment for microbial growth. Similarly, Sautour *et al.* (2012) suggest that *F. oxyporum* could be a secondary colonizer in biofilms, and it is therefore well adapted to the water environment. It has been reported that *Fusarium* species have a greater ability to form biofilms than other genera (Short *et al.* 2011). According to the literature (Harding *et al.* 2009), yeast-like molds such as *A. pullulans* can also form biofilms. Furthermore, *Alternaria alternata*, *Aspergillus clavatus*, *Aspergillus fumigatus*, *Aureobasidium* sp., *Chaetomium globosum*, *Cladosporium cladosporioides*, *Exophiala* sp., *Penicillium chrysogenum*, *Penicillium commune*, and *Penicillium polonicum* were detected in the current study. These fungal species are generally known as causative agents of allergy and respiratory diseases, and their exposure via bioaerosol or dermal contact may present potential health risks.

CONCLUSIONS

The present study advances the understanding of fungal contamination in man-made water systems connected to municipal water supplies. Although the average culturable fungal concentration was below 100 CFU/100 mL in the municipal water systems of Istanbul, Turkey, the mycotoxigenic *Aspergillus flavus* and *Aspergillus westerdijkiae* were isolated from hospital water samples. In addition, *Aureobasidium pullulans*, *Alternaria alternata*, *Aspergillus fumigatus*, and *Fusarium oxysporum* were detected in the water systems. It is important to have a control system to prevent these fungi and this should be checked regularly; therefore, chlorine disinfection conditions should be well managed. The results of this study are important since they determine the mycological quality of drinking and municipal water, as well as the need for adequate fungal-prevention measures and the development of necessary strategies.

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