The occurrence of fungi in drinking water has received increased attention in the last decades, and fungi are now generally accepted as drinking water contaminants. The knowledge about the occurrence and diversity of fungi in water has increased considerably from a low knowledge base. However, the relevance of waterborne fungi for water quality and human health is poorly understood and still conflicting. Scientific reports on effective treatment against fungi in water are also few. This article presents a review of the literature on fungal water studies, including some general results, and considerations of significance, limits, contradictions, precautions, and practical consequences.

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in contact with air (Kirk et al. 2001). These fungi can also enter drinking water from various locations, although this is considered an ‘unnatural’ habitat for them. The knowledge of the occurrence of fungi in drinking water was limited, but has increased due to the various studies performed.

The history of fungal water studies

The issue of fungi in drinking water appears to have arisen in the 1960s and 1970s, when analyses of water due to problems, such as foul taste and odour, and reports of health episodes caused by contaminated water were reported (Bays et al. 1970; Burman 1965; Metzger et al. 1976). First of all, these analyses were intended for investigating the presence of Actinomycetes or Cyanobacteria, which were already known to be able to cause problems in drinking water. However, significant amounts of microfungi were also recovered from the water samples, leading to speculation as to whether fungi could be causing the experienced problems. During the 1980s and 1990s, more cases of health problems caused by fungal-contaminated drinking water were reported from Finland and Sweden (Åslund 1984; Muittari et al. 1980), and fungi in drinking water were investigated in several countries worldwide (Åkerstrand 1984; Arvanitidou et al. 1999; Franková 1993; Franková & Horecka 1995; Hinzelin & Block 1985; Lahti 1993; Nagy & Olson 1982, 1985; Niemi et al. 1982; Ormerod 1987; Rosenzweig et al. 1986; West 1986; Zacheuς & Martikainen 1995). In addition, results from studies of taste and odour problems in drinking water indicated the occurrence of fungi as an important causative factor for these sensoric changes (Montiel et al. 1999; Nystrom et al. 1992). Common for many of the early studies was that they were undertaken in response to a water contamination problem. Thus fungi were never the main object for the analyses.

In the last decade, several more studies have resulted in increased knowledge on the occurrence of fungi in drinking water (Anaissie et al. 2001, 2002b, 2003; Arvanitidou et al. 2000; Gonçalves et al. 2006a; Göttlich et al. 2002; Hageskal et al. 2006, 2007, 2008; Hageskal 2007; Hapcioglu et al. 2005; Kanzler et al. 2007; Kelley et al. 2003; Kennedy & Williams 2007; Panagopoulos et al. 2002; Pires-Gonçalves et al. 2008; Varo et al. 2007; Warris et al. 2001; Warris et al. 2002). Some general results for all the studies performed are, for example, that the recovery of fungi varied between 7.5–89 % positive samples, and that the levels of fungi in the samples varied considerably in the various investigations. Fungi have been reported from all types of water, from raw water to treated water, and from heavily polluted water to distilled or ultra-pure water. Fungi have also been reported from bottled drinking water (Cabral & Fernández 2002; Fujikawa et al. 1997; Ribeiro et al. 2006). Statistically, it has been established that the odds for fungal recovery are three times higher in surfaces-sourced water compared with ground-sourced water, and fungi are more commonly recovered from cold water and shower water than from hot tap water (Hageskal et al. 2007).

Significance and health aspects

The fungi recovered from water in the studies performed include some yeast, but are mostly filamentous fungi (moulds). A wide diversity of mould species has been isolated from drinking water. Among these are potentially pathogenic, allergenic, and toxicogenic species. For instance, Aspergillus fumigatus was recovered from 49 % of the investigated taps at Rikshospitalet University Hospital in Oslo, in a study performed by Warris and colleagues (2001). A. fumigatus has been one of the most significant fungal pathogen causing infections in immunocompromised patients in hospitals, and the infection rates are increasing (Marr et al. 2002; White 2005). Because of the increasing frequencies of severely immunocompromised patients, hospitals are facing a greater challenge with respect to opportunistic fungal infections (Denning 2006).

The occurrence of potentially pathogenic species, such as A. fumigatus, in drinking water has lead to speculations whether hospital water systems may serve as a transmission route for fungal infections. Several studies have focused on analysing hospital water systems with respect to the presence of fungi (Anaissie et al. 2001, 2003, Arvanitidou et al. 1999, 2000; Hapcioglu et al. 2005; Kanzler et al. 2007; Kennedy & Williams 2007; Panagopoulos et al. 2002; Pires-Gonçalves et al. 2008; Warris et al. 2001, 2002). The results indicate that hospital water may contain a wide diversity of fungi, including potential pathogens. The hypothesis is that fungi in water are aerosolized into air when water passes installations, such as taps and showers, and thus introduced to severely immunocompromised patients with a high risk of fungal infections. Air levels of Fusarium and Aspergillus were found to increase in hospital environments after running showers multiple times (Anaissie et al. 2002b; Warris et al. 2001).

Penicillium species have been frequently recovered from water in the various studies performed. Several of the species in both genus Penicillium and Aspergillus are known to produce mycotoxins in other substrates, such as food and beverages (Moreau 1979; Pitt & Hocking 1999). Interestingly, detection of aflatoxins produced by A. flavus in water from a cold water storage tank was demonstrated by Paterson and colleagues (1997). In addition, in vitro studies by Kelley et al. (2003) concluded that mycotoxins and other metabolites can be produced by fungi in water. Mycotoxins produced in water will of course be extremely diluted, and are perhaps of minor concern. Nevertheless, water is occasionally stored in cisterns or reservoirs, or even in bottles, for prolonged periods. In such cases, the concentrations of mycotoxins may increase. Large amounts of water are consumed daily, and daily intake over many years of even small amounts of mycotoxins may be hazardous to human health. In addition, fungi and fungal toxins have been considered as potential biological weapons, as mycotoxins, such as aflatoxins or ergot alkaloids, can be purified and deliberately spread in water by nefarious actions (Paterson 2006).

When it comes to the allergic potential of fungi in water, three cases have been reported from Sweden and Finland. An outbreak of skin irritations in humans in the city of Råbacka in Sweden was reported by Åslund (1984). The symptoms were associated with taking showers and baths, and analyses of the water resulted in recovery of 77 up to 3100 CFU 100 ml⁻¹ water of the fungi Phialophora richardiae. Two case reports from Finland indicated fungal contaminated water as the source of hypersensitivity pneumonitis. Metzger and...
colleagues (1976) indicated that elevated levels of the fungi *Aureobasidium pullulans* in water from a home sauna were causing hypersensitivity pneumonitis, and the symptoms were referred to as ‘sauna-takers disease’. Muittari *et al.* (1980) reported an outbreak of symptoms similar to hypersensitivity pneumonitis after taking saunas, baths, and showers, involving more than 100 people. Symptoms were even reported after laundering and dishwashing. The subsequent investigation indicated that the epidemic was caused by water contaminated by microorganisms, such as *Aspergillus fumigatus*, *Mucor*, *Absidia*, *Candida*, coliform bacteria, and other bacterial species. The implication of fungi such as *Aspergillus*, *Penicillium* and *Cladosporium* with allergy, asthma and other respiratory problems has been widely investigated with respect to indoor environments (Denning *et al.* 2006; Straus 2004). Several reports have implied that various fungal genera may contribute to the growing epidemic of allergy and asthma observed in the human population (Green *et al.* 2003; Hogaboam *et al.* 2005; Jaakkola *et al.* 2002; Kauffman & van der Heide 2003; Lugauskas *et al.* 2004; Schwab & Straus 2004). For instance, *Trichoderma viride*, which was found to be the most dominant species in Norwegian drinking water (Hageskal *et al.* 2006), has been associated with asthma in children living in water-damaged homes (Vesper *et al.* 2006). The allergic potential of fungi in drinking water has not been studies, and would be pure speculation, although the possibility cannot be totally rejected. Large epidemiological studies are needed to investigate such correlations.

Several microorganisms can cause taste and odour problems in drinking water, and such sensoric problems have been connected to the presence of fungi. Several fungal species, such as, e.g. *Chaetomium globosum* (Kikuchi *et al.* 1981), have been found to produce geosmin, a compound often associated with earthy odour and taste in drinking water (Paterson *et al.* 2007). In addition, fungi may produce a variety of other compounds with distinctive off-flavours and tastes (Ezeonu *et al.* 1994; Kaminski *et al.* 1974; Mattheis & Roberts 1992; Montiel *et al.* 1999; Nyström & Nyström 1992). However, the recovery of fungi from a water source does not necessarily mean that their presence in water is causing the sensoric problem. Odours and foul-tasting compounds may also be produced terrestrially or in biofilms in piping systems and then washed into aquatic environments. For the same reason, the lack of fungal recovery from a water source does not mean that they are not involved in causing the sensoric problems.

Fungi have the ability to grow attached to a substrate, forming part of microbial biofilms on pipe surfaces, debris, or sediments. They are likely to become established where forming part of microbial biofilms on pipe surfaces, debris, they are not involved in causing the sensoric problems. Odours and foul-tasting compounds may also be produced of fungi from a water source does not necessarily mean that in biofilms on water and wastewater pipe surfaces (Doggett 2000; Hendrickx *et al.* 2002; Kelley *et al.* 2003; Nagy & Olson 1985; Paterson *et al.* 2006; Skaar & Østensvik 2005). Fungi established in biofilms in water systems may be protected and are more resistant to water treatment, and fungi may also colonize filters in treatment plants, and consequently affect the water treatment. The occurrence and distribution of filamentous fungi was investigated in a Norwegian study, and the results indicated that several species of filamentous fungi have the ability to establish in biofilms or sediments in water distribution systems (Hageskal *et al.* 2006). The ecology of fungi in biofilms has been studied only to a small degree, and further research should aim to investigate the character of fungi in biofilms.

**Limits in the methodology**

A critical point with respect to the study of fungi in water is how the analyses are performed. Most studies conducted apply culturing methods for analysing fungi in water. The main disadvantage of culturing fungi is that it requires the intermediate and often time-consuming step of obtaining each organism in isolation on appropriate media. There is no international standard method described for analyses of fungi in drinking water, hence several isolation procedures are used in the various studies preformed. Membrane filter techniques are mostly employed, applying volumes of 10–100 ml water (Arai *et al.* 2002b; Arvanitidou *et al.* 1999; Doggett 2000; Gonçalves *et al.* 2006b; Gonçalves *et al.* 2006a; Hageskal *et al.* 2007; Hapcioglu *et al.* 2005; Hendrickx *et al.* 2002; Hinzelin & Block 1985; Kanzler *et al.* 2007; Kelley *et al.* 2003; Nagy & Olson 1982; 1985; Niemi *et al.* 1982; Ormerod 1987; Fires-Gonçalves *et al.* 2008; Varo *et al.* 2007; Warris *et al.* 2001; Zachos & Martikainen 1995). Another technique performed is direct plate-spread with volumes of 0.1–1 ml water (Franková 1993; Franková & Horčík 1995; Gottlich *et al.* 2002). Centrifugation of water to obtain the fungal propagules or direct microscope observation are also applicable methods (Mara & Horan 2006). These different isolation procedures may result in different detection limits of fungi in the water.

The isolation medium also varies between the investigations performed. High nutritional media are often used, such as Sabouraud dextrose agar (Hapcioglu *et al.* 2005), Sabouraud glucose agar (Kanzler *et al.* 2007), and malt extract agar (Niemi *et al.* 1982). Others use media that inhibit overgrowth of fast-growing fungi, such as dichloran 18 % glycerol agar (DG18) (Hageskal *et al.* 2007). DG18 is now recommended as a general medium for the isolation and enumeration of fungi in foods with high water activity (aw > 0.90) (Samson *et al.* 2004), and may, therefore, also be suitable for water analyses. Half-strength corn meal agar (CMA/2) is an example of the use of a low nutritional medium (Gonçalves *et al.* 2006a). The use of different media may result in selectivity towards some fungal species and loss of others. Kinsey and colleagues (1999) recommended a combination of both high and low nutritional media, to obtain the complete cross-section of fungi present in water. Although we have to keep in mind that there will always be fungi that will go undetected. The incubation temperature is also important for what fungal species are obtained. The incubating temperatures range from 20–37 °C in the studies preformed, and will consequently result in recovery of different genera and species.

In order to assess the contribution of fungi to problems in drinking water the quantification is essential. Quantification of fungi in water is mostly assessed by counting total fungal colonies on agar plates, referred to as colony-forming units (CFU) per volume of water sample investigated (Mara & Horan 2006). Due to the nature of filamentous fungi, the data generated in this way may be difficult to interpret. Fungi have
a tendency to be unevenly distributed in water, which may cause difficulty in ensuring representative samples. A frequent problem in quantifying fungi is overgrowth of the filter, which can occur very quickly. Therefore, it is very important to monitor the plates and remove new colonies as soon as they appear. Another problem is that not all fungi are able to grow under laboratory conditions. In consequence, the currently used quantification methods will never provide the precise number of fungi, but may give a fair indication of the level of fungi present in water. Chemical methods, such as analyses of ergosterol for quantification of total fungal biomass, as suggested by Kelley & Paterson (2003), could be useful as supplemental methods.

The identification of fungi has mainly been based on morphological identification keys. This identification is subjective, relying on the knowledge and experience of the individual researcher, and also on good and updated identification keys. A frequent problem is that some fungi never sporulate, thus cannot be identified morphologically. DNA sequencing is not dependent on sporulating fungi or even viable fungi, and is a good supplement to morphological identification. Hence, this method relies on the accuracy of public databases, such as GenBank. Analysis of fungal metabolites could also contribute to ensure correct identification. In many of the studies performed, the fungal colonies have only been identified to genus rather than to the species level. This makes the consideration of the occurrence of species difficult, as species of the same genera may have very different characters with respect to, for example, toxin production, pathogenicity, and allergenic potential. Identification to the species level should preferably be done, at least for the most dominant species.

In consequence, these limits in the methodology makes comparisons between the different studies difficult, and may explain much of the variation in the results obtained. Another crucial limitation of fungal water studies is that the information regarding the acceptable or normal levels of fungi in drinking water is limited. Further research is needed to increase this knowledge. Almost without exceptions, regulations for the occurrence of fungi in drinking water do not exist. To the authors’ knowledge, only the Swedish water regulation authority (Anon. 2003) includes specifications on fungi. The methods for analysing fungi in drinking water should be standardized.

Contradictions in interpreting the significance

The main contradictions of fungal water studies are basically the divided opinions with respect to interpreting the importance of the fungal results. How important is the occurrence of fungi in drinking water when it comes to health? Some researchers state that nosocomial aspergillosis is waterborne (Anaissie & Costa 2001), and recommend that hospitalized patients with high risk of fungal infection should avoid exposure to hospital water and use sterile water instead (Anaissie et al. 2002a). Others state that the problem with fungi in water is substantially overestimated, and requires further evidence before acting (Hunter 2003).

Common for most fungal infections is the incidence of invasive disease in a variety of immunocompromising conditions. According to a review by Denning (2006), patients at high risk of fungal infections include the use of corticosteroids; patients suffering from pulmonary disorders, diabetes mellitus, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), cystic fibrosis, cancer, and patients who have undergone gastrointestinal or cardiac surgery, and organ transplants. It has been estimated that the number of immunocompromised individuals will continue to increase in the future. Factors responsible for this rise include the increase in the elderly population in countries with advanced medical technology, increased incidence of many cancers, and increased numbers of transplantations (Perfiroth et al. 2007).

Pathogens resistance to antifungal drugs continue to emerge (Kauffman 2004; Marr et al. 2002; Pfaller et al. 2006; White 2005), presenting challenges in the treatment of fungal infections. Despite all diagnostic and therapeutic efforts, the outcome of such infections is often fatal. In fact, the mortalities are as high as 50–100%, and nearly 100% in untreated cases (Denning 1998, 2006; VandenBergh et al. 1999). Infections due to fungi other than Aspergillus and Candida species are particularly lethal (Husain et al. 2003; Pfaller et al. 2006). The treatment of fungal infections is often prolonged and is also very expensive. Therefore, preventive measures may be of major importance.

The source of fungal infections is not yet determined, but inhalation of airborne fungal spores is suggested to play a crucial role in the epidemiology (Perfiroth et al. 2007). However, extended air filtration has not had the desired effect in decreasing the number of infections. Genotyping of environmental and clinical fungal strains have indicated genetic relatedness between clinical strains and water-related strains (Anaissie et al. 2002b; Warris et al. 2003), whereas others have indicated that it may not be possible to determine one exact source of infection, thus several sources, both nosocomial and community acquired, are possible (Menotti et al. 2005). Although water as a possible source of fungal infections is controversial, different researchers seem to agree on one point that proper epidemiological studies to determine the real contribution of water supplies to the fungal disease burden should be developed. An important issue that merits consideration is, nevertheless, whether fungi in water can just be ignored, or whether precautions should be considered in the case of vulnerable groups. In future, preventive measures may become more important than ever, and the occurrence of fungi in drinking water may require extended regulations. Further research is needed to investigate measures that may be appropriate.

Precautionary measures

In the United States, the Centers for Disease Control and Prevention (CDC 2000) have developed recommendations for the prevention of nosocomial infections that also includes hospital-acquired aspergillosis. The recommendations are as follows: (1) to minimize exposure of high-risk patients to potential sources of Aspergillus species, (2) to minimize activities that may aerosolize Aspergillus and other fungi, and (3) to eliminate the source of aspergilli. No specific practical
measures with respect to water were suggested, leaving the hospitals with as good as no recommendation. It is important though, that these recommendations require consideration of the hospital water systems. According to the review on aspergillosis by Denning (2006), there are several precautionary measures related to air quality that may reduce the incidence of aspergillosis in hospitalized, high-risk patients. These measures include the use of high-efficiency particulate air filtration and laminar air-flow in protective areas, complete separation of construction and clinical areas, and prevention of exposure to unsterilized pepper or spices, and potted plants or shrubs. Interestingly, the review also states that attention should be paid to routine maintenance and cleaning of showers and water systems.

Other precautionary measures in the case of high-risk patients have been suggested with respect to water quality, mainly by Anaissie and colleagues (2001, 2002b, 2003). First, it was recommended that hospital water should be tested for the presence of disease-related fungi. High-risk patients should avoid exposure to hospital water and use sterile water for drinking, not only because of the danger of fungal infections but also due to other serious waterborne pathogens. Anaissie et al. (2003) suggested that high-risk patients should avoid showers, and that these patients should use sterile sponges for bed-baths instead. It was also suggested that shower facilities should be thoroughly cleaned to prevent fungal aerosols, and that education of both patients and healthcare workers should be emphasized.

With respect to the rest of the community, the significance of fungi in drinking water has scarcely been evaluated. Two contradicting aspects are of importance when considering fungal water studies with respect to the community. First, we have the request for publishing important scientific results, and that the public have a right to know what is in the water they are consuming daily. Second, we do not want to create unnecessary concern in the population. In light of the knowledge obtained from fungal water studies, and the insufficient epidemiological knowledge about health impacts, it seems like a good idea to take precautionary measures for high-risk patients. Other possible health impacts with respect to the rest of the population are poorly understood, and require further studies.

**Practical consequences of fungi in water**

At present, very few practical measures have been implemented based on the knowledge from the various studies of fungi in water. This is basically due to the need for increased knowledge about the significance of the presence of fungi in drinking water for human health. The exceptions are some precautionary recommendations related to high-risk patients in hospitals, as previously mentioned. As a consequence of episodes of water problems caused by fungi, the analysis of fungi was implemented in Swedish drinking water regulation from 1993 (Anonymous 2003). However, this is not an analysis required on a routine basis, thus fungal analyses of water are conducted only in cases of complaints of taste and odour problems. The limit for the occurrence of fungi in water is 100 CFU per 100 ml water according to the Swedish regulation authority.

A starting place could be to monitor the water distribution systems to obtain baseline information regarding fungi in drinking water. Special emphasis should be given to water systems in healthcare units, where it will be important to obtain information on the presence and frequencies of fungi, and what species that are obtained. If future analyses of water are to include fungi, this will require both better methods and new knowledge to establish adequate parameters. A natural solution to fungi in drinking water would be water treatment. However, the various fungal water studies performed establish that fungi can survive both treatment and disinfection, and that most of the current water treatment methods are not sufficient against fungi. The control strategies and efficiency of water treatment against fungi has been studied only to a small degree, and the results are not consistent. For example, chemical coagulation was reported to be the most effective treatment method by Niemi et al. (1982), whereas in a study by Kelley and colleagues (2003) sand filtration was reported to give better removal of fungi than coagulation with iron. Chlorination used for disinfection of water was found to be insufficient (Franková & Horecká 1995; Nagy & Olson 1985), whereas the use of chlorine dioxide and ozone were reported as the most effective water treatment methods against fungal spores by Kelley et al. (2003).

A frequently used water-treatment method is UV radiation. With respect to fungi in drinking water, UV may have a positive effect, as indicated by Kanzler et al. (2007). However, as the UV sensitivity of fungi often is related to pigmentation, fungi with pigmented spores, such as Aspergillus and Penicillium, have better protection against radiation and are less sensitive to UV (Waipara 1998). This was also experienced in the study by Kelley et al. (2003) where melanised, thick-walled species predominated after water treatment. The effect of dose of UV should be tested on fungi in drinking water. A recently published study on the impact of copper and silver ionization on fungal colonization of hospital water systems indicated that this treatment method could be usable as an in-house treatment in hospitals (Pedro-Botet et al. 2007). Internal water treatment inside hospitals may be a good solution to the fungal problem. Also, food production plants and other establishments where water quality is essential could benefit from extended water treatment, as many of the fungal species recovered from drinking water are common contaminants of food and beverages. Yet, it should be noted that fungi may also establish in biofilms in internal water systems. So-called 'point of use water filtration' on taps and showers in, for example hospital wards, may also be a practical way to reduce the risk of aerosolizing waterborne fungi.

Although few practical measures are implemented at present, monitoring the water for the presence of fungi, adequate water treatment, and regulation may be required in the future, at least with respect to ensuring adequate water quality in healthcare institutions.

**Conclusions**

Studies of fungi in drinking water have demonstrated that fungi are relatively common in water distribution systems. Species of pathogenic, allergenic, and toxigenic concern are
isolated from water, sometimes in high concentrations. Fungi in water may be aerosolized into air, and introduced to immunocompromised patients, and sensoric changes have been associated with the occurrence of fungi in drinking water systems. The main limitations of fungal water studies lie within the methodology. The differences in methodology limit the consistency in the studies preformed, and make direct comparisons almost impossible. Culturing methods may limit the accuracy of the results and are time-consuming, thus better and more standardized methods for analyses should be designed. Identification of fungi to the species level is difficult, and may require a polyphasic approach, implementing morphology and molecular techniques, as well as metabolite analyses. In future, new molecular and spectral methods may prove to be usable tools for analyses of fungi in water.

The possible health impacts of fungi in water are still contradictory, although precautionary recommendations and measures implemented in the case of high-risk patients now include the elimination of waterborne fungi. In future, monitoring of water systems for the present of fungi may be required, especially in hospital water systems. Adequate water treatment could be a solution, and further studies are required, both with respect to establishing accurate methodologies and to investigate the effects of water treatment against fungi in water. In addition, the water suppliers need to be informed about the different aspects of fungi in water. Epidemiological studies should also be conducted to determine the health significance of fungal-contaminated drinking water. Until knowledge about the significance of fungi in water is obtained, controversy will most certainly remain. In the meantime, we should be concerned about fungi in drinking water for the same reasons as for air-borne fungi, because increased levels may reduce the quality of drinking water, and constitute a potential health risk.

If the microbiological quality of drinking water is to include fungi, implementing fungal parameters in the water regulations may be required. Fungi are a difficult group to examine, and fungal water studies require experience and caution. However, that should not mean that fungal contamination of drinking water can be ignored. As fungi may influence the water quality in several ways, the mycobionta of drinking water should be considered when the microbiological safety and quality of drinking water is assessed.

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