

EXECUTIVE SUMMARY

Fungi are eukaryotic, heterotrophic organisms, including both single-celled yeasts and multi-cellular filamentous fungi. Many fungal species can survive in oligotrophic environments, through scavenging nutrients from the substrate which they colonise, or the air or water in which they live. Fungi also produce secondary metabolites, some of which are toxins. Some of the fungal species and the metabolites they produce are human pathogens or allergens.

Fungi can enter drinking water distribution systems through several contamination pathways, including treatment breakthrough, deficiencies in stored water facilities cross-connections, mains breaks and intrusions, and during mains installation and maintenance. Once introduced, fungal species can become established on the inner surfaces of pipes, including interaction and reaction with sealings and coatings, and biofilms within distribution systems, or can be suspended in the water. Water companies in England and Wales have in place procedures to minimise the risk of microbial contamination.

The results of sample analysis from customer taps and other points within distribution systems often reveal higher numbers of fungi than the analysis of samples following treatment, prior to entry into the distribution system. Such increases through the distribution system could be due to two reasons: i) the fungi that remain present after treatment multiply within the system or that fungi that were only partially inactivated later recover, and ii) fungi enter the system via pathways of secondary contamination. Accumulation of fungi in stored water at the consumer end, such as in water tanks, has also been observed. For example, higher numbers of colony forming units of *Aspergillus* have been found in hospital water storage tanks than in the municipal water supply.

A number of different methods of analysing drinking water samples are used, including culture, measurement of ergosterol, quantitative PCR, gene markers and probes, protein probes, direct observation and mass spectrometry. There is currently no international standard specifically for the measurement of fungi in drinking water, and there is no widespread adoption of other relevant standards. Therefore, differences in analysis methods limit the extent to which results can be compared between studies. Furthermore, the most commonly used unit of quantification is numbers of Colony Forming Units (CFUs). However, this measure does not necessarily give an accurate representation of the number of fungi present in a sample, as not all species can be detected using culturing methods. It is also likely that one colony is formed of many different fungal structures, such as hyphae, conidia, conidiophores, from different "individuals" clumped together into one CFU.

Relatively few studies have investigated the fungi found in treated drinking water. The numbers of fungi found in the existing studies range from 1 CFU per litre to 5000 CFU per litre. Of the sixty-five genera that have been isolated in the studies analysed during this review, the majority were filamentous fungi. The most commonly isolated genera were *Penicillium*, *Cladosporium*, *Aspergillus*, *Phialophora* and *Acremonium*.

A number of factors influence the ecology of fungal taxa in drinking water distribution systems. Fungi are more likely to be isolated from surface-water derived drinking water than from that derived from groundwater. This may be related to the larger amounts of organic matter in surface water. Differences in acidity and calcium content may also account for some of the variation. Fungi were also more likely to be isolated from cold water than hot water, although this depends on the species considered and their optimum temperature range. Associations between fungi and bacteria are also relevant, in order to determine if fungal numbers correlate with commonly measured bacterial parameters of drinking water quality. However, there is no consensus in the literature of whether such a correlation exists.

Biofilms are an important habitat for fungi in drinking water. Their development is influenced by many factors including temperature, nutrient concentration, pipe material and water flow rate. However, how exactly such factors affect biofilm development and specifically the role of fungi in biofilms is not well known.

Water treatment appears to reduce the number of fungi in water, without removing all of them. Melanised species are particularly able to resist water treatment. Different treatment processes have different removal efficiencies, although it is not agreed which process is the most efficient method.

Many of the fungi that have been isolated from treated drinking water are known to be pathogenic, particularly *Aspergillus* and *Candida*. Although healthy individuals may suffer from superficial or localised fungal infections caused by these taxa, there is little evidence that their pathogenicity arises from their presence in drinking water. More severe invasive infections are limited to those with immune deficiency, due to for example HIV/AIDS, chemotherapy, immunosuppressive therapy following transplants, or other underlying health conditions, such as cystic fibrosis or diabetes mellitus. Such invasive infections carry a high mortality rate, estimated at between 50 and 100%, depending on the species involved. The extent to which infections arise from at-risk individuals is not well known. The continuing rise of *Aspergillus* infections in at-risk individuals despite hospital-based measures to control airborne fungal spores suggests that another environmental source exists. A small number of studies have linked the genotype of fungi recovered from patients to that of fungi from hospital water supplies. The significance of exposure via drinking the water, as opposed to washing with it, has not been specifically studied. Aerosolisation of fungi during showering or from running taps has received more attention; numbers of airborne fungi have been found to increase after running taps or showers. Infections caused by *Candida* species

are also significant, and while this genus has been isolated from drinking water the significance of exposure via drinking water is not known.

Fungi have also been linked to allergic disease, including worsening of asthma symptoms, hypersensitivity pneumonitis and skin irritation. Fungi known to provoke allergic responses in susceptible individuals, such as *Alternaria* spp., *Aspergillus*, spp., *Cladosporium* spp. and *Penicillium* spp., have been isolated from drinking water. Symptoms have arisen due to exposure when showering, bathing or using saunas, or from exposure to water-damaged buildings.

Some fungi, including *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp. and *Claviceps* spp. are known to produce mycotoxins such as patulin, aflatoxins and zearalenone. It is thought that concentrations of mycotoxins in drinking water are low due to being diluted. No reports of disease caused by mycotoxins in drinking water have been identified.

Indirect health impacts may arise from association with other pathogens. For example, colonisation of the respiratory tract with *Candida* spp. increases the risk of ventilator-associated pneumonia from *Pseudomonas aeruginosa*. Biocorrosion of pipes by fungal species may represent a second indirect health impact. This process can lead to increased metal concentrations in drinking water and corrosion tubercles also provide habitat for fungi.

Secondary metabolites produced by fungi, particularly those growing in localised pockets near the consumer end may be responsible for altering the taste and odour of drinking water. It is thought that the threshold level for numbers of fungi that can cause such issues may be around 10^2 - 10^3 CFU l⁻¹. While problems with taste and odour do not necessarily imply a health risk they are often perceived as such by the consumer.

Due to the relative lack of literature on the topic of fungi in drinking water, there are a number of aspects that remain poorly understood. Research needs include a need to determine the importance of drinking water as the environmental source of fungal infection in vulnerable or at-risk population groups. Greater knowledge on the importance of ingestion as opposed to inhalation or skin contact as exposure pathways for fungi in drinking water will ensure that mitigation measures for at-risk patients are appropriate. Finally, greater understanding of the effect of the analytical method on the results obtained and development of a standard method would facilitate further research into fungi in drinking water.

- Fungi present in drinking water may cause severe fungal infections in immunosuppressed patients. In a small number of studies, drinking water supplies have been found to be the source of infection, although the pathway of infection (drinking vs. inhalation of aerosolised spores while showering) is uncertain
- Additional research would be required to further investigate the link between fungi in drinking water and infections in immunosuppressed patients, address its frequency from an epidemiological viewpoint and determine the fungal species and quantity in water that may cause such infections.
- The present risk of health impact for the general population is thought to be low based on current knowledge. Therefore current procedures for water system maintenance or water monitoring and treatment might be sufficient.
- The literature should be reviewed periodically in order to take account of potential environmental or procedural changes, such as climate change or altered water treatment processes.
- If future scientific results suggest an increase in risk, pilot epidemiological studies and surveillance may be justified.
- Further research and monitoring (if needed) would be facilitated by the use of a simpler and quicker method of fungal quantification and identification than culture.
- Greater knowledge of the associations between fungi and bacteria would help to ascertain whether commonly measured bacterial parameters of water quality correlate with fungi presence.