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Diversity and Significance of Mold Species in Norwegian Drinking Water▽

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In order to determine the occurrence, distribution, and significance of mold species in groundwater- and surface water-derived drinking water in Norway, molds isolated from 273 water samples were identified. Samples of raw water, treated water, and water from private homes and hospital installations were analyzed by incubation of 100-ml membrane-filtered samples on dichloran–18% glycerol agar. The total count (number of CFU per 100 ml) of fungal species and the species diversity within each sample were determined. The identification of mold species was based on morphological and molecular methods. In total, 94 mold species belonging to 30 genera were identified. The mycobiota was dominated by species of *Penicillium*, *Trichoderma*, and *Aspergillus*, with some of them occurring throughout the drinking water system. Several of the same species as isolated from water may have the potential to cause allergic reactions or disease in humans. Other species are common contaminants of food and beverages, and some may cause unwanted changes in the taste or smell of water. The present results indicate that the mycobiota of water should be considered when the microbiological safety and quality of drinking water are assessed. In fact, molds in drinking water should possibly be included in the Norwegian water supply and drinking water regulations.

Exposure to filamentous fungi may have a wide variety of health consequences in humans, and there is an increasing awareness of molds as a cause of human allergies and infections (12, 20). It is generally accepted that the main route of fungal infection is inhalation of mold conidia from contaminated indoor air. However, several studies have suggested that exposure through inhalation of aerosolized molds from water can occur (1, 61) and genetic relatedness between clinical mold strains and water-related mold strains has been reported (4, 62).

Investigations have shown that water distribution systems may disseminate potentially allergenic, toxigenic, and opportunistic fungal species to hospitals and private homes (34). In addition, studies have revealed species that may produce unwanted flavors and odors in water (10, 46) and species of technical concern that have the ability to oxidize pipe surfaces in the water distribution network (19). Reports from Sweden and Finland have indicated allergic and respiratory health effects of fungi resident in water (9, 41, 43).

A wide variety of species have been isolated from water in various investigations. Some of the mold species isolated from water samples are known to be strongly allergenic skin irritants or may cause infections in immunosuppressed individuals such as AIDS, cancer, and organ transplant patients and persons with asthma or various respiratory problems (12, 14, 64, 67). Mold spores and hyphal fragments may be aerosolized in indoor air when contaminated water passes trough showerheads, taps, or toilet cisterns. This could result in respiratory exposure to potentially harmful species. Several mold species may survive disinfection and water treatment and could thus contaminate the water that reaches consumers. A few investigations have also reported molds to be residents of biofilms in water pipe systems (15, 34, 44). Fragments of biofilm may be released into the water stream from time to time, resulting in increased contamination. Molds established in biofilms may also act as conidial reservoirs.

Relatively little attention has been paid to the occurrence and characteristics of mold species in water distribution systems in Norway. Microfungi are not included in the Norwegian drinking water regulations (6), although a standard method for analyzing microfungi in water was published in 1991 (5). Warris et al. (61) identified filamentous fungi belonging to 13 different genera in water samples from taps, showers, and the main pipe at the Rikshospitalet University Hospital of Oslo, Norway, but only three *Aspergillus* spp. were identified to the species level. Additional studies are needed to determine which mold species reside in drinking water and to what extent they may survive water treatment and contaminate drinking water that reaches consumers.

In a previous study on the occurrence of molds in drinking water (28), the likelihood of recovering molds was found to be three times greater in surface water-derived than in groundwater-derived water samples. It was also indicated that molds are more likely to be detected in cold water and in showers than in hot water. No significant seasonal variations in mold recovery were observed. However, none of the molds were identified to the species level in the aforementioned study. Therefore, data on the occurrence and distribution of individual mold species throughout the water system are needed, both in groundwater-derived and in surface water-derived water supply systems. The aim of the present study was to identify

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and determine the diversity, occurrence, and distribution of mold species in public drinking water in Norway and to consider the possible significance of the species identified for water quality and human health.

MATERIALS AND METHODS

Water samples. The sampling and fungal isolation procedures used were described in a previous report on the occurrence of molds in drinking water (28). Briefly, 273 water samples were collected from 14 water supplies, of which 10 had surface water sources ($n = 195$) and 4 had underground water sources ($n = 78$). Each water supply was sampled three times. Sample points included raw water and treated water at the treatment plants and hot- and cold-water taps and showers in hospitals and private homes attached to each supply network.

Isolation of molds. The isolation procedure used was based on a Norwegian standard method for analysis of microfungi in water (5), with some modifications as previously described (28). Briefly, 100-ml water samples were membrane filtered and the filters were incubated on dichloran–18% glycerol agar (29) in darkness at 20°C ± 1°C for up to 2 weeks. The number of colonies was determined and expressed as the number of CFU per 100-ml water sample. The isolation frequency (percent) for each species was calculated by dividing the number of positive samples by the total number of samples. Concentrations of each species were expressed as minimum and maximum numbers of CFU per 100 ml. For isolation of pure single colonies, positive cultures were subcultured on potato dextrose agar (Oxoid, Basingstoke, United Kingdom) or malt extract agar (Oxoid) and incubated at 25°C ± 1°C for 7 days. Representative isolates were stored on potato dextrose agar slants at 4°C.

Identification of molds. For identification, the molds were subcultured on suitable agar medium as described by Samson et al. (56). Whenever possible, the molds were phenotypically identified to the species level by macroscopic and microscopic characteristics by employing light microscopy (12, 16–18, 35, 50, 51, 53–57). Slide preparations were stained with lactofuchsin, with or without alcohol, in lactic acid or in distilled water. To ensure correct identification of closely related species, some *Penicillium* species were, in addition to morphological identification, confirmed by detection of indole metabolites by a filter paper method (39). Briefly, a piece of filter paper dipped in Ehrlich reagent (56) was placed on an agar plug with mycelia. In the case of indole metabolite production, a violet ring appeared within 10 min.

Sequencing. A few isolates that could not be identified morphologically were identified by sequencing of the rRNA gene internal transcribed spacer region (ITS), comprising ITS1, 5.8S, and ITS2. The *Fusarium* isolates were identified by sequencing parts of translation elongation factor 1 alpha (TEF-1α).

DNA was extracted from mycelium and spores by the cetyltrimethylammonium bromide protocol described by Gardes and Bruns (23), with some modifications: (i) scaling to a starting volume of 600 μl cetyltrimethylammonium bromide and (ii) a single step of freezing (−80°C, 15 min) before incubation at 65°C.

PCR amplification of ITS and TEF-1α with the primers ITS1-ITS4 (65) and EF1-EF2 (47), respectively, was conducted on a PTC-0200 (Peltier Thermal Cycler; MJ Research, Waltham, MA) with each primer at 1.7 μM, 2 μl genomic DNA, PuReTaq Ready-To-Go PCR beads (Amersham Biosciences, United Kingdom), and milliQ water adjusted to a final volume of 25 μl. The PCR protocol consisted of initial denaturation at 95°C for 10 min; 38 cycles of 95°C for 1 min, 55°C for 45 s, and 72°C for 1 min; and a final elongation of 72°C for 5 min. Purified PCR products were sequenced in both directions with the primers previously mentioned by DYEnamicET dye terminator chemistry (Amersham Biosciences) and run on a MEGABACE 1000 (Amersham Biosciences). Assembly and manual editing of the sequence chromatograms were conducted in Contig Express, Vector NTI Advance (Invitrogen, Frederick, Maryland).

Identifications based on sequences. Strains of *Fusarium* were identified by sequence similarity searches by using TEF-1α sequences in the FUSARIUM-SEQ v.1.0 database (24). All of the other sequenced strains were identified by similarity searches with ITS sequences in EMBL/GenBank.

RESULTS

Mold diversity. In total, 686 fungal colonies were isolated from water samples and cultivated for identification. Some of the isolates were sterile, and others did not survive cultivation and thus could not be identified. Nevertheless, 94 different species belonging to 30 genera were identified (Table 1). As many as 24 species were isolated from groundwater-derived samples, and 89 species were isolated from surface water-derived samples. Eight species were molecularly confirmed, and isolates and EMBL/GenBank accession numbers are presented in Table 1. The sequences were all between 98% and 100% similar to the database sequences.

Occurrence of species. The mycobiota was dominated by species of *Penicillium*, but *Trichoderma* and *Aspergillus* species were also frequently isolated, some of them throughout the drinking water distribution system. The most frequent species was *Penicillium montanense*, followed by *P. spinulosum* and *Aspergillus ustus*. Of the dematiaceous fungi, *Phialophora fastigiata* and *Phoma* species were often isolated. *P. montanense* was the dominant species in the surface water-derived samples, while *A. ustus* was the most frequently isolated species in groundwater-derived samples. The *Trichoderma* isolates could only be identified to the genus level.

The minimum and maximum concentrations of the individual species are given in Table 1. Again, species of *Penicillium*, *Aspergillus*, and *Trichoderma* were among those with the highest maximum number of CFU per 100-ml sample. Of all of the *Penicillium* species isolated, *P. montanense* had the highest maximum concentration, followed by *P. spinulosum*. A maximum concentration of 16 CFU/100 ml was observed for *A. ustus*. In addition, *Acremonium butyri*, *Cladosporium cladosporioides*, and *Beauveria brongniartii* were among the species with the highest maximum concentrations. A high concentration of *Fusarium dimerum* was unexpectedly recovered from one groundwater-derived shower site.

Distribution of species. The distribution of different species found at the different sampling points is presented in Table 2. In general, no large differences could be observed in the number of different species recovered from raw water compared to treated water. With the exception of hot water from taps in surface water systems, where a reduction in the number of species was observed, the species diversity was more or less maintained throughout the water system installations. Only five species were isolated from the hot-water sampling sites: *Aspergillus fumigatus*, *A. ustus*, *Phialophora verrucosa*, *Phoma* sp., and *Trichoderma* sp. No significant seasonal differences in species diversity could be observed.

The genus *Penicillium* was found to be particularly widespread in the surface water samples, and 37 different *Penicillium* species were identified (Table 1). They occurred frequently at all sampling points except the hot-water taps. *Penicillium* species were recovered from approximately 41% of the surface water samples and 5% of the groundwater samples. Considering all of the water samples, a decrease of 22% in *Penicillium* recovery could be observed in the treated water samples compared to the raw water samples (Fig. 1), while no large difference was observed in samples from cold-water taps and showers compared to treated water.

The genus *Aspergillus* was represented by five species, two of which, *A. ustus* and *A. fumigatus*, were present in samples throughout the water distribution system (Fig. 1). *A. ustus* was particularly frequently isolated from groundwater-derived samples, especially from the hot-water tap samples. *A. ustus* was recovered from 16% of the hot-water tap samples, while 12% of the raw water and only 7% of the treated water samples yielded *A. ustus*. The clinically important species *A. fumigatus*

TABLE 1. Mold species isolated from Norwegian water samples

Mold species	No. of positive samples (n = 273)	% Positive samples		Min-max range[a] (CFU/100 ml)
		Surface water (n = 195)	Groundwater (n = 78)	
Absidia corymbifera (Cohn) Saccardo et Trotter	1	0.5		2
Absidia cylindrospora Hagem	3	1.5		1
Absidia glauca Hagem	1	0.5		1
Acremonium butyri (J. F. H. Beyma) W. Gams	7	2.6	2.6	1–26
Acremonium sp.	1	0.5		1
Acremonium strictum W. Gams	4	1.5	1.3	1–14
Arthrinium phaeospermum (Corda) M. B. Ellis	1	0.5		2
Aspergillus clavatus Desmazières	1		1.3	1
Aspergillus fumigatus Fresenius	15	7.2	1.3	1
Aspergillus niger Tieghem	2	1		1
Aspergillus sydowii (Bain. et Sart.) Thom et Church	1	0.5		1
Aspergillus ustus (Bainier) Thom et Church[b]	26	6.7	16.7	1–16
Aureobasidium pullulans (de Bary) G. Arnaud	6	2.6	1.3	1–3
Beauveria bassiana (Balsamo-Crivelli) Vuillemin	7	3.6		1–2
Beauveria brongniartii (Saccardo) Petch	11	5.6		1–13
Botrytis cinerea Persoon	1	0.5		1
Botrytis elliptica (Berkeley) Cooke[c]	4	2.1		1–2
Byssochlamys nivea Westling	2	1		1–2
Ceratocystis fimbriata (Ellis et Halsted) Saccardo	2	1		1–3
Chaetomium globosum Kunze ex Fries	3	1.5		1–5
Chaetomium sp.	1		1.3	1
Chrysonilia sp.	2	0.5	1.3	1
Chrysosporium pannorum (Link) S. Hughes	1	0.5		1
Cladosporium cladosporioides (Fres.) G. A. de Vries	15	7.7		1–14
Cladosporium herbarum (Persoon) Link	7	3.6		1–2
Cladosporium sphaerospermum Penzig	2	1		1
Epicoccum nigrum Link	3	1	1.3	1–2
Eupenicillium cinnamopurpureum Scott et Stolk	1	0.5		1
Fusarium dimerum Penzig[c]	1		1.3	100
Fusarium oxysporum Schlechtendal[c]	4	1	2.6	1–6
Fusarium sp.[c]	1	0.5		1
Geotrichum sp.	3	1.5		1–2
Lecythophora hoffmannii (Beyma) Gams et McGinnis	4	1.5	1.3	1–3
Leucostoma persoonii (Nitschke) Höhnel[c]	7	3.6		1–4
Monascus ruber Tieghem	7	3.6		1–5
Mucor azygosporus R. K. Benjamin	1	0.5		1
Mucor circinelloides Tieghem	2	1		1
Mucor hiemalis Wehmer	12	6.2		1–5
Mucor plumbeus Bonorden	2	1		1–2
Paecilomyces carneus (Duché et Heim) Brown et Smith	4	1	2.6	1–5
Paecilomyces farinosus (Holmskjold) Brown et Smith	10	4.6	1.3	1–5
Paecilomyces lilacinus (Thom) Samson	1		1.3	4
Paecilomyces variotii Bainier	2	1		1–2
Penicillium brevicompactum Dierckx	15	7.2	1.3	1–7
Penicillium canescens Sopp	8	4.1		1–6
Penicillium chrysogenum Thom	1	0.5		2
Penicillium citrinum Thom	5	2.6		1–4
Penicillium expansum Link	3	1.5		1–5
Penicillium fellutanum Biourge	4	2.1		1–7
Penicillium glabrum (Wehmer) Westling	6	3.1		1–4
Penicillium implicatum Biourge	2	1		1
Penicillium inflatum Stolk et Malla	1	0.5		1
Penicillium janczewskii K. M. Zalessky	15	7.7		1–3
Penicillium janthinellum Biourge	1	0.5		2
Penicillium jensenii K. M. Zalessky	2	1		1–2
Penicillium kojigenum G. Smith[c]	3	1.5		1–4
Penicillium lividum Westling	2	1		4–5
Penicillium megasporum Orpurt et Fenne	2	1		1–3
Penicillium melinii Thom	10	5.1		1–5
Penicillium miczynskii K. M. Zalessky	8	4.1		1–5
Penicillium montanense M. Christensen et Backus	30	14.9	1.3	1–13
Penicillium olsonii Bainier et Sartory	1	0.5		1
Penicillium oxalicum Currie et Thom	1	0.5		4
Penicillium paxilli Bainier	1	0.5		1
Penicillium piscarium Westling	1	0.5		1

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TABLE 1—*Continued*

Mold species	No. of positive samples (n = 273)	% Positive samples		Min-max range[a] (CFU/100 ml)
		Surface water (n = 195)	Groundwater (n = 78)	
Penicillium pulvillorum Turfitt	1	0.5		1
Penicillium purpurogenum Stoll sensu Raper et Thom	1	0.5		1
Penicillium raistrickii G. Smith	3	1.5		1–3
Penicillium restrictum J. C. Gilman et E. V. Abbott	1	0.5		1
Penicillium roseopurpureum Dierckx	1	0.5		2
Penicillium simplicissimum (Oudemans) Thom	6	3.1		1–5
Penicillium solitum Westling	1	0.5		1
Penicillium soppii K. M. Zalessky	8	4.1		1–10
Penicillium sp.	2	1		1
Penicillium spinulosum Thom	26	13.3	1.3	1–12
Penicillium steckii K. M. Zalessky	1	0.5		1
Penicillium swiecickii K. M. Zales[c]	1	0.5		1
Penicillium thomii Maire	4	2.1		1–5
Penicillium verrucosum Dierckx	2	0.5	1.3	1
Penicillium westlingii K. M. Zalessky	1	0.5		3
Phialophora cyclaminis J. F. H. Beyma	1	0.5		1
Phialophora fastigiata (Lagerberg et Melin) Conant	22	9.2	5.1	1–9
Phialophora malorum (Kidd et Beaumont) McColloch	5	1.5	2.6	1
Phialophora melinii (Nannfeldt) Conant	1	0.5		2
Phialophora sp.	2	1		1–2
Phialophora verrucosa Medlar	1	0.5		4
Phoma glomerata (Corda) Wollenweber et Hochapfel	17	8.7		1–10
Phoma sp.[c]	22	9.2	5.1	1–8
Pseudogymnoascus roseus Raillo	1	0.5		1
Scopulariopsis brevicaulis (Saccardo) Bainier	1	0.5		2
Scopulariopsis fusca Zach	1	0.5		2
Staphylotrichum sp.	3	1.5		2
Trichoderma sp.	72	36.9		1–12
Verticillium lecanii (Zimmermann) Viégas	2	0.5	1.3	1–2

[a] Minimum and maximum numbers of CFU per 100-ml water sample, calculated on the basis of the positive samples only. Where the minimum and maximum values are the same, only one value is given.
[b] *A. ustus* was morphologically confirmed by CABI Bioscience, Egham, Surrey, United Kingdom (reference no. IMI 391614).
[c] Species confirmed by sequencing. Accession numbers: AM236581 to AM236588 and AM236787 to AM236789.

was mainly isolated from surface water and was found only on one occasion in a groundwater-derived shower water sample. In surface water-derived samples, this species was isolated from all sample points but only once in a sample from a hot-water tap.

The genus *Trichoderma* was only isolated from surface water samples and from nearly 40% of them. *Trichoderma* species occurred in all of the samples from all of the sampling points in surface water-derived water systems. The raw water samples were more often positive than the treated water samples, with almost 20% lower recovery in treated water (Fig. 1). Further, *Trichoderma* species were isolated from samples collected throughout the water system, except from hot-water taps, where only two recoveries were made.

TABLE 2. Distributions of different species recovered at the sampling points

Sampling point	No. of samples	Total no. of species	No. of species in:	
			Surface water	Groundwater
Raw water	42	51	48	3
Treated water	42	51	43	8
Private home				
Cold-water tap	42	46	43	3
Hot-water tap	42	7	5	2
Shower	42	44	37	7
Hospital				
Cold-water tap	21	36	29	7
Hot-water tap	21	3	2	1
Shower	21	27	22	5

DISCUSSION

The present investigation indicates that Norwegian municipal drinking water may be an important contributor to the transmission of a wide variety of mold species to water consumers. Identification to the species level has provided important information about which mold species may be isolated from drinking water in Norway, at what frequencies the different species occur, their concentrations in the samples, and finally the distribution of the various molds in different parts of the water supply system.

In surface water-derived water systems, the diversity of species isolated from private homes and hospitals was lower than that of species from raw water samples. This finding confirms the expectation that not all of the species that gain access to the water system are able to survive water treatment or establish themselves in the system for prolonged periods. However, it was a surprise that the treated water samples harbored so many different species. In contrast, the groundwater-derived

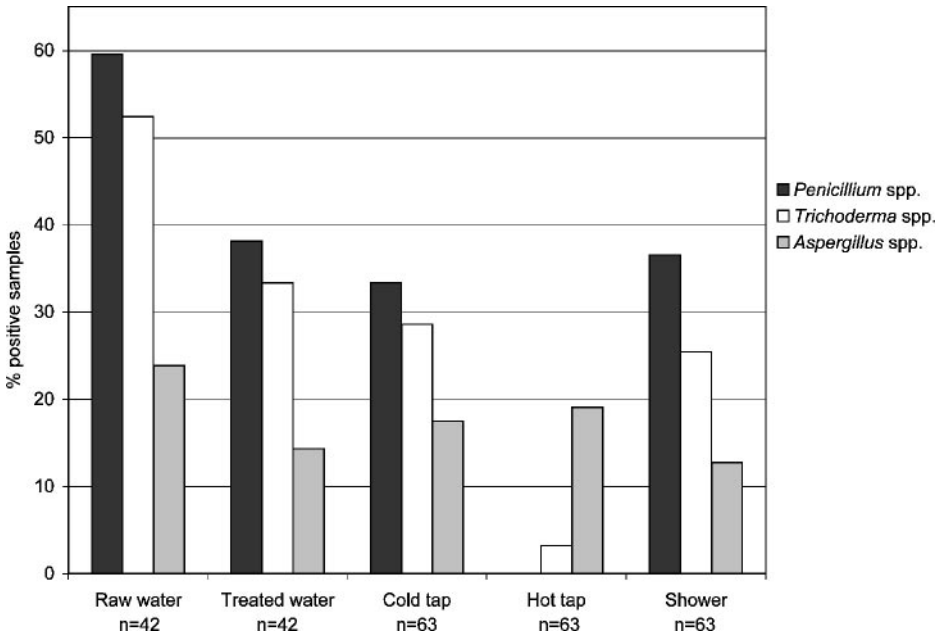

FIG. 1. Trends in the distribution of the three genera with the highest isolation frequencies. The water system installations (cold-water taps, hot-water taps, and showers) represent both private homes and hospitals. n, number of samples.

samples provided the opposite results. Here, the diversity of species increased from raw water to treated water and was maintained throughout the water network, including the hot-water taps. This finding suggests contamination or active growth somewhere in the water system, possibly in biofilms on pipe surfaces. Heating of water seems to kill most of the molds, although some species do survive, as observed in the hot-water tap samples. Thermophilic species may even benefit from these conditions, resulting in growth in warming tanks or hot-water pipes.

In the present study, the total number of species isolated from groundwater was much lower than the number of species isolated from surface water. Although this difference may reflect the fact that more surface water-derived samples were analyzed, the result is in accordance with the significant differences in mold recovery observed between surface water and groundwater, as demonstrated previously (28). Also, the results are generally in agreement with those reported from other studies (21, 44, 66) but contradict the assumption of Warris et al. (63) that groundwater does not contain molds.

The genera *Penicillium*, *Trichoderma*, and *Aspergillus* were particularly often isolated, with a wide diversity of species. Several of these species may be allergenic or cause infections in humans. In addition, other demonstrated genera, such as *Absidia*, *Acremonium*, *Mucor*, and *Paecilomyces*, also include potentially pathogenic species (12, 26, 56). The results presented in the present report are, of course, not evidence that waterborne molds are involved in disease. However, it is important to be aware that several of the same species which are of clinical concern are also present in water. Considering potential problems related to the molds isolated from water may contribute to increased knowledge in the field of clinically important species. All further speculations on water-transmitted molds affecting human health are based on relating the mold species in the present study to the reported literature.

Penicillium species were especially abundantly distributed and clearly have the ability to survive water treatment and contaminate water reaching various network installations. Only heating of water seems to inhibit the recovery of viable *Penicillium* spores or hyphae. The implication of *Penicillium* species in allergy, asthma, or other respiratory problems has been a subject of several studies world wide (reviewed in reference 58). Strong associations between *Penicillium* spp. and health problems were also reported by Cooley et al. (11). Hence, many of the species isolated in the present investigation may have allergic potential if susceptible individuals are exposed. Furthermore, several of the demonstrated *Penicillium* species have been reported to be active mycotoxin producers (22, 42, 56). This fact raises the question of potential mycotoxin production in water, and further investigations into this problem are merited. The genus *Penicillium* also includes common contaminants of food and beverages (52, 56), several of which were recovered in this study. It is not unlikely that water can be the route of transmission for mold contamination and spoilage of foods. To reveal such correlations, further investigations are required.

The results from our study are consistent with the findings of Arvanitidou et al. (7, 8) that *Aspergillus* is one of the more commonly isolated genera in water. *A. fumigatus* and *A. niger* are common allergens and may cause opportunistic invasive infections in hospitalized immunocompromised patients (12, 13). *A. fumigatus* was found on several occasions during this study, both at the waterworks and in water system installations such as showers. This finding is in support of previous studies conducted at the Rikshospitalet University Hospital, Oslo, Norway, where Warris et al. (61) established that *A. fumigatus*

occurred frequently in the water. Further, a genotypic relatedness between clinical and water-related isolates was recognized (62). *A. fumigatus* has been one of the most significant fungal pathogens causing health problems over the last decades, and studies of a wet route of transmission are increasing, providing further evidence of the existence of the water source theory (2, 3, 62).

In the present study, *A. ustus* was frequently isolated throughout drinking water systems, particularly in groundwater-derived samples but also in surface water-derived samples. This species had one of the highest maximum concentrations obtained in the water samples tested. *A. ustus* was often isolated from hot-water samples and thus seems to have the ability to adapt to and survive at high temperatures. Supporting this suggestion, the species was abundant in water from installations on arctic Svalbard, where water pipelines are heated because of permafrost (G. Hageskal and S. B. Rønning, unpublished results). *A. ustus* has not, to our knowledge, been isolated from water previously, and the finding of this fungus is particularly interesting because it has been reported from infections in humans and appears to be an emerging opportunistic pathogen in immunosuppressed patients (12, 25, 49). It is noteworthy that *A. ustus* was frequently isolated from hospital samples.

An outbreak of invasive infections caused by *A. ustus* was recently reported, where a common source of infection was suggested (48). Clinical isolates from six patients were genetically similar, but an exact source of infection was not determined. Analyses of the water system may have been merited in this outbreak, since this may have provided a common source of infection of all six patients. Differences in the time of infection may be explained by the biofilm theory, as it is hypothesized that biofilms are continuously built up, detached, and rebuilt, resulting in increased concentrations of *A. ustus* from time to time. Since *A. ustus* seems to be able to establish itself in heated-water installations, the hospital warming tank could have been the source of the fungal infections and water samples should probably be investigated.

Most *Trichoderma* species are soilborne and are characterized by rapidly growing colonies that have a great potential for spore production. The genus includes species reported to cause mycoses and allergy in humans (12, 31, 36, 59). Toxin production has also been reported, and some species can be preservative resistant in food products (45, 56). In this study, *Trichoderma* species were only isolated from surface water systems. They were found at all sampling points of these systems but were only rarely obtained from hot-water taps. This may indicate that *Trichoderma* species are not present in underground water sources and that these species are not particularly thermotolerant. The different *Trichoderma* species were difficult to identify to the species level on the basis of morphological characteristics only because the few morphological characteristics available are variable, leading to overlap among species. Molecular analysis-based characterization of species in the genus *Trichoderma* is also complicated, as species concepts are subject to change. Sequencing of the ITS region often fails to distinguish between closely related taxa, because more than one species may share the same ITS sequence. Extensive molecular work has to be implemented to assess the different *Trichoderma* species recovered. If molecular methods allow one to distinguish between *Trichoderma* species, one would expect several more species to be added to the list in Table 1. Consequently, the list of dominant species in Norwegian drinking water may have to be revised since *Trichoderma* species were present in so many samples.

The fundamental question for water consumers will obviously be the significance of the presence of all of these mold species in water. Firstly, mold-contaminated water will probably be most important in hospitals and other health care institutions, where immunocompromised patients with, e.g., AIDS, cancer, or organ transplants undergo treatment. Because of the increasing frequencies of severely immunocompromised patients, hospitals are facing a greater challenge with respect to opportunistic fungal infections (40, 64). Contaminated water may be a potential problem if the mold conidia or small hyphal fragments are aerosolized into the environment and subsequently inhaled. Such aerosol formations can occur when water passes through installations like taps or showers. The level of *A. fumigatus* in air was observed to increase in a patient's bathroom after the shower was run for 10 min multiple times (61).

Most of the recovered fungal species were only isolated as a few colonies per 100-ml water sample. However, some species did occur at maximum concentrations between 10 and 26 colonies per 100-ml sample and even at 100 colonies per 100-ml sample. The species recovered at the highest maximum concentrations were generally the same species that were most frequently isolated from the water. This may indicate that some mold species have certain properties that make them able to multiply in water systems. Although no significant seasonal differences in species diversity could be observed, the concentrations of individual species seemed to vary over time. This could be explained by the biofilm theory, as previously mentioned. It was observed that the concentration of the same mold species could vary from 1 to nearly 30 colonies between two analyses. Concentrations of nearly 30 CFU of the same species in a 100-ml sample could represent a temporary water treatment malfunction or problems in the distribution system or system installations.

It is unlikely that the occurrence of molds in water at the concentrations observed in this study would cause disease in healthy individuals. However, if the right conditions are present and regrowth of molds occurs in water systems, exposure of humans to large amounts of potentially harmful mold species could become a problem. Several of the molds are potential toxin producers, and exposure to small amounts of toxins for several years may have negative effects on the immune system. In the present study, a groundwater-derived sample from a shower contained a high concentration of *F. dimerum* (100 CFU/100 ml). This result clearly indicates biofilm formation in this particular shower, since the species was not isolated from the other sampling sites in this private home. It should also be noted that *F. dimerum* has been reported from infections in humans (37, 60).

When it comes to respiratory symptoms and allergies, little is known about the implications of molds in water. A few studies from Sweden and Finland have reported allergic reactions directly caused by mold-contaminated water (9, 41, 43). Several reports have implied that various fungal genera, including several of those presently identified, may be causative factors

in the growing epidemic of allergy and asthma observed in the human population (27, 30, 32, 33, 38). The evidence linking molds like the *Aspergillus*, *Cladosporium*, and *Penicillium* species with severe asthma was recently reviewed and found to be strong (14). The role of *Penicillium* and *Aspergillus* species in sick-building syndrome was reviewed by Schwab and Straus (58). They concluded that these genera play a major role in allergies and respiratory diseases in humans. The theory that mold exposure via water may cause respiratory symptoms or allergic reactions in susceptible individuals cannot be rejected, and the present species identification results have revealed the same species present in water as those reported in the aforementioned literature. However, additional studies are needed before such correlations can be established.

Water-related problems like off flavor and odor have been connected to the presence of molds. Members of the genera *Phialophora*, *Acremonium*, and *Penicillium* were found to be responsible for bad taste and odor of water (46). Since species belonging to these genera were present in our investigation, smell or taste problems that may occur in Norwegian drinking water could be related to the presence of molds.

The results obtained in the present investigation indicate that a wide variety of mold species is present in all parts of the water distribution systems in Norway, in both surface water-derived and groundwater-derived water. Potentially harmful fungi were isolated on several occasions. Piped drinking water can offer a transmission route for molds. If regrowth in distribution systems or installations in homes and hospitals occurs, increased concentrations of undesirable species may constitute a potential health hazard for humans and reduce water quality. Our results indicate that the mycobiota of water should be kept under surveillance in terms of assessing the microbiological safety and quality of drinking water and that perhaps analyses of molds should be included in the Norwegian drinking water regulations.

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