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Potential transmission pathways of clinically relevant fungi in indoor swimming pool facilities

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ABSTRACT

Possible transmission pathways of fungi in indoor swimming pool facilities were assessed through fungal counting in different areas of the facilities and typing of the collected fungal isolates. Air, water and surface samples were collected from seven different indoor swimming pool facilities. Fungal species were identified based on their internal transcribed spacer (ITS) sequences. Maximum fungal concentrations of 6.2 CFU/cm^2, 1.39 CFU/100 mL, and 202 CFU/m^3 were found on surfaces, in water and air, respectively. In total, 458 isolates were obtained, belonging to 111 fungal species, of which 50 species were clinically relevant. *Phialophora oxyspora* (13.3%) and *Trichosporon dohaense* (5.0%) were the most frequently isolated species and were merely detected on floors, as were the dermatophytes, *Trichophyton interdigitale* and *T. rubrum*. *Penicillium* spp. and *Aspergillus* spp. were the dominant fungi in water and air. No typical patterns of fungal concentrations along the preferential pathways of pool visitors were observed, however, sites where pool visitors converge while moving from one room (e.g. dressing room) to another (e.g. shower room) and walking barefoot displayed the highest fungal concentrations thus posing the highest risk of contamination. The dispersal of fungi on floors is most likely facilitated by the pool visitors and cleaning tools. Clinically relevant fungi, including the ones rarely identified in nature, were widely detected on floors, in water and in air, as well as on cleaning tools and flexibeams. Preventive measures such as cleaning should minimize the prevalence of clinically relevant fungi in swimming pool facilities since these potentially pose health risks to those vulnerable for infections.

1. Introduction

Studies have shown that swimming pool facilities have contributed to the spread of fungal infections (Gentles and Evans, 1973; Seebacher et al., 2008). Epidemiological studies of fungal infections and/or diseases related to swimming pools have been conducted for decades, mostly focusing on tinea pedis and onychomycosis caused by dermatophytes (Ali-Shtayeh et al., 2003; Detandt and Nolard, 1988, 1995; English and Gibson, 1959; Gentles and Evans, 1973; Gudnadóttir et al., 1999). Fungal skin and nail infections are mainly caused by dermatophytes although some cases caused by non-dermatophytes have also been reported (Ellabib et al., 2002; Lateur et al., 2003; Morales-Cardona et al., 2014; Sharma and Sharma, 2012; Thomas et al., 2010). Besides dermatophytes, other clinically relevant fungi such as *Fusarium* spp., *Aspergillus* spp., and *Candida* spp., were detected on surfaces, in water and in air inside swimming pool facilities (Aho and Hirn, 1980; Brandi et al., 2007; Buot et al., 2010; Jankowski et al., 2017; Maghazy et al., 1989; Viegas et al., 2011). Clinically relevant fungal species include fungal species which have been implicated in human cases of superficial (e.g piedra, otitis externa), cutaneous (e.g. ringworm, onychomycosis), and subcutaneous (e.g. mycetoma) mycoses, as well as deep mycoses (e.g. fungemia). The immune system in healthy humans is generally able to resist infections from exposure to most fungi, however, patients with immunodeficiency are more susceptible to fungal infections (Brown et al., 2012).

In swimming pool facilities, fungal skin and nail infections (e.g. tinea pedis, onychomycosis) are prevalent among pool visitors (Detandt and Nolard, 1995; Kamihama et al., 1997; Shemer et al., 2016). Surfaces in swimming pool facilities may contain skin fragments from infected persons and thus the infection may spread when infected skin

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fragments adhere to e.g. the feet of thus far uninfected individuals while walking over contaminated surfaces (English and Gibson, 1959). Our previous study (Ekowati et al., 2017) demonstrated that fungi were ubiquitously present on surfaces and in water in an indoor swimming pool facility, with higher fungal counts on floors where people walked barefoot compared to other surfaces such as benches and diving platforms. Particularly floors near pools appeared to be prone to fungal contamination, with clinically relevant fungi being present, including *Aspergillus fumigatus*, *Fusarium solani* complex, *Purpureocillium lilacinum*, and *Candida parapsilosis*.

The focus of this study was to identify possible transmission pathways in seven different indoor swimming pool facilities by comparing the fungal populations and concentrations on floors where people walk barefoot, in pool water and in air in the facilities. The presence of fungi on cleaning equipment and teaching aids was also investigated in order to observe their role in facilitating the dispersal of fungi in indoor swimming pool facilities.

2. Materials and methods

2.1. Sampling locations

Sampling was carried out in seven swimming pool facilities (A-G) within the same province in the Netherlands. In total, 59 samples were collected from floors in swimming pool facilities, whereas seven water samples and seven air samples were taken. In each facility, six samples were taken from floor surfaces in different areas in the facility, following the visitors' pathway from the dressing rooms to the swimming hall, one water sample was taken from the pool closest to the shower room and one air sample was taken in the swimming hall closest to the sampled pool (Table 1). In four of the pool facilities (A, B, C, D), two swimming pools were located in the same swimming hall hence one additional sample was taken from the floor close to the second swimming pool. One sample was collected from the surface of one of the flexibeams (foam teaching aids) in each swimming pool facility. When the equipment was available, samples were taken from the cleaning tools (mop, scrubber and wiper) used to clean the floors in the facility.

Water quality parameters were measured in water samples collected from the examined pools. Temperature and pH were measured using a portable probe (pH meter 3310, WTW, Germany). Free chlorine levels were measured using DPD free chlorine reagent powder pillows and a portable colorimeter DR890 (Hach, USA). Total organic carbon (TOC) was measured using a TOC-L analyser (Shimadzu, Japan).

2.2. Sample collection and processing

Ten litres of water were collected from the pools using plastic containers, which were previously cleaned using chlorine tablets (Suma Tab D4 Tab, Diversey, the Netherlands) according to the manufacturer's recommendation, and subsequently thoroughly rinsed with demineralized water. Sodium thiosulfate (final concentration 0.2 mM) was added to the water samples to quench residual chlorine. The water samples were transported to the laboratory at ambient temperature and subsequently stored at 4 °C until further analysis. Sample volumes of 2 L, 1 L and 2 × 0.5 L of pool water from each sampling location were filtered through 0.45 µm pore size membrane filters (Millipore, no. EZHAWG474, the Netherlands). Membrane filters were placed on MEA (Malt Extract Agar) and SDA (Sabouraud Dextrose Agar) plates, which were prepared as described by Ekowati et al. (2017).

Each surface sample was taken by applying Replicate Organism Detection and Counting (RODAC) plates filled with MEA and SDA. RODAC plates were applied by pressing the plates gently on the surface for 10 s.

Air samples were collected using two simultaneously running AirPort MD8 (Sartorius Stedim Biotech, Germany) air samplers, each sampling for culture either on MEA or on SDA. The sample volume was 500 L at a sampling speed of 40 L/min. The air was filtered through 8 µm pore size cellulose nitrate filters (Sartorius Stedim Biotech no. 11301–80——ALN). Immediately after air sample collection, the filters were placed on MEA and SDA plates.

The limit of detection for the culture methods is 1 CFU per analysed volume (for water and air samples) or analysed area (for surface samples).

2.3. Cultivation and isolation of fungi

MEA and SDA plates were incubated in the dark at 24 °C. After 7 days of incubation, the number of colonies was counted and was expressed as the number of colony forming units (CFU)/100 mL for water samples, CFU/m^3 for air samples and CFU/cm^2 for surface samples. In some cases where overgrowth or too many colonies were observed after 7 days of incubation, the plates were not counted and were not included in the calculation of concentrations, however, some loose colonies were picked for sub-culturing and typing.

From each sample, up to five colonies from both MEA and SDA were selected for isolation and identification. The selected colonies were the ones closest to the centre of the plates or membrane filters and loose individual colonies. The colonies were picked and directly cultured on MEA slants at 24 °C for 3 days and subsequently stored at 10 °C.

2.4. DNA extraction and identification

DNA was extracted following the Quick CTAB extraction as described by Zhou et al. (2014). DNA samples were stored at −20 °C until further use.

Amplification of the internal transcribed spacer (ITS) followed by sequencing was performed to identify fungal species. Primers ITS1 or ITS5 and ITS4 were used to amplify DNA (White et al., 1990). The total

Table 1
Sampling sites in each of the studied swimming pool facilities.

Pool facility	Site number	Sampling site[a]	Description of sampling site	Sample matrix	Sample code
A, B, C, D, E, F, G	1	Dressing room (pathway)	Floor	Surface	DR
	2	Entrance from dressing room to shower room	Floor	Surface	DR-SH
	3	Shower room	Floor	Surface	SH
	4	Entrance from shower room to swimming hall	Floor	Surface	SH-P
	5	Swimming hall (in front of pool ladder)	Floor	Surface	P-LA
	6	Swimming hall (preferential pathway)	Floor	Surface	P-PP
		Flexibeam	Foam teaching aid	Surface	FB
		Swimming pool	Water	Water	Water
		Swimming hall	Air	Air	Air
A, B, C, D	7	Swimming hall (pathway close to the 2nd pool)	Floor	Surface	EXT
A, C, E		Mop/scrubber	Cleaning tool	Surface	CT-MS
C, D, E		Wiper	Cleaning tool	Surface	CT-W

[a] Locations of the sampling sites 1–7 are described in Supplementary Material Fig. S1.

volume of 25 μL PCR mixture contained 2.5 μL PCR buffer (10xNH_4^+), 1 μL $MgCl_2$ (50 mM), 2.5 μL dNTP (10 μM), 1 μL of each primer (10 μM), 1.5 μL DMSO, 1 μL Bioline Taq polymerase (0.5U/μL) (Bioline, UK), 13.5 μL sterile demineralized water, and 1 μL DNA template. Amplification of fungal DNA and sequencing of PCR products were done as described by Ekowati et al. (2017).

The forward and reverse sequences were assembled using SeqMan Pro from Lasergene software (DNASTAR, USA) and checked against the GenBank database. The fungal species were identified based on the closest results obtained with the BLAST algorithm (BLASTN 2.7.0) with a similarity of at least 98%. In some cases, with results of less than 98% similarity, the genus, family or order name was assigned to the isolates concerned, based on the highest similarity found in BLASTN.

2.5. Phylogenetic tree

All sequences were aligned using online version multiple sequence alignment program, MAFFT version 7 (Katoh et al., 2017; Katoh and Standley, 2013). Phylogenetic analyses were conducted using MEGA version 7 (Kumar et al., 2016) with Maximum Likelihood method using 1000 bootstrap replications.

2.6. Statistical analysis

Statistical analysis to compare the fungal concentrations was carried out by using a non-parametric Kruskal-Wallis test ($\alpha = 0.01$) followed by post hoc Dunn's multiple comparison test. The comparisons were done for the different sampling sites in the swimming pool facilities (Table 1) and for the different pool facilities (A-G).

3. Results

3.1. Water quality parameters and cleaning regimes

The measured water quality parameters are shown in Table 2. Free chlorine and pH levels were within the range of standard requirements for swimming pool water quality in the Netherlands: $6.8 \leq pH \leq 7.8$ and free chlorine 0.5–1.5 $mgCl_2$/L (Bhvbz, 2011).

A questionnaire regarding the cleaning regime in swimming pool facilities was filled in by the pool managers. The floors inside all the facilities were cleaned at least once a day using a floor scrubbing machine, usually after closing hours. Some areas in some facilities were cleaned more frequently: pool facility D cleaned the shower room and the floors in the dressing/locker rooms about 3–4 times per day, pool facility C cleaned the dressing/locker room floors twice a day, and pool facility F cleaned the shower room twice a day. The products used for cleaning or disinfecting purposes in each facility varied in their chemical content, such as phosphoric acid, sodium hydroxide, hydrogen peroxide, and a mixture of alkali substances.

3.2. Fungal concentrations in water, air and surface samples

Fungi were detected in water from all the sampled swimming pools. Overall, fungal concentrations in water samples from the different pools cultured on MEA and SDA ranged between 0 and 1.53 CFU/100 mL (Fig. 1A, Supplementary Material Table S1). The highest concentration was detected in the water of pool D. The fungal counts in air samples cultured on MEA and SDA ranged between 0 and 202 CFU/m^3 with the highest count in air in pool facility E (Fig. 1B, Supplementary Material Table S1).

Table 2
Water quality in different swimming pools.

Pool facility	pH	Temperature (°C)	Free chlorine (mg/L)	TOC (mg/L)
A	7.43	28.0	1.30	2.91
B	7.41	28.0	0.98	3.11
C	7.10	30.0	1.04	3.42
D	7.07	29.0	0.98	2.75
E	7.33	27.5	0.75	2.51
F	7.40	28.0	0.96	1.52
G	7.31	30.4	1.03	2.80

For comparison of fungal concentrations on floors at different sampling sites in seven pool facilities, concentrations on MEA are displayed in Fig. 2 (count data are available in Supplementary Material Table S1). Higher fungal concentrations were observed on floors at the sites where people leave the dressing rooms and enter the shower room (DR-SH; (2)), as well as the sites where people leave the shower room and enter the swimming hall (SH-P; (4)) (Fig. 2, Supplementary Material Fig. S1). Almost no fungi were observed on the shower floors except in pool facility A. Fungal concentrations on the surfaces of flexibeams ranged between 0 and 0.20 CFU/cm^2 (Fig. 2). Fungal concentrations in the additional samples from the floors in the swim halls of facility A-D ranged between 0 and 3.56 CFU/cm^2 and on the surfaces of cleaning tools were between 0.32 and 2.96 CFU/cm^2 (Fig. 2).

Statistical comparison was performed on fungal concentrations on MEA only since this medium is a general purpose culture medium and thus yielded more representative fungal counts than SDA, a (semi-)selective medium. No significant differences ($H(6) = 1.5960$, $p = 0.95$) were observed in the fungal concentrations between pool facilities (comparing only floor samples, except the floor samples taken near the second pool in facility A-D designated EXT), However, there were significant differences ($H(5) = 21.1123$, $p = 0.0008$) when comparing the fungal concentrations on floors between the different sampling sites. Further analysis using the post hoc Dunn's multiple comparison test revealed that the fungal concentrations on shower floors (SH) were significantly lower than the concentrations found on the floors at the exit of the dressing rooms leading to the shower room (DR-SH) and the exit of the shower room leading to the swimming hall (SH-P), while the rest of pairwise comparisons did not show significant differences.

3.3. Isolated fungal species

A total of 458 isolates obtained from the seven pool facilities, were identified to the species level based on their ITS sequences. Some fungal species were difficult to identify to the species level based on their ITS sequences, and were therefore identified up to the genus, species complex or section level.

The isolates comprised of 46 isolates from water, 40 isolates from air, and 372 isolates from surfaces. Fungal DNA identification resulted in 111 fungal species: 73 species were detected only on MEA and 18 species were from SDA, while 20 species were isolated from both media. Based on sampling sites, 27 species were isolated from water, 21 from air and 88 from surfaces. Among the identified species, 11, 10 and 68 fungal species, respectively, originated from water, air and surfaces only and 22 species were recovered from at least two different environmental matrices. The number of fungal species and isolates in samples from the studied swimming pool facilities can be found in Supplementary Material Table S2.

Phialophora oxyspora was the most frequently isolated fungal species (13.3%, 61/458). It was detected mostly on surfaces, rarely in water and was never detected in air samples in any of the pool facilities. Another frequently isolated fungal species, *Trichosporon dohaense* (5.0%, 23/458) was detected only in samples from floors, whereas fungal species in the *Penicillium* section *Chrysogena* (4.6%, 21/458) were isolated from water, air and floors. Fungal species isolated in this study are listed in Supplementary Material Table S3.

3.4. Clinically relevant fungal species

Fifty of the isolated fungal species (45%, 50/111) were considered clinically relevant. Of the total number of 458 isolates, 316 isolates

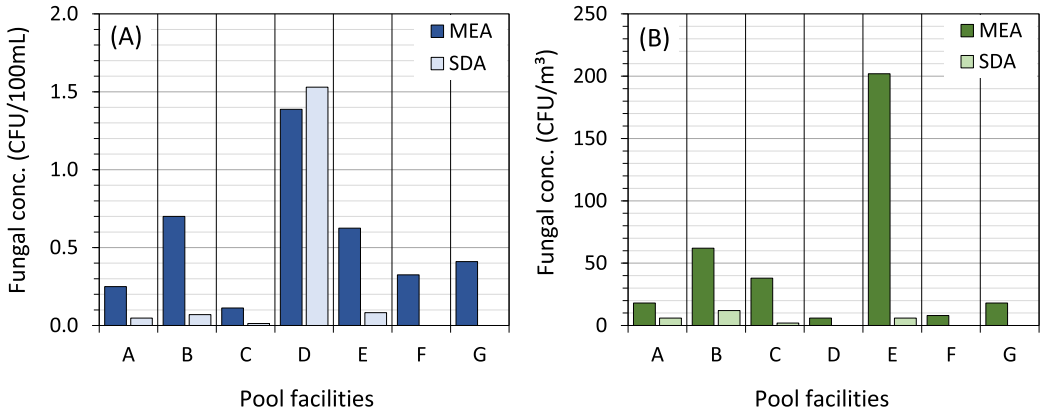

Fig. 1. Fungal concentrations in water (A) and air (B) samples.

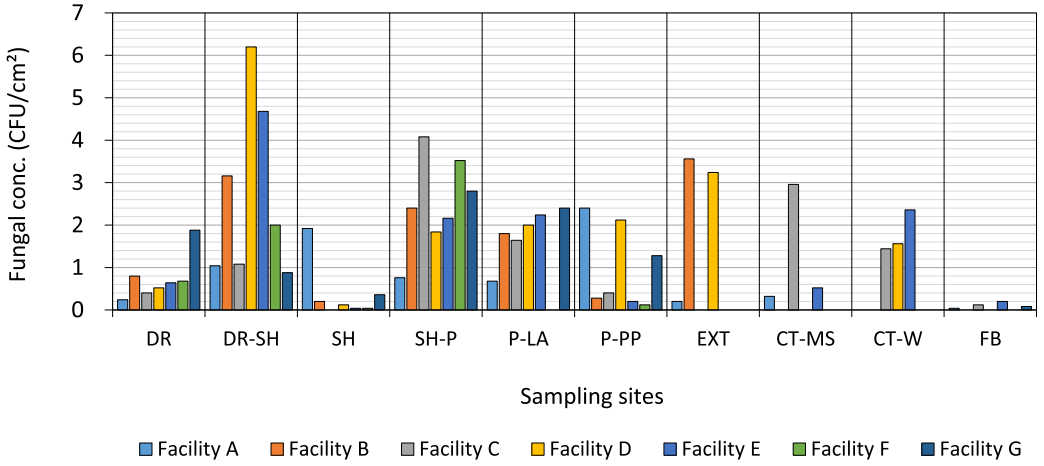

Fig. 2. Fungal concentrations on surfaces, in samples cultured on MEA. DR: dressing room, DR-SH: dressing room to shower room, SH: shower room, SH-P: shower room to swimming hall, P-LA: pool ladder, P-PP: preferential pathway, FB: flexibeam, EXT: second pool pathway, CT-MS: mop/scrubber, CT-W: wiper.

(69%) belonged to clinically relevant species (35 isolates from water, 29 isolates from air and 252 isolates from surfaces). Based on the number of fungal species, 59% (16/27), 67% (14/21) and 46% (41/88) of the species from water, air and surfaces, respectively, were clinically relevant.

Phialophora oxyspora was not only the most frequently isolated fungal species, but also the most commonly isolated clinically relevant fungal species (Supplementary Material Table S3). Additionally, clinically relevant fungal species in the genera *Penicillium* and *Aspergillus* were also frequently detected on surfaces, in water and in air with overall occurrences of 12% (53/458) and 9% (42/458), respectively. Five percent of the isolates (22/458) belonged to five clinically relevant species within the genus *Exophiala* which were detected on surfaces.

Although rarely isolated, some fungal species belonging to risk group 2, were detected at different sampling sites in different pool facilities. *Candida auris* was detected in water samples from pool facilities A and D, *Scedosporium boydii* which was isolated from water samples in pool facilities A and G, *Rhinocladiella similis* was isolated from a cleaning tool in pool facility D and *Exophiala dermatitidis* was isolated once from pool facility A.

Two dermatophyte species were isolated from both floors and water. *Trichophyton interdigitale* (1.3%, 6/458) was isolated from the floors in the dressing rooms in facility F, the dressing room exit leading to shower room in facility A and the swimming hall in facilities A and F, as well as from pool water in facility B. *Trichophyton rubrum* (0.4%, 2/458) was isolated from the floors in the shower room in facility B and the floors in the swimming hall of facility F.

3.5. Distribution of fungi in swimming pool facilities

Regardless of the sampling sites, 40 fungal species were isolated from at least two different pool facilities. *Phialophora oxyspora* was detected in all pool facilities except pool facility A. *Trichosporon dohaense*, *Rhodotorula mucilaginosa* and *Purpureocillium lilacinum* were present in five pool facilities. *Penicillium*, *Aspergillus*, *Cutaneotrichosporon*, *Fusarium*, and *Exophiala* were the most frequent fungal genera observed in the different pool facilities.

Phylogenetic trees were constructed based on the fungal species detected in each pool facility (Fig. 3, Supplementary Material Figs. S2–S7). The markers in different colours represent different sampling sites (floor, water, air, teaching aid and cleaning tool). If the same fungal species was isolated more than once in one sampling site, only one sequence was used to construct the phylogenetic tree. Fig. 3 shows the phylogenetic tree of the fungal species isolated from pool facility E representing that which displayed most species diversity. From a total of 74 isolates and 37 fungal species, sections and species complexes found in this pool facility, six fungal species/sections/complexes were isolated from samples taken from two different sampling sites. *Cladosporium cladosporioides* complex was detected on floors, in water and in air. *Cladosporium herbarum* complex was isolated from floors and a teaching aid. *Phoma* spp. were detected in air, on floors and on a cleaning tool. *Alternaria infectoria* were isolated from water, floors, and

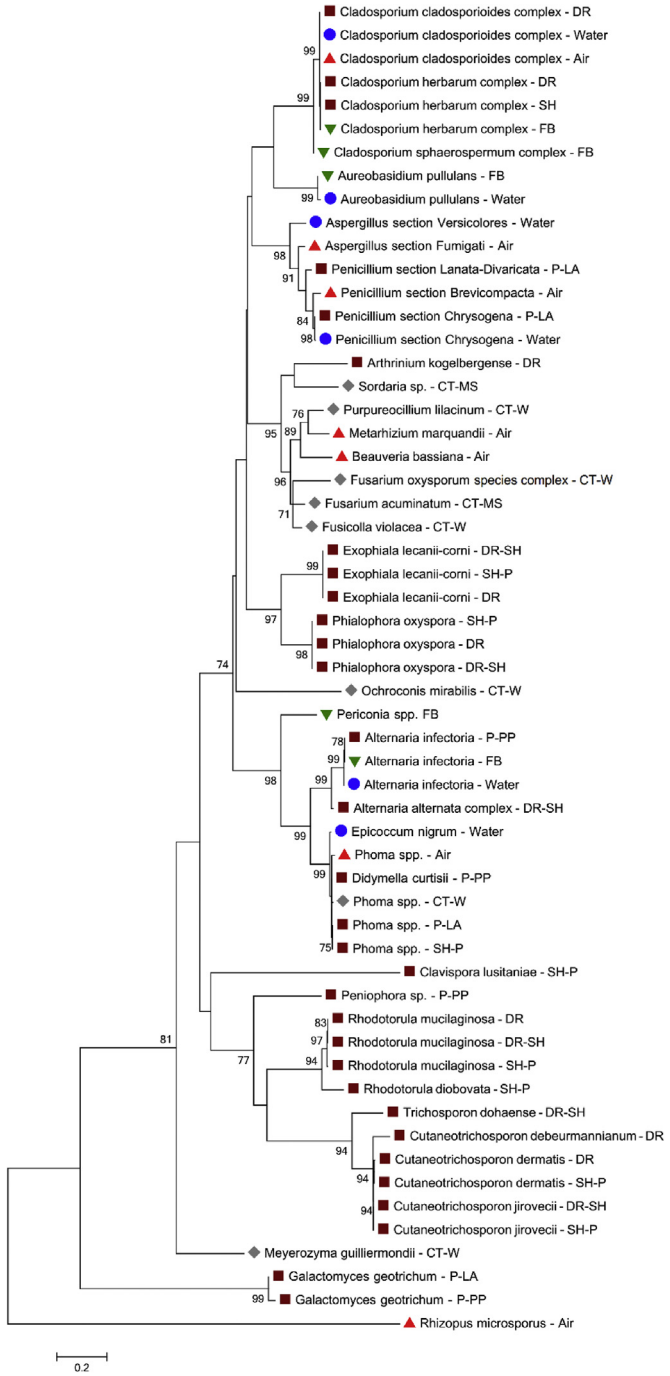

Fig. 3. Maximum likelihood tree based on ITS sequences of fungal isolates from pool facility E. (■: floor, ▲: air, ●: water, ▼: teaching aid, ◆: cleaning tool).

a teaching aid. *Penicillium* section *Chrysogena* was isolated from water and floors and *Aureobasidium pullulans* was isolated from water and a teaching aid.

Examining the phylogenetic trees from all the pool facilities (data not shown except for pool facility E) revealed that in total, 12 fungal species (and sections and species complexes) were detected in the combination of water, air and/or surfaces (either floor, teaching aid or cleaning tool). In the studied pool facilities, some corresponding fungal species (and sections and species complexes) were detected in water, in air and on surfaces except in pool A and D (Table 3). While most of the corresponding species (and sections and species complexes) were isolated from two different environments, only a species belonging to *Cladosporium cladosporioides* complex was detected in air, in water and on surfaces (in pool facility E).

From the ten fungal species detected on the surfaces of the sampled teaching aids (i.e. flexibeams), seven species were clinically relevant and six species were also detected either in the water or on the floors in the corresponding facility.

Samples collected from six cleaning tools (mops, scrubbers and wipers) used in four different facilities resulted in 21 fungal species; 15 of them were clinically relevant species. Seven fungal species isolated from the cleaning tools were also isolated from floor surfaces. At least one fungal species isolated from cleaning tools in each facility was also found on floor samples in that facility (Table 3).

4. Discussion

4.1. Clinically relevant fungi in swimming pool environments

Phialophora oxyspora, the most frequently isolated fungal species in this study, was isolated from six out of seven pool facilities studied. The pathogenicity of this species is considered low since it was only occasionally isolated from cutaneous samples (de Hoog et al., 2015). Fungal species belonging to the genera *Penicillium* (7/7) and *Aspergillus* (6/7) dominated the fungal species isolated from air samples. The presence of these fungi in indoor air samples from swimming pool facilities was also demonstrated by Brandi et al. (2007) and Viegas et al. (2011). *Aspergillus* spp., especially *A. fumigatus*, are known to cause deep mycoses in immunocompromised humans while cases of human infections by *Penicillium* spp. other than *P. marnefeii*, are rare (de Hoog et al., 2015). Clinically relevant species belonging to the genus *Exophiala* were detected in five pool facilities. *Exophiala oligosperma* and *Exophiala dermatitidis* were previously detected in swimming pools and saunas and were associated with systemic mycoses (de Hoog et al., 2000, 2003; Matos et al., 2002; Zeng et al., 2007). Other clinically relevant fungal species which were isolated in five pool facilities were *Rhodotorula mucilaginosa* and *Purpureocillium lilacinum*. Both were reported to be involved in cases of catheter-related fungemia and cutaneous infections (de Hoog et al., 2015). *Candida auris* was unexpectedly isolated from water samples in two different facilities. In recent years, *Candida auris* has been recognised as fungal pathogen associated with invasive infections, causing outbreaks in healthcare settings around the world (Chowdhary et al., 2016; ECDC, 2018). *Trichophyton interdigitale* (3/7) and *T. rubrum* (2/7), causative agents of human nail and skin infections (Weitzman and Summerbell, 1995), were rarely isolated in the current study.

Although most of the isolated clinically relevant fungi are opportunistic pathogens, it is important to be aware of their presence, especially in public facilities such as swimming pools, where many people interact and use the same space and equipment. The presence of clinically relevant fungal species in the studied swimming pool facilities suggests a possible health risk for immunocompromised persons such as children, pregnant women, and the elderly or with otherwise suppressed immune systems.

4.2. Transmission pathways

The spread of fungal skin and nail infections is facilitated by direct contact with infected skin fragments (e.g. on surfaces and in water) or skin-to-skin contact (English and Gibson, 1959). Deep fungal infections in immunocompromised patients are acquired through the respiratory tract, the gastrointestinal tract, or intravascular devices (de Hoog et al., 2015; Walsh and Dixon, 1996). In swimming pool facilities, contaminated surfaces, water, and air may contribute to the transmission of fungal infections to susceptible visitors.

4.2.1. Floors

In none of the studied pool facilities, consistent patterns were observed for fungal concentrations on the floor surfaces of the visitors'

Table 3
Fungal species detected in or on at least two environments: water, air, floor, teaching aid, and cleaning tool in the same facility.

Pool facilities	Fungal species/species complex/section	Environmental matrices				
		Air	Water	Surface		
				Floor	Teaching aid	Cleaning tool
A	*Cutaneotrichosporon dermatis*			X		X
	Penicillium section *Fasciculata*			X		X
	Rhodotorula mucilaginosa			X		X
B	*Aspergillus* section *Versicolores*	X	X			n/a
	Penicillium section *Chrysogena*	X	X			
	Trichophyton interdigitale		X	X		
C	*Aspergillus* section *Nigri*			X	X	
	Cyphellophora sp.			X		X
	Exophiala oligosperma			X		X
	Penicillium section *Brevicompacta*	X	X			
	Phialophora oxyspora		X	X	X	
D	*Fusarium oxysporum* species complex			X		X
	Phialophora oxyspora			X	X	
	Phoma spp.			X		X
E	*Cladosporium cladosporioides* complex	X	X	X		
	Cladosporium herbarum complex			X	X	
	Phoma spp.	X		X		X
	Alternaria infectoria		X	X	X	
	Penicillium section Chrysogena		X	X		
	Aureobasidium pullulans		X		X	
F	*Alternaria alternata* complex		X	X		n/a
G	*Cladosporium herbarum* complex	X		X		
	Rhodotorula mucilaginosa	X		X		n/a
	Penicillium section Chrysogena		X	X		

n/a. no samples from cleaning tools in that pool facility.

pathways from the dressing rooms up to the swimming hall. However, the results showed that the fungal concentrations on the floors in the shower room were the lowest in all facilities except pool facility A. In pool facility A, the shower room samples were taken from the floor in the middle of the shower room, for logistic reasons, whereas in the other pool facilities the samples were taken from floors under the shower heads. The deviating result in facility A indicates that the flow of water from the showers in the other facilities most likely flushed away the fungal material and resulted in lower fungal counts. The observed higher fungal concentrations on floors where people move from one part of the facility to another, like from the dressing room to the shower room and from the shower room to the swimming hall, can be explained by people converging at narrower exits and entrances.

The presence of *Trichophyton interdigitale* and *Trichophyton rubrum* on floors from the dressing rooms leading to the swimming hall, demonstrated the spread of dermatophytes which can potentially cause fungal skin infections. Moreover, the abundance of species like *Phialophora oxyspora*, *Trichosporon dohaense*, and *Cutaneotrichosporon debeurmannianum* on floors in different areas in the pool facilities suggests that pool visitors facilitated the dispersal of these fungi inside the facility.

4.2.2. Cleaning tools

Fungi were also detected on the mops, scrubbers and wipers used to clean the floors in the facilities. Some of the fungi isolated from the cleaning tools were also isolated from the floors. The tools may have become contaminated with these species while being used for the cleaning of contaminated floors, which subsequently gave the possibility that the use of these contaminated cleaning tools resulted in (re) contamination of floors and dissemination of fungi to other areas inside the pool facilities. Using a single-use cleaning tool or using a properly cleaned or disinfected tool or using several type of disinfectants could be good options to avoid recontamination of floors, especially when the pool staff uses a mop or a wiper to remove water from the floors without using cleaning solutions. However, further study is needed to investigate the effectiveness of different interventions. As considerations, some fungi could survive after cleaning due to ineffective disinfectants (Bobichon et al., 1993; Pap et al., 2006) and cleaning or disinfecting a large area using a single mop could even facilitate the dissemination of pathogens (Exner et al., 2004).

4.2.3. Teaching aids

Both in our previous study (Ekowati et al., 2017) and in the current study, fungi were detected on the surface of flexibeams from different swimming pool facilities. While in use, flexibeams are in contact with water and swimmers for at least 30 min up to 2 h (information from the questionnaire, Schets et al. (2014)). Since fungal contamination of the surface of flexibeams may occur, proper drying and cleaning after use of these and other foam teaching aids are necessary, to avoid possible infection of the users and contamination of the pool water. It is also recommended to avoid the growth of pathogenic bacteria such as *Pseudomonas aeruginosa* (Schets et al., 2014).

4.2.4. Indoor air

Many studies have been done on the presence of fungi in indoor air in residential settings but only limited data can be found for swimming pool facilities. A study by Brandi et al. (2007) obtained 32.7 CFU/m^3 and 48.2 CFU/m^3 of fungal loads in air samples in swimming halls and in locker rooms, respectively. The fungal concentrations in this study were in a similar range as in Brandi et al. (2007). However, one air sample from facility E showed a much higher concentration compared to other air samples (202 CFU/m^3) for which we have no explanation

based on the available characteristics and information on facility E. Rao et al. (1996) summarized quantitative standards and guidelines for fungal concentrations in indoor air from different institutions and research studies. WHO (1988) and Health Canada (1995) mentioned that fungal concentrations up to 150 CFU/m^3 in mixture species as acceptable, while some research mentioned in Rao et al. (1996) proposed higher values. In a more recent document, the Portuguese legislation on indoor air quality (Decree Law no. 79/2006, April 4, 2006), the maximum limit for fungal contamination is set at 500 CFU/m^3. Although different methods were used to quantify the fungal contamination in the guidelines summarized by Rao et al. (1996), such as different culture media and sampling methods, based on the guidelines values mentioned, the fungal concentrations in the air in the here studied swimming pool facilities can be considered as acceptable. For carrying out a health assessment on indoor air, a more extensive sampling campaign is required, taking into account not only fungal concentrations, but also the fungal species present. The influence of outdoor air should not be overlooked.

4.2.5. Pool water

Direct contact with contaminated pool water is unlikely to cause skin infections since the infected skin fragments in pool water may not be able to attach firmly enough to the swimmers' skin (English and Gibson, 1959). Ear infections, such as otitis externa, are commonly contracted by swimmers (Bernius and Perlin, 2006). Some cases of otitis externa caused by *Aspergillus* spp. and *Candida* spp. associated with swimming activities have been reported (Dorko et al., 2004; Ozcan et al., 2003). Cases of fungal infections due to ingestion of contaminated water have not been reported. However, some cases of aspergillosis in the gastrointestinal tract (Eggimann et al., 2006; Myoken et al., 2001; Prescott et al., 1992) and a case of mucormycosis due to ingestion of naturopathic medicine (Oliver et al., 1996) have been described, which suggests that ingestion may be a potential route of transmission. In a study of fungi in drinking water, Hageskal et al. (2009) mentioned that consumption of fungi-contaminated drinking water has thus far not led to acute disease.

4.3. Reoccurring fungal species in an indoor swimming pool facility

More than half of the fungal species detected in pool facility D in this study, were the same species as detected in the previous study (Ekowati et al., 2017), and nearly 80% of these reoccurring species were clinically relevant fungal species. Frequently isolated fungal species in both studies; *Phialophora oxyspora* was previously isolated from plant materials and human skin (Feng et al., 2014); *Trichosporon dohaense* and *Cutaneotrichosporon debeurmannianum* were previously isolated from clinical samples (Abdel-Sater et al., 2016; Nath et al., 2017); *Phoma* spp. are common soil fungi; and *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp., and *Alternaria* spp. are commonly found in outdoor and indoor air (Flannigan, 1997) and were also detected on different sites of the skin of healthy humans (Findley et al., 2013). The occurrence of these fungi suggests that the fungal population in pool facility D is influenced by the outdoor environment and pool visitors.

4.4. Preventive measures

The fungal concentrations observed at different sites in the studied swimming pool facilities only suggest that there is a possibility of infections. The existing guidelines recommend the maximum limits of fungal concentrations, but there are no guideline or limit values that indicate a specific concentration which can pose a risk of fungal infections. In general, the guidelines for indoor environments provide suggestions for prevention and remediation of fungal contamination.

Indoor fungal growth is associated with humidity of indoor air which can be controlled by ventilation and air conditioning (WHO, 2009). Ventilation plays a major role in removing and reducing fungal materials in indoor air. However, if it is not well maintained, ventilation could be the source of fungal contamination. The ventilation in indoor swimming pool facilities should be adequate to remove and dilute the pollutants in indoor air.

In general, swimming pools have to apply disinfection to prevent the microbial contamination of pool water. Chlorination is the most commonly used disinfectant in swimming pool practices. However, some fungal spores have a higher resistance to chlorine compared to indicator microorganisms, such as *Escherichia coli* (Ma and Bibby, 2017; Pereira et al., 2013). The application of alternative disinfection technologies which are able to inactivate chlorine resistant pathogens, such as UV and ozone, could be valuable in reducing fungal contamination in pool water.

The spread of fungal infections through direct contact with contaminated surfaces in swimming pool facilities can be avoided by taking some preventive measures. In this study, the information about the cleaning regime in each facility obtained from the pool managers was not sufficient enough to assess the effect of different cleaning regimes on the fungal concentration on surfaces. Watanabe et al. (2000) showed that washing feet with soap and subsequent wiping dry with a towel significantly reduced skin contamination. Gentles et al. (1974) demonstrated that by applying an intervention such as supplying and encouraging pool visitors to use foot powder could reduce the incidence of tinea pedis. The WHO Guideline for Safe Recreational Water Environments volume 2 (WHO, 2006) emphasises public education about fungal disease (e.g. tinea pedis), the importance of limiting contact to infected people and medical treatment. Other preventive measures were mentioned by Al-Doory and Ramsey (1987) such as pre-swim showers, wearing sandals and frequent cleaning of surfaces. The latter indicates the importance of having a regular check and thorough cleaning of the swimming pool environments, especially in favourable places that support fungal growth while being aware of possible fungal spread by contaminated cleaning tools.

5. Conclusions

In this study, possible transmission pathways in swimming pool facilities were identified from the exposure sites by means of fungal counts and typing. Although, there were no typical patterns of fungal concentrations along the preferential pathways, it can be concluded that sites where all pool visitors converge because they have to walk from one room (e.g. dressing room) to another (e.g. shower room) posed the highest risk of contamination, indicated by higher concentrations of fungi at these particular sites. The fungal species identified along the most likely transmission pathways, suggest that the dispersal of fungi on floors is most likely facilitated by the pool visitors and cleaning tools. Clinically relevant fungi, including the ones rarely identified in nature, were widely present on floors, in water and in air in the studied swimming pool facilities. Their presence potentially poses a health risk to those vulnerable, and thus their prevalence should be minimized by implementing preventive measures.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2018.07.013.

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