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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.