International Journal of Hygiene and Environmental Health 220 (2017) 1152–1160

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Clinically relevant fungi in water and on surfaces in an indoor swimming pool facility

Yuli Ekowati[a,*], Anne D. van Diepeningen[b,f], Giuliana Ferrero[a], Maria D. Kennedy[a,d], Ana Maria de Roda Husman[c,e], Franciska M. Schets[c]

[a] IHE Delft Institute for Water Education, Westvest 7, 2611 AX Delft, The Netherlands
[b] Westerdijk Fungal Biodiversity Institute, P.O. Box 85167, 3508 AD Utrecht, The Netherlands
[c] National Institute for Public Health and the Environment, Centre for Zoonoses and Environmental Microbiology, P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[d] Delft University of Technology, Stevinweg 1, 2628 CN Delft, The Netherlands
[e] Utrecht University, Faculty of Veterinary Medicine, Institute for Risk Assessment Sciences, Domplein 29, 3512 JE, The Netherlands
[f] Wageningen Plant Research, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 8 May 2017
Received in revised form 7 July 2017
Accepted 7 July 2017

Keywords:
Clinical fungi
Occurrence
Spatial distribution
Diversity
Exposure
Cleaning

ABSTRACT

The density of fungal contamination and the fungal diversity in an indoor swimming pool facility were assessed. A total of 16 surface samples and 6 water samples were analysed by using a combination of different (semi-) selective culture media. Isolated fungal colonies were identified to the genus or species level by sequencing of the internal transcribed spacer (ITS). The highest fungal counts in water and on surfaces were in the recreational pool (17 CFU/100 mL) and on a flexibeam (5.8 CFU/cm^2), respectively as compared with low counts (<0.1 CFU/cm^2) on the diving platform, bench tops and walls. The 357 obtained isolates belonged to 79 species and species complexes, 42 of which known as clinically relevant. *Phialophora oxyspora* (13.7%) and *Phoma* spp. (12.3%) were the most frequently identified groups. We demonstrated that despite chlorine treatment and regular cleaning of surfaces both water and surfaces were commonly infested with fungi, including many clinically relevant species.

1. Introduction

Both filamentous and yeast-like fungi are ubiquitous in natural and man-made environments and some fungal species are known to cause diseases in plants, animals, and/or humans (Fisher et al., 2012; San-Blas and Calderone, 2008). Of all the estimated millions of fungal species, approximately 600 species are known as obligate or opportunistic pathogens (de Hoog et al., 2015). Human mycoses vary from relatively innocent, superficial infections in otherwise healthy people (e.g. ringworm and onychomycosis) to life-threatening invasive deep and disseminated infections (e.g. candidiasis, aspergillosis) especially in immunocompromised individuals. Most human mycoses are caused by opportunistic pathogens with a common occurrence in the environment (e.g. *Candida* spp., *Cryptococcus neoformans*, and *Aspergillus* spp.).

Fungal infections in humans appear to be increasing worldwide (Brandt and Park, 2013; Brown et al., 2012; Dufresne et al., 2017). This may be partially due to better detection methods, but the increasing number of immunocompromised patients can also play a role (Low and Rotstein, 2011). In addition, there are concerns about the effects of climate change on pathogenic fungi: rising temperatures may increase the growth of waterborne pathogens, including fungi (de Roda Husman and Schets, 2010; van der Wielen et al., 2014). Other concerns are about the emergence of resistance to antifungal agents in pathogenic fungi over the past decades (Alcazar-Fuoli and Mellado, 2014; Gago et al., 2016).

In public facilities like swimming pools, people can get exposed to pathogens. The risk of acquiring infections in swimming pools is often associated with microbial contamination of the water due to faecal matter, non-faecal human shedding, and inadequate disinfection. Direct contact with contaminated surfaces and inhalation of air are also potential routes of exposure to pathogens. Studies on fungal contamination in swimming pool environments (water, surface and air) by Brandi et al. (2007) and Viegas et al. (2011) demonstrated the common occurrence of clinically relevant fungi of the genera *Cladosporium*, *Aspergillus*, *Fusarium* and *Trichophyton*.

* Corresponding author.
E-mail address: y.ekowati@un-ihe.org (Y. Ekowati).

http://dx.doi.org/10.1016/j.ijheh.2017.07.002
1438-4639/

The species within these genera are known to be associated with human fungal infections (de Hoog et al., 2015). Although it is clear that fungi can affect human health, guidelines to prevent fungal contamination in swimming pool environments are limited. The dermatophytic, *Trichophyton* spp. and *Epidermophyton floccosum*, which are known to cause athlete's foot, are the only fungal species considered as potential microbial hazards in the WHO guidelines for safe recreational water (WHO, 2006).

To date, most studies in swimming pool facilities focused on specific fungal species (e.g. *Candida albicans* (Sato et al., 1995)), fungal genera (e.g. *Fusarium* (Buot et al., 2010)), fungal groups (e.g. dermatophytes (Ali-Shtayeh et al., 2003; Detandt and Nolard, 1995; Hilmarsdottir et al., 2005)) or specific fungal infections (e.g. tinea pedis (Kamihama et al., 1997; Shemer et al., 2016)). Some studies assessed the total fungal contamination in swimming pool environments from different samples collected at different sites throughout swimming pools (Brandi et al., 2007; Viegas et al., 2011). However, fungal identification in most of these studies only went as far as the genus level and was based on morphologic characteristics.

In this study, the occurrence of different fungal species in water and on surfaces in an indoor swimming pool facility was investigated, focusing on clinically relevant fungal species. We defined clinically relevant fungal species as fungal species which are known to have caused infection and/or disease in humans. The purpose of this study was to broaden the knowledge on the fungal community in the swimming pool environment, in relation to a better understanding of the exposure routes of fungal infections in swimming pools. The diversity of fungal species was assessed by applying different sampling methods and culture media. Fungal identification to the genus level and where possible to the species level, was based on ITS sequencing.

2. Materials and methods

2.1. Sampling locations and water quality parameters

The sampling was carried out in one swimming pool facility in the Netherlands. The facility comprised of six separate pools with different sizes: pool A with a pool size of 375 m^2, pool B with a pool size of 50 m^2, pool C and D: separate pools with a total pool size of 300 m^2, pool E with a pool size of 135 m^2, and pool F with a pool size of 4.5 m^2. The sampling locations were chosen such that they represented the sites where exposure to the possibly present fungi would be most likely through direct contact with surfaces or water (Table 1).

Water quality parameters were measured in water samples collected from each pool. The pH was measured using a portable probe (pH meter 3310, WTW, Germany). Free chlorine levels were measured using DPD free chlorine powder pillows (Hach, USA). Total organic carbon (TOC) was measured using a TOC-L analyser (Shimadzu, Japan).

2.2. Sample collection and preparation

Water samples were collected from the six pools using plastic containers with a volume of 20 L, which were previously cleaned using chlorine tablets (Suma Tab D4 Tab, Diversey, the Netherlands) according to the manufacturer's recommendation. The volume collected from each pool was 20 L and in each container sodium thiosulfate (final concentration 0.2 mM) was added 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 different (semi)-selective culture media (see 2.3 Sample cultivation).

Each surface sample was taken by applying Replicate Organism Detection and Counting (RODAC) plates and Enviro swabs (3 M, the Netherlands) on adjacent surfaces. RODAC plates with different kinds of culture media (see 2.3 Sample cultivation) were applied by pressing the plates gently on the surface for 10 s (Nivens et al., 2009). Swab samples were collected by swabbing the surface inside a sterile sampling template 10 × 10 cm^2 (Copan Diagnostics, USA) by moving the swab back and forth across the surface with horizontal and vertical strokes (8–10 strokes per direction) covering the entire surface. Swabs were washed with 20 mL of 0.1% peptone saline, which was added to the tubes with the swabs followed by vigorous mixing by vortexing for 1 min. Subsequently, the swabs were pressed firmly against the wall of the tube and removed from the solution. The extracted solution in the tubes was homogenized by gentle shaking by hand for 5–10 s. Sample volumes of 0.1 mL, 0.5 mL and 2 × 1 mL were spread over the surface of different (semi)-selective media (see 2.3 Sample cultivation).

2.3. Sample cultivation

For all samples, the following culture media were used:

a Malt Extract Agar (MEA) (Oxoid, Thermo Fisher Scientific, UK) with penicillin and streptomycin, which allows general fungal growth. MEA was prepared by dissolving 50 g malt extract agar (Oxoid) in 1 L distilled water. The pH was set at 5.4 ± 0.2 and the medium was sterilized at 115 °C for 10 min. Penicillin and streptomycin were added to a final concentration of 50 mg/L after cooling to 55–60 °C.

b Pentachloronitrobenzene medium (PCNB) with rose Bengal (van Wyk et al., 1986), streptomycin and chloramphenicol was used to select for *Fusarium* and other PCNB tolerant species. PCNB was prepared by dissolving 25 g agar, 15 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 g PCNB, 0.05 g Rose Bengal, and 0.05 g chloramphenicol in 1 L of distilled water and the mixture was sterilized at 121 °C for 20 min. Streptomycin was added to a final concentration of 50 mg/L after cooling to 55–60 °C.

c Erythritol-chloramphenicol agar (ECA) with streptomycin was used to select for black yeasts (de Hoog and Haase, 1993). ECA was prepared by dissolving 25 g agar, 6.7 g yeast nitrogen base, 10 g *meso*-erythritol and 0.05 g chloramphenicol in 1 L of distilled water and the mixture was sterilized at 121 °C for 20 min. Streptomycin was added to a final concentration of 50 mg/L after cooling to 55–60 °C.

d Sabouraud dextrose agar (SDA) (Oxoid, Thermo Fisher Scientific, UK) with cycloheximide and chloramphenicol (Kamihama et al., 1997) was used to select for dermatophytes and other pathogenic fungi. SDA was prepared by dissolving 65 g Sabouraud dextrose agar (Oxoid) in 1 L of distilled water and the pH was set at 5.6 ± 0.2. 500 mg/L of cycloheximide and 50 mg/L of chloramphenicol were added before sterilization at 121 °C for 15 min.

All plates were incubated in the dark: ECA, PCNB, and SDA plates at 24 °C and MEA plates both at 24 °C and at 40 °C (MEA40). MEA40 was used to select for thermophilic fungi like the human pathogen *Aspergillus fumigatus*. 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 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.