International Biodeterioration & Biodegradation 117 (2017) 115–122

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Indoor fungal contamination of traditional public baths (Hammams)

Leyla Benammar [a], Taha Menasria [a, b, *], Amel Chergui [a], Soumia Benfiala [a], Ammar Ayachi [c]

[a] Department of Microbiology and Biochemistry, Faculty of Natural and Life Sciences, University of Batna 2, Batna 05078, Algeria
[b] Department of Applied Biology, Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, Tebessa 12002, Algeria
[c] Veterinary and Agricultural Sciences Institute, Department of Veterinary Sciences, University of Batna 1, Batna 05000, Algeria

ARTICLE INFO

Article history:
Received 17 September 2016
Accepted 7 December 2016
Available online 19 December 2016

Keywords:
Indoor environment
Public baths
Hammam
Fungal flora
Candida albicans
Dermatophyte

ABSTRACT

This study was carried out to provide an overview of the fungal load in Algerian traditional baths (Hammams) as well as to isolate and identify the main pathogenic fungi. Over a period of four months, ten baths were examined and screened for fungal contamination from several parts of the hot steamy rooms (floor, wall, door, air and marble massage platform). In total, 7157 fungi isolates were recovered from the surveyed Hammams and the most abundant molds were *Penicillium* spp. (45.12%) followed by *Aspergillus* spp. (28.80%). In addition, molds flora in traditional baths was characterized by a large number of hydrophilic species like *Cladosporium, Fusarium, Rhizopus, Mucor* and *Alternaria*. Eight candida-like appeared frequently (*C. albicans, C. glabrata, C. tropicalis, C. lipolytica, Geotrichum* sp., *Trichosporon* sp., *Rhodotorula* sp. and *Cryptococcus* sp.) of which *C. albicans* was the common isolated yeast (35.14%). The results indicate a significant difference ($p = 0.007$) in species richness between molds and yeasts and their distribution varied significantly among sampled positions in baths. ANCOVA revealed a significant increase in fungal loads related to the average number of customers and mean opening year of the Hammams, in contrast with locality (favored or popular district). This study indicates that Hammams present a potential source of pathogenic fungi which may impose a real threat on public health.

1. Introduction

Traditional baths, known as Hammams or Moorish baths are a widespread culture in Maghreb countries including Algeria, Middle East (mainly Syria) and Turkey (Bastuji-Garin et al., 2002; Ouaffak et al., 2003; Goksugur et al., 2006; Benouis et al., 2008; Cherif-Seffadj, 2009; Boggs, 2010). They are highly frequented public places not only for personal hygiene but because Hammams were considered for a long time as a cultural heritage originally attached to religious and familiar rituals. Formerly, traditional bath was adapted to the precepts of Islam, which calls for meticulous hygiene and regular ablutions for prayer, performed as “Ghusl” (full body cleansing) or “Wudu” (washing some parts of the body before each prayers, five times per day). In most cases, Hammam is visited frequently by women in order to recover from childbirth or illness, as well it is considered as a real beauty corners related to different provided cares such as; hair removal, dyeing, application of henna, scrubbing, massage and body care. In Islamic countries, women and men alternatively use Hammams and it is strictly forbidden for the simultaneous use, according to Islamic rulings.

Traditional baths are in general structured as enclosed place consisting of a series of rooms; Bit Skhouna (hot steamy room), Bit al Wasta (warm room), Bit al Barda (cold room) and al Bit El Q'ad (the rest room) where people get wiping and dressing (Ouaffak et al., 2003; Cherif-Seffadj, 2009). Nowadays, with the emergence of private or individual baths and the progress of therapeutic virtues of balneotherapy, usefulness of Hammams extends to be much more associated with relaxation, well-being and even to treat or prevent several dermatological and rheumatological illness (Hannuksela and Ellahham, 2001; Matz et al., 2003; Verhagen et al., 2003; Bender et al., 2005).

Fungal pathogens are recognized among leading agents that causes human skin infections, accounting more than 25% of affected population worldwide (Havlickova et al., 2008). Up to date, over 400 pathogenic fungi species have been reported and some of these are widely distributed in damp location such as swimming pools, saunas, baths including Hammams, which were responsible

* Corresponding author. Departement of Applied Biology, Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, Tebessa 12002, Algeria.
E-mail address: tahamenasria@hotmail.com (T. Menasria).

http://dx.doi.org/10.1016/j.ibiod.2016.12.004
0964-8305/

for several infections (Chabasse et al., 2009; Matos et al., 2002; Brandi et al., 2007). Hammams are highly suspected in mycosis transmission, considering the high level of temperature and humidity for long periods promoting fungal development (Havlickova et al., 2008; Moriyama et al., 1992; Hamada and Abe, 2010). It was reported that cutaneous infections specially induced by dermatophytes were mostly contracted in such places particularly *Tinea pedis*, *Tinea capitis* and *Onychomycosis* (Kamihama et al., 1997; Gudnadottir et al., 1999). In most cases superficial fungal infections are contagious and may tend to recurrence (Kawachi et al., 2010).

A part from, temperature and humidity which contributing to fungi survival, poor hygiene and uncontrolled people attendance with fungal skin infections, adnexa, and mucosae are certainly remaining among the major sources of molds and yeast dissemination in traditional public baths. Many scientific reports deal with the indoor mycobiota of various public and private buildings (Hunter et al., 1988; Gilleberg et al., 1998; Picco and Rodol, 2000; Gniadek et al., 2005; Nunes et al., 2013). However and despite the increased importance of fungal contamination studies in traditional hot steamy baths as part of preventive medicine against mycosis, no researches are available on the incidence of indoor fungal diversity and contaminants in Hammams in Algeria and North Africa. Therefore, a quantitative and qualitative study was conducted to determine and better understand the current distribution of indoor fungal flora of traditional baths in a medium sized city with approximately 320,000 inhabitants (Batna northeastern Algeria).

2. Materials and methods

2.1. Sample collection

Samples were collected from ten different Hammams in Batna city (Northeast Algeria) over a period of four months (from February up to May 2015). Hammams were selected randomly including eight used alternatively by men and women and two others used only by women. For each Hammam, two sets of samples were carried out before cleaning time on five different places including floor, wall, door, air, and marble massage platform of caladarium (hot steamy room). The first set was intended for the enumeration of total fungal flora, while the second used for the isolation.

For surface sampling, 10 cm^2 selected sections was swabbed and swabs expected for enumeration were placed in their tubes with sterile saline, whereas those used for isolation in dry tubes (Hamada and Abe, 2010). The air sampling has been carried out using settle plate method (Napoli et al., 2012).

2.2. Fungal enumeration

After vortexing for two minutes, tenfold dilution series were carried out and aliquots of 0.5 ml of each dilution was spread immediately on Sabouraud chloramphenicol medium (*BioRad*) followed by incubation at 25 °C for 6–8 days. Fungal colonies were then counted and levels are expressed as logarithmic scale of colony format units ($\log_{10}$ CFU/cm^2) (Hamada and Fujita, 2000). All experiments were performed in duplicate.

2.3. Fungal isolation and identification

Fungal flora was isolated by direct inoculation on three different media, Sabouraud agar, Sabouraud agar supplemented with 0.1% chloramphenicol and Sabouraud chloramphenicol actidione agar. The plates were incubated at 25 °C for 4 weeks, and were observed every other day for fungal growth. Positive cultures were subcultured and stored on Sabouraud agar slopes at 4 °C. Selected molds and dermatophytes isolates were identified basing on macro/microscopic characteristics using Lactophenol Cotton Bleu (LPCB) slide mount and urease test when necessary. While, yeasts were identifying using several conventional approaches including macromorphology, germ tube test, Dalmau plate culture, urease test, sensitivity to actidione and India ink staining. Further identification of yeasts was achieved using API 20C AUX system (Biomérieux).

2.4. Survey and questionnaire

After obtaining the agreement, direct interviews were conducted with the owners of Hammams from which sampling was performed, in order to seek information about their knowledge of fungi, their propagation, possibility of fungal survival in Hammams and the way in which they cause infection. A preliminary investigation was also carried aiming to correlate the data with possible fungal contamination.

The questionnaire targeted different factors: location of Hammams, opening year, possible renovations, average daily users, cleaning frequency, cleaned places, and products used for cleaning and disinfection.

2.5. Data analysis

Fungal α-diversity was calculated in terms of Shannon's diversity index $H' = -\sum (p_i \times \ln p_i)$ and evenness ($E = H'/\log S$) according to sampling position, nature and localization of Hammams where species richness (S) is the total number of identified fungal species and p_i correspond to the proportion of individuals of total species represented by *i*th species (Magurran, 2004). Similarities between molds and yeasts assemblages were compared using a Student's *t*-test with $\alpha = 0.05$.

Response frequencies were calculated as the percentage of respondents to the total of surveyed Hammam's owners. Pearson's Chi-squared test (χ^2) was performed to test the statistical significance of differences between groups of each surveyed question.

Analysis of covariance (ANCOVA) was computed to test the effect of factors mean opening year, average customer, cleaning frequency, locality, nature, sampling position and their interactions on fungi load recovered from Hammams. In addition, analysis of variance (ANOVA) was performed testing the effect of sampling position and surveyed Hammams on fungal load. Multiple comparisons of means with Tukey's post hoc analysis were conducted to distinguish homogeneous and heterogeneous groups among different variables. Descriptive statistics (mean, standard deviation and quartiles) were given as box plots to describe the effect of sampling position, nature and localization of Hammams on total and specified fungal loads. All computations were performed using XLSTAT (Addinsoft's, 2014).

3. Results and discussion

3.1. Fungal load and factor affecting their interaction

All studied Hammams yielded positive results for fungal contamination, which is consistent with conditions of fungus growth in particular physical environmental factors including humidity and temperature which considering determinants not only for growth initiation, but to stimulate fungi colonization (Kavanagh, 2011).

The total fungal load depending on sampling sites indicate highly significant difference ($p = 0.003$) (Table 1), showing that the doors and marble massage platforms had the highest rates of