Effect Types of Culture Media on Isolation of Fungi from Indoor Swimming Pools

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***Abstract*—The present study was conducted to study the level of fungal contamination in indoor public swimming pools in Duhok city. A total of 230 samples (50) from pool water and 180 samples from indoor environments were collected from five indoor public swimming pools during September 2014 to February 2015. All samples were examined for the detection of fungi using three different culture media (Potato Dextrose Agar [PDA], Malt Extract Agar [MEA], and Sabouraud's Dextrose Agar [SDA]). A high dominant percentage (44%) of fungi was isolated on PDA, followed by MEA (30%) and SDA (26%). The percentages of fungal contamination of five indoor swimming pools in decreasing manner were 33.5% in swimming pool 4, 27% in swimming pool 1, 25% in swimming pool 5, and 7% in swimming pools 3 and 2. The aims of the present study were to determine the level of fungal contamination in five public swimming pools in Duhok city and to study the effect of different culture media on growth of fungal isolates.**

***Index Terms*–Culture media Duhok, Swimming pools, Yeasts.**

I. Introduction

Fungi are eukaryotic, heterotrophic organisms which may be multicellular (molds) or unicellular (Yeasts). They are ubiquitous and exist as saprobes, mutualisms, or parasites include molds, yeasts, yeast-like fungi, and dimorphic fungi.

The basic morphological structure of filamentous fungi is the hypha, and a web of intertwined hyphae is called a mycelium. The basic form of a unicellular fungus is the yeast cell. Dimorphic fungi usually occur as yeasts in the parasitic stage and as mycelia in the saprophytic stage [1,2].

Fungi have been identified as the most important contaminants of indoor air in swimming pools, and they introduced to the pool environments through fecal matter, vomit, mucus, saliva, and skin contact. Commonly isolated fungi from these environments include *Aspergillus* sp., *Alternaria* sp., *Aureobasidium* sp., *Penicillium* sp., *Cladosporium* sp., *Epicoccum* sp., *Mucor* sp., *Rhizopus* sp., and dermatophytes [3]. The exposure to those fungi has been reported to cause several types of human diseases such as irritation, infections, and allergies [4-6].

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II. Materials and Methods

A. Sample Collection

Fifty water samples and 180 indoor samples were collected from five indoor public swimming pools in Duhok city, Kurdistan Region of Iraq during September 2014 to January 2015. The samples were taken twice per month for each swimming pool. Water samples were collected in sterilized bottles and 1 ml of sodium thiosulfate was added to neutralize the chlorine residual in each sample [7]. Indoor samples from bathrooms, dressing rooms, dried sauna rooms, walls, and floor around the pools were taken using sterile cotton swabs. All samples were transferred immediately to the Faculty of Science/Mycological Research Laboratory.

In this method, 1 ml of each sample was added to six empty Petri dishes: Three plates for Potato Dextrose Agar (PDA), two plates for Malt Extract Agar (MEA), and one plate for Sabouraud Dextrose Agar (SDA) and poured with 20 ml of specified type of molten culture media. Then, the plates (3 PDA, 1 MEA) were shaken gently and incubated at 25°C for 5–7 days and the (1 SDA, 1 MEA) plates at 37°C for 1 week, and they were checked at frequent intervals.

B. Technique of Fungal Isolation from Indoor Samples

Swab rubbed inoculate

The spreading method was used for isolating fungi from indoor environments according to the method described by Viegas *et al.* [8]. Each swab was spread over six Petri dishes containing three types of culture media (PDA, SDA, and MEA). Three plates of PDA and one of MEA were incubated at 25°C for 5–7 days, whereas the plates of SDA and MEA were incubated at 37°C for 1 week and were checked at frequent intervals.

C. Fungal Identification

All species identification was done by colony characterization and microscopic examination using KOH, lactophenol, and lactophenol with cotton blue staining.

Characterization was done according to the keys and descriptions provided by authors in reference [9-21].

D. Media Used for Identification of Candida Species

Chromogenic Candida agar (Rapid Labs Ltd., Essex, UK)

Clinically important *Candida* species were identified using this selective medium which contains chromogenic substrates that detect specific enzymes of some *Candida* species. This selective medium is light sensitive and prepared by dissolving 45.5 g in 1 L of distilled water; stirred until completely dissolved, then autoclaved at 121°C for 15 min, and poured in Petri dishes and kept in refrigerator.

Sabouraud's dextrose agar with 6.5% NaCl

This is prepared by dissolving 65 g of SDA in 1 L of distilled water with the addition of 6.5% NaCl. The solution was completely dissolved and autoclaved at 121°C for 15 min and then poured in Petri dishes and kept in a refrigerator.

III. Results and Discussion

A. Influence of Culture Media on the Percentage of Fungal Isolates

The fungal genera were isolated using three types of culture media PDA, MEA, and SDA as shown in Table I. The highest number of fungi was obtained from PDA (44%) followed by MEA (30%), whereas the lowest number of isolated fungi obtained from SDA (26%). 41 species of fungi were isolated on PDA; this number was expected because PDA is a selective medium for luxuriant growth of yeasts and molds. Potato infusion encourages mold sporulation and pigment production even in some dermatophytes [22]. Furthermore, PDA enhances the production of reproductive

TABLE I
Fungi Isolated by Different Culture Media

No	Fungal isolates	Potato Dextrose Agar	Sabouraud Dextrose Agar	Malt Extract Agar
1	*Absidia* sp.	+	+	—
2	*Acremonium* sp.	+	—	—
3	*Alternaria* sp.	+	—	+
4	*Arthrinium* sp.	+	—	—
5	*Aspergillus* sp.	+	+	+
6	*Aureobasidium* sp.	+	+	+
7	*Bipolaris* sp.	+	—	—
8	*Candida* sp.	+	+	+
9	*Chaetomium* sp.	+	—	—
10	*Chrysosporium* sp.	+	+	+
11	*Cladophialophora* sp.	+	—	+
12	*Cladosporium* sp.	+	—	+
13	*Curvularia* sp.	+	—	—
14	*Drechslera* sp.	+	—	+
15	*Epicoccum* sp.	+	—	—
16	*Epidermophyton* sp.	—	—	+
17	*Eurotium* sp.	+	+	—
18	*Exophiala* sp.	+	+	+
19	*Fusarium* sp.	+	+	+
20	*Gliocladium* sp.	+	—	+
21	*Geotrichum* sp.	+	+	—
22	*Microsporum* sp.	+	—	—
23	*Monascus* sp.	+	+	+
24	*Mucor* sp.	+	+	+
25	*Myrothecium* sp.	+	+	+
26	*Neosartorya fischeri*	—	+	+
27	*Neoscytalidium* sp.	+	+	+
28	*Paecilomyces* sp.	+	—	+
29	*Penicillium* sp.	+	+	+
30	*Phialophora* sp.	+	+	—
31	*Phoma* sp.	+	+	+
32	*Rhizomucor* sp.	—	+	+
33	*Rhizopus* sp.	+	—	—
34	*Rhodotorula* sp.	+	+	+
35	*Scedosporium* sp.	—	—	+
36	*Scopulariopsis* sp.	+	—	+
37	*Stachybotrys* sp.	+	—	—

(Contd...)

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TABLE I
(Continued)

No	Fungal isolates	Potato Dextrose Agar	Sabouraud Dextrose Agar	Malt Extract Agar
38	*Stemphylium* sp.	+	—	—
39	Sterile mycelium (colored)	+	+	+
40	Sterile mycelium (hyaline)	+	+	+
41	*Talaromyces* sp.	—	+	—
42	*Trichoderma* sp.	+	—	—
43	*Trichophyton* sp.	+	—	—
44	*Trichosporon* sp.	—	+	—
45	*Ulocladium* sp.	+	—	+
46	*Verticillium* sp.	+	—	—
47	Yeasts (non-identified)	+	+	+
	Total	41 (44%)	24 (26%)	28 (30%)

structures (conidia) and colony color when other media fail [20].

Twenty-eight species were isolated on MEA, it is a broad-spectrum growth medium commonly used for mold culture in indoor environment. Many studies have revealed that the MEA agar recipe provides excellent morphological growth characters over a broad range of features for a wide selection of organisms [23].

Twenty-four species were isolated on SDA; it is useful for the cultivation of fungi particularly those associated with skin infections; however, SDA has limitations as a medium for the primary isolation of fungi directly from samples [24,25].

The data revealed that the type of culture medium plays a vital role in the dominance of fungi; this may be due to the effect of the media component and the assimilation of dissolved materials in such media, in addition to the ability of many fungi to grow in a wide range of temperature (10-45°C) [26].

B. Fungal Contamination in Five Indoor Public Swimming Pools

The genera of molds and yeasts that have been isolated from five indoor public swimming pools using pour plate method, hair baiting method, and swab spreading method are shown in Table II.

The percentages of fungal contamination in five indoor swimming pools were 33.5% in swimming pool 4, 27% in swimming pool 1, 25% in swimming pool 5, and 7 % in swimming pool 3 and 2.

A total of 44 genera were identified, and similar results were obtained from the study of Mohammad and Habeb [27] who reported *Aspergillus* sp., *Trichophyton* sp., and *Fusarium* sp. as the most frequent isolated fungi in Baghdad swimming pools. Furthermore, Bello *et al.* [7] who studied in Nigeria swimming pools mentioned that the most frequent isolates were *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Aspergillus* sp., and *Candida* sp.

In this study, the highest numbers of fungal isolates were obtained from indoor swimming pool 4, 1, and 5, whereas the lowest number was detected in indoor swimming pool 2 and 3 also and revealed that both saprophytic and opportunistic fungi are existed in water and environmental pools which is in agreement with reference [28].

The recent results are similar to the study of Viegas *et al.* [8] who indicated that 37 different species of molds were isolated from surface water. Moreover, similar results were obtained by from the studies of Nanbakhsh *et al.* [29] and Papadopoulou *et al.* [30] who isolated different types of fungi with different percentages from indoor swimming pools in Iran and Greece. Recent results showed that the fungus contamination in those five indoor swimming pools could be an alarm for the induction of infections and allergy, especially for immunocompromised hosts. Many researchers have shown that the sanitary quality of indoor public swimming pools is a concern for the swimmers due to swallowing of and contact with water. Moreover, it has been shown that swimming pools contribute to the spread of fungi and other organisms such as protozoa, bacteria and viruses because of their common usage [31,32].

The higher number of fungi isolated in swimming pool 4 may be related to the large number of swimmers and low level of residual chlorine, whereas swimming pool 2 and 3 with the lowest number of swimmers and high level of residual chlorine had the lowest number of fungi isolated. The results showed that the swimming pools (4, 1, and 5) were more contaminated than swimming pool (2 and 3), in these swimming pools, bather density was presumably be higher, and thus, the contribution of fecal pollution and other pathogens by the bathers may also higher. In addition, the sanitary condition of the bather would be more difficult to control [33].

The results of this study indicate that the pH of indoor swimming pools in Dohuk Province is suitable for fungal growth which explains the reason behind the high degree of fungal contamination found on this study [34].

IV. Conclusion

Types of media affect numbers and genera isolated of fungi, and these fungi may be a source of infection to the people visiting indoor public swimming pools, especially those with lower immunity.

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Table II
Types of Fungi Isolated in Five Swimming Pools

No	Fungal isolates	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5
1	*Absidia* sp.	—	—	—	+	+
2	*Acremonium* sp.	+	—	—	+	—
3	*Alternaria* sp.	+	+	+	+	+
4	*Arthirinium* sp.	—	—	—	—	+
5	*Aspergillus* sp.	+	+	+	+	+
6	*Aureobasidium* sp.	+	+	+	+	+
7	*Bipolaris* sp.	—	—	—	+	—
8	*Candida* sp.	+	+	+	+	+
9	*Chaetomium* sp.	—	+	—	—	+
10	*Chrysosporium* sp.	+	+	—	+	—
11	*Cladophialophora* sp.	+	—	+	—	—
12	*Cladosporium* sp.	+	+	+	+	+
13	*Curvularia* sp.	+	+	—	+	—
14	*Drechslera* sp.	+	+	—	+	—
15	*Epicoccum* sp.	+	+	—	—	—
16	*Epidermophyton* sp.	+	+	—	—	—
17	*Eurotium* sp.	+	—	—	—	+
18	*Exophiala* sp.	+	+	—	+	+
19	*Fusarium* sp.	+	—	+	+	+
20	*Cliaocladium* sp.	+	+	—	+	+
21	*Geotrichum* sp.	—	—	+	+	+
22	*Microsporum* sp.	+	+	—	—	—
23	*Monascus* sp.	+	—	—	+	—
24	*Mucor* sp.	+	+	+	—	—
25	*Myrothecium* sp.	+	—	+	+	+
26	*Neosartorya fischeri*	+	—	—	+	—
27	*Neoscytalidium* sp.	+	+	+	+	+
28	*Paecilomyces* sp.	—	—	—	—	+
29	*Penicillium* sp.	+	+	+	+	+
30	*Phialophora* sp.	+	+	—	—	—
31	*Phoma* sp.	+	+	—	+	+
32	*Rhizomucor* sp.	—	—	—	—	+
33	*Rhizopus* sp.	+	+	—	—	—
34	*Rhodotorula* sp.	+	+	+	+	+
35	*Scedosporium* sp.	+	+	—	—	—
36	*Scopulariopsis* sp.	—	—	—	—	+
37	*Stachybotrys* sp.	+	+	—	—	—
38	*Stemphylium* sp.	—	—	—	+	—
39	Sterile mycelium (colored)	+	+	+	+	+
40	Sterile mycelium (hyaline)	+	+	+	+	+
41	*Talaromyces* sp.	+	—	—	—	—
42	*Trichoderma* sp.	+	+	—	+	+
43	*Trichophyton* sp.	+	—	—	—	—
44	*Trichosporon* sp.	+	—	—	—	—
45	*Ulocladium* sp.	+	+	—	+	+
46	*Verticillum* sp.	—	—	—	—	+
47	Yeasts (non-identified(	+	+	+	+	+
	Percentage	27%	7%	7%	33.5%	25%

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