Epidemiological Study of Keratinophilic Fungi in Baghdad Swimming Pools

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Abstract:

The present study was included the isolation of Keratinophilic fungi from water samples taken from 19 indoor public swimming pools in Baghdad during four season (summer ,fall ,spring ,and winter). Isolation period was September 2011and September 2012 . Samples were collected from different places in swimming pool . Sixteen keratinophilic fungal species, belonging to twenty –one genera were isolated using Surface Dilution Plating (SDP) and Hair Bait Technique (HBT) . *Aspergillus* genus was the most frequent and occurrence (19.84%), followed by *Trichophyton* (11.60%) and *Fusarium* (7.59%) . The most common fungal infection occurred in summer season (42.16%) , and the most frequent month manifestation of species occurred in August (17%) .

Key words: Keratinophilic fungi , Swimming pools

Introduction:

Keratinophilic fungi are a group of fungi that colonize various keratinous substrates . There are a great number of micro-fungi, potential or conditional pathogens that may be contacted swimming pool but there is no epidemiological evidence about transmission of pathogens by this way [1]. The widespread use of water and other natural surface water for recreational purposes in recent years has brought to the question a problem of exposure to potential risk of health of bathers, swimmers and people who do some other ways of recreation on water . Investigating samples of water during four season, found all samples contaminated by particles of filamentous fungi . Frequent presence of filamentous fungi like *Penicillium, Aspergillus, Fusarium, Cladosporium* and *Rhizopus , Scopulariopsis, Trichophyton* and *Crysosporium* with the frequent presence of people, especially in July and August cause superficial fungal infections of hair, nails and skin. . Infection of the skin of feet, mostly among toes, so called Tinea pedis is characterised by symptoms that involve ulceration, holes and cuts of skin with strong scab [2] . *In vitro* experiment shows that it is necessary approximately 3-4 hours for fungi to initiate the infection. This infection often happens swimmers and it is considered as a relatively benign. The only source of dermatomycota in swimming pools and baths are the infected swimmers , so in monitoring of this kind of fungal infection and in the control of expanding of this disease, education is very important [3]. The occurrence and distribution patterns of dermatophytes and keratinophilic fungi in aquatic habitats depend on many ecological, physical and chemical factors particularly pH, dissolved oxygen concentrations, temperature, heavy metals, and organic matter especially keratinaceous substances [4,5]. Polluted water habitats can be sources of environmental contamination and disease. People exposed to such

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contaminated environments are likely to contract fungal. Some keratinophilic species such as *T. mentagrophytes*, *M. audounii* and *M. canis* isolated from polluted habitats can cause superfacial mycoses. Others such as *M. gypseum*, *T. tonsurans*, *T. ajelloi*, *S. brevicaulis*, and *G. candidum* cause opportunistic mycoses . Swimming pools have also been established to be considered the sources of tinea pedis infection [6] . Faecal matter is introduced into the water when a person has an accidental faecal release (through the release of diarrheic stool into the water) or when residual faecal material on swimmers' bodies is washed into the pool [7] . Non-faecal human shedding (e.g. from vomit, mucus, saliva or skin) in the swimming pool is also a potential source of pathogenic organisms. In addition, infected users can directly contaminate pool waters with pathogens , which may lead to skin infections in other patrons, who come in contact with the contaminated water. In addition, certain living keratinophilic fungi can grow in pool waters, in pool components or facilities (including heating, ventilation and air-systems) or on other wet surfaces within the facility to a point at which some of them may cause a variety of respiratory, dermal or central nervous system infections or diseases [8] .

Materials and Methods:

Collected Samples:

- Samples were taken from 19 indoor public swimming pools of Baghdad City and investigated during four season (summer ,fall ,spring , winter).
- Samples were collected two times per month taken from 4 places in each location as follow :-

A. Water surface near the margin

B. 50 cm depth from Water surface near the margin

C. water surface in the middle of the pool

D. 50 cm depth from Water surface in the middle of the pool .

- Water samples were taken from the swimming pools and foot-washing sink. To neutralize the chlorine residual, sodium thiosulfate was added in the sampling 1.5 liter in dark bottles of water collection [9] .
- All samples were transferred to the laboratory, where they were passed through milipore filters with 0.45 µm size. Filtrates were cultured on Mycosel agar (Sabouraud's dextrose agar with Chloramphenicol and Cycloheximide) . The plates were incubated at 25°C for 4 weeks, and examined at frequent intervals.

Isolation methods:

- **Hair Baiting Technique**

The keratinophilic nature of these fungi makes it possible to isolate them from water by implanting hair, the 'hair baiting' technique initially developed by Vanbreuseghem R., a Belgian mycologist in 1952. Since then, a number of modifications have been developed, but the basic principle remains the same i.e. use of natural keratin substrate as baits to recover these fungi from water

I. Half fill sterile Petri dishes with the sterile soil .

II. Spread short (2-3 cm) strands of sterilized defatted human hair over the surface of the soil and 0.5 ml of water sample (Swimming pools) [10] .

- **Growth on polished rice grains**

I. The medium is prepared in 12 ml flasks by mixing

II. One portion raw unfortified rice grains and 3 portions water or 8,0 g of rice grains and 125 ml of distilled water.

III. Autoclave the rice-water mixture for 15 minutes.

Inoculate the surface of rice, with human hairs and incubate the sample for 2 weeks at 25 to 30°C .

- ***In Vitro* Hair Perforation Test**

1. Place short strands of hair in petri dishes and autoclave the dishes at 121°Cfor 10 minute .
2. added 25 ml of sterile distilled water and 2 or 3 drops of 10% sterilized yeast extract.
3. Inoculate these plates with several fragments of the test fungus collected last from Swimming pools that have been grown on SDA .
4. Incubate the plates at 25°C .

examine them at regular intervals over a period of 21 days [11] .

Fungi identification

Isolation and identification of fungi were achieved by macroscopic colony characterization and microscopic examination. Dermatophyte confirmation was undertaken by complementary tests such as (HBT) .

Results:

The present study comprehends isolation of Keratinophilic fungi from water samples of 19 indoor public swimming pools in Baghdad, the investigation of which had been effected during four seasons (summer, fall, spring, and winter). Isolation period was carried between September 2011and September 2012. Results of isolation showed that 60 keratinophilic fungal species were found belonging to 21genera, the diagnosis of which was established by clinical findings confirmed by Surface dilution plating (SDP), Hair Baiting Techniques [12], Rice grains test ,and Hair *in vitro* test for Dermatophytes, . The results of the survey for keratinophilic fungal genera indicate that different types of fungi among the more frequent isolated genera were *Aspergillus* (15.76%) ,*Trichophyton* (11.41%) and *Fusarium* (8.15%) . (Table 1)

Table (1): Frequency and occurrence of Fungal genera isolated from Swimming pool

Genus	Occurrence%	Frequency%
1-*Alternaria*	4.34%	5.09%
2- *Aspergillus*	15.76%	19.84%
3-*Cephaliophora*	1.63%	1.62%
4-*Chrysosporium*	3.88%	3.37%
5-*Cladosporium*	5.97%	5.53%
6-*Curvularia*	4.43%	6.63%
7-*Exophiala*	1.08%	2.16%
8-*Fusarium*	8.15%	7.59%
9-*Geotrichum*	1.63%	2.06%
10-*Hypomyces*	2.17%	1.62%
11-*Microsporum*	9.23%	6.29%
12-*Mucor*	4.89%	4.12%
13-*Penicillium*	9.78%	6.72%
14-*Phialophora*	1.08%	1.08%
15-*Phoma*	3.80%	3.57%
16-*Rhizopus*	1.63%	1.51%
17-*Scopulariopsis*	3.80%	3.90%
18-*Sterile fungi*	0.54%	0.79%
19-*Trichoderma*	3.26%	4.01%
20-*Trichophyton*	11.41%	11.60%
21-*Ulocladium*	1.63%	1.19%

Keratinophilic Fungal species Isolated From Swimming Pools during the year

The survey of swimming pool samples revealed that 60 species of keratinphilic fungi were found in the water (Table 2): 11 species represent dermatophytes of Keratinophilic fungi which belong to the genera; *Aspegillus(A.parasiticus, A.ochraceus , A.flavipes, A.flavus ,A.niveus, A.versicolor, A.terreus, A.niger, A.fumigatus).* Eight species of *Penicillium* isolated (*P.cyclopium, P.glabrum,P.griseofulvum,P.lividum,P. rubrum,P.rugulosum,P.brevicompactu m,Penic-illium sp.*) .Genus *Fusarium* including Five species (*F.moniliforum , F.solani, F .oxysporum, F. poae, F. sporotrichioides*) .Regarding dermathophytes many species were isolated from pools , including genus *Trichophyton* which was identified. Six species (*T.mentagrophytes ,T.rubrum ,T.tonsurans ,T.interdigitale ,T.verrucosum ,T.violaceum*) , as well as the genus *Microsporum* which was identified, five species (*M.audouinii, M.canis, M.equinum, M.gypseum,*

M.nanum). Genus *Cladosporium* including (*C.herbarum*, *C.cladosporioides*, *C. sfiaerospermum*) .Also two species of *Alternaria* were identified (*A. altrnata*, *A.tenuissima*) ,and two species of *Phoma* (*P.eupyrena* , *P.glomerata*) , *Trichoderma* (*T.harzianum* , *T. longibbrachiatum*) ,and Mucor (*M.circinelloides*, *M.racemosus*) .Other Keratinophilic fungal species were isolated from pools (*Rhizopus* , *Exophiala jeanselmei*, *Geotrichium candidum*, *Cephaliophora tropica*, *Hypomyces chrysospermus*, *Phialophora*, *Ulocladium* , *Chrysosporium Curvelaria*, *Sterile fungi*).

Table (2): Distribution of Keratinophilic Fungal species during the year :-

Month	Location	Types of fungi	No.
January	Palestine Hotel	*A.flavus;A.niveus;A.versicolor;A,niger;C.herbarum;C.cladosporioides; A.altrnata; T.harzianum; Chrysosporium; Curvelaria*	10
February	Palestine Hotel	*Curvelaria ;M.audouinii; A.altrnata;*	3
March	Palestine Hotel	*A.flavus; P.cyclopium; M.racemosus; R. stolonifer ; T.mentagrophytes; Ulocladium*	6
April	Palestine Hotel	*A.flavus; A.niger ;M.canis ;T.tonsurans ;C.cladosporioides Chrysosporium*	12
	Al-Jaderia	*;A.tenuissima ; P.glabrum ;H.chrysospermus ;T.longibranchiatum ; Curvelaria ,Sterile fungi*	
May	Al-Yarmuk	*A.terreus ; P.griseofulvum ;M.circinelloides ;R stolonifer*	13
	Al-Adel	*;M.nanum ;T.verrucosum ; T .violaceum ;c.sfiaerospermum ;Penicillium sp ;G.candidum ;Phialophora ;C.tropica ; Ulocladium*	
June	Al-Rafidain	*A.parasiticus ; ;F.moniliforum ;F.poae ;M.audouinii; P.lividum ;T.rubrum ;Phoma ; H.chrysospermus*	17
	Al-Adel	*; M.canis,A.fumigatus G.candidum ; T.longibranchiatum ; Chrysosporium ; Curvelaria*	
July	Al-Yarmuk	*A.parasiticus ;A.flavipes ;S.brumptii ;S.brevicaulis ; M.audouinii ;M.gypseum ; T.mentagrophytes; C.cladosporioides;*	20
	Al-Adel	*T.rubrum ;T.interdigitale ;C. herbarum ;;E.jeanselmei ; A.altrnata ;P.eupyrena ; Ulocladium; Curvelaria; A.flavus ; A.niveus ; P.rubrum*	
August	Al-Rafidain	*;A.ochraceus ;; A.niger ; P.rugulosum ; F.moniliforum ;F.solani ; M.circinelloides ; M.audouinii ;M.equinum ; T.mentagrophytes; Chrysosporium ; A.tenuissima*	25
	Al-Yarmuk	*A.terreus ;A.parasiticus T.rubrum ; T.tonsurans ; C. herbarum ; A.altrnata ; P.eupyrena ; G.candidum ; Phialophora ; H.chrysospermus ; T.longibranchiatum ; Curvelaria ;T.harzianum; F.oxysporum*	
September	Al-Rafidain	*; A.niveus ; P.brevicompactum ; P.glabrum ; F.solani ;R. stolonifer; Sterile fungi*	12
	Al-Yarmuk	*M.gypseum ; T.violaceum ; Chrysosporium ; T.harzianum ; Curvelaria ; A.versicolor*	
November	Palatine Hotel	*A.parasiticus ; A.ochraceus ; A.flavipes ; A.flavus ; A.niger ; P.griseofulvum ; F.moniliforum ; M.circinelloides ;Rhizopus; S.brevicaulis ; C. herbarum ; C.cladosporioides ; A.altrnata ; C.tropica ; Chrysosporium ; P.rugulosum , P.brevicompactum,P.sp.*	18
December	Palestine Hotel	*P.rubrum ;F. sporotrichioides ; S.brevicaulis ; E.jeanselmei ; A.altrnata ;*	11
	Al-Jaderia	*P.eupyrena ;P. glomerata ; H.chrysospermus ; Curvelaria ; F.moniliforum; S.brumptii*	
			147

A total of 147 species were collected from different parts of pools during the year. The most frequent manifestation of species appears in summer especially during the months: August (17%) and July (14%) when the most common fungal cutaneous infection found at public swimming pools especially in warm climates. The results of winter season are less comparing with those of summer ones especially in February (2.04%) because bath users are less as seen figures (1).

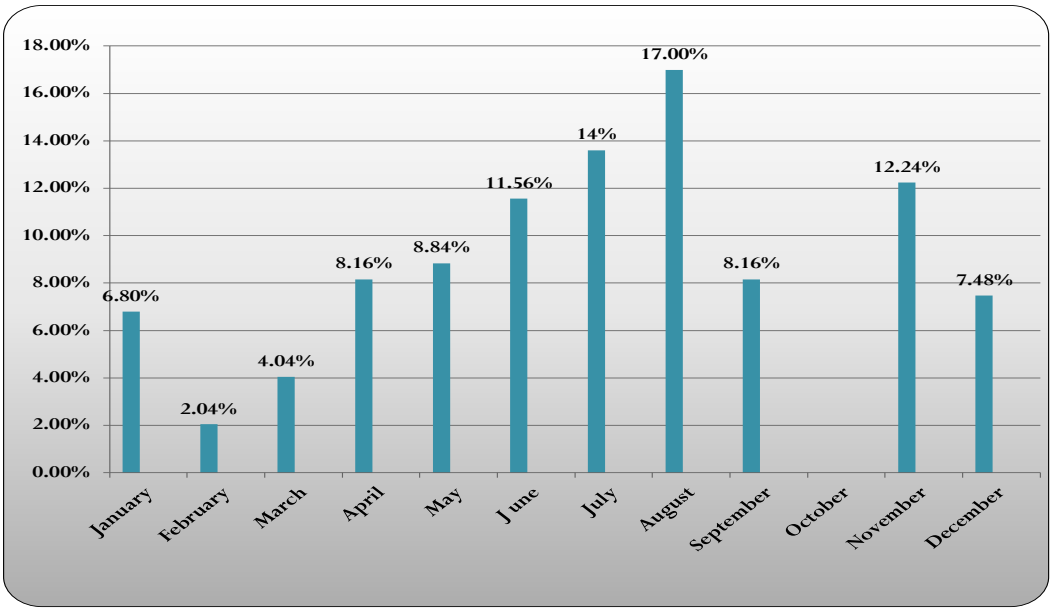

Fig.(1) : Distribution of isolated Keratinophilic Fungi from Swimming Pools during the year

Keratinophilic species isolated from four places for each Pool

The results reveal that the highest number of fungal species found in water surface near the margin (57 species) followed by the fungi of water surface in the middle of the pool (37 species), whereas the number of fungi in 50 cm distance from water surface near the margin and 50 cm distance from water surface in the middle of the pool were 27 and 26 species respectively (table 3) .

Table (3): Distribution of Keratinophilic species isolated from four places for each location In Pools during the year

Location	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	No.
A	5	1	1	6	7	7	9	10	5	-	5	1	57
B	4	-	1	3	2	2	2	1	3	-	4	4	26
C	-	1	1	1	3	6	6	9	3	-	6	1	37
D	1	1	3	2	1	2	3	5	1	-	3	5	27
	10	3	6	12	13	17	20	25	12	-	18	11	147

A. Water surface near the margin
B. 50 cm distance from Water surface near the margin
C. water surface in the middle of the pool
D. 50 cm distance from Water surface in the middle of the pool

Occurrence of Keratinophilic Fungi in Swimming pools during the Seasons

The seasonal variations have an effect over the operation of fungal isolation during collecting time and sites which is almost identical. Maximum incidence of fungal isolates was found during summer season (42.16%) for the four collecting sites, when the number of swimmers using the pools increases in this season and selected ecological factors on these fungi in relation to species diversity and population densities were also considered. A minimum incidence of fungal isolates was detected during winter (16.32%) ,when the number of swimmers using the pools is less in amount than that of summer and condition of grown fungal are poor in winter . (Fig. 3).

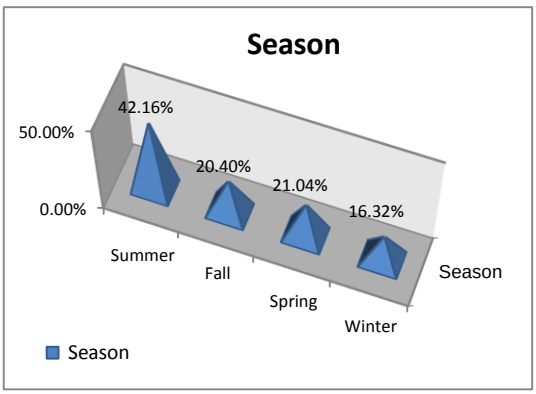

Fig.(3):Occurrence of Keratinophilic Fungi during Four Seasons

Discussion:

Different studies indicated that swimming pools may play an important role as a potential resources for spreading of pathogenic and/or potentially pathogenic fungi to human populations . This is more important in tropical distinct especially during summer, because of more bather users.Swimming pools have been surveyed for keratinophilic fungi and other fungi elsewhere and showed that species of dermatophyes were rarely reported [13]. Eleven species of dermatophytes were found in swimming pools samples from Baghdad pools and they were mostly recovered from Surface dilution plating (SDP) and Hair Baiting Technique (HBT), the sterile rice grain test is useful in distinguishing between atypical isolates of *Microsporum canis* from *M. audouinii* [11]. Other keratinophilic fungal species recovered from swimming pools , in this study, it has been also reported as water common saprophytes and as opportunistic fungi [14] .Among the keratinophilic fungi, species of *Aspergillus* (represented by 9 species) were the most frequent in swimming pools samples . Members of this genus were also reported from water elsewhere . Some species of this genus are known as pathogenic and capable of degrading human hair and feathers , *F.moniliforum, T.mentagrophytes, M.audouinii, A. altrnata, P.eupyrena , P.glomerata , Geotrichium candidum , A.terreus, A.niger, A.fumigatus* and *Chrysosporium* have been recorded as a common species in polluted waters [12]. This survey indicated that this fungus was frequently occurred in swimming pools samples baited with different keratin substrates. The dermatophyte *Trichophyton* was previously found with a high incidence (11.41%) causing tinea infection [15]. The use of different keratin substrates (human hair, feathers, nail) rendered a divers keratinophilic species that able to colonize and degrade one or more of keratin substrates. Keratin degradation ability by fungi is related to the chemical nature of keratin source as well as to the nature of the enzymes produced by these fungi [9]. There was a similarity pattern among the ecological parameters of studied sites and over collecting times. Although the temperature dropped down into (11-16

C) in winter mainly in February, however, number of fungal isolates was recovered from swimming pools samples collected from the four sites. Swimming pools samples collected in August and July, where the temperatures elevated (32-39 C), also showed high occurrence of fungi .The seasonal fluctuation trend of total fungal isolates in swimming pools .

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دراسة وبائية للفطريات المحبة للكراتين في أحواض السباحة في مدينة بغداد

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الخلاصة :

تضمنت هذه الدراسة عزل وتشخيص الفطريات المحبة للكراتين من أحواض السباحة حيث أخذت عينات من 19 حوض منتشرة مناطق مختلفة من بغداد . تم التقصي عن هذه الفطريات خلال فصول السنة المختلفة (الصيف ،الخريف ، الربيع ،والشتاء) . فترة العزل كانت بين أيلول 2011 وأيلول 2012 . جمعت العينات من مواقع مختلفة من المسبح.عزلت وشخص 60 نوعا فطريا محبا للكراتين تابعا إلى 21 جنسا ، طريقة العزل باستخدام تقنية الشعرة وطريقة التخافيف على الطبق. كان جنس *Aspergillus* هو الأكثر تكرارا والأكثر تواجدا بنسبة (19.84%) ،تبعة جنس *Trichophyton* بنسبة تكرار (11.60%) ،ثم جنس*Fusarium* (7.59%) . الإصابات الفطرية الأكثر شيوعا حدثت خلال فصل الصيف بنسبة (42.16%) .وقد سجلت أعلى نسبة لها خلال شهر آب (17%) .