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Original Paper

Fungal Contamination of Public Outdoor Swimming Pools in Awka, Nigeria

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The fungal contamination of some public outdoor swimming pool in Awka was studied before and after use by swimming their suitability for use by bathers. The average fungal counts were 0cfu/ml-8cfu/ml and 1cfu/ml-15cfu/ml before and after use respectively. Trichophyton Rubrum (28.1%), Trichophyton Concentricum (18.8%), Trichophyton Mentagrophytes (37.5%) and Mocrosporium Canis (15.6%) were detected before use while Trichophyton Rubrum (20.7%), Trichophyton Concentricum (17.2%), Trichophyton Mentagrophytes (31.1%), Microsporium Canis (13.8%), Penicillium Marneffei (3.4%), Trichophyton Soudanense (8.6%) and trichophyton Violaceum (5.2% was Isolated after use. Candida Albicas (29.2%), Candida Glabrata (41.7%), Rhodotorula mucilaginosa (35.0%), rhodotorula Mucilaginosa (20.0%), Cryptocous Neoformans (7.5%) and Rhodotorula Glutinis (12.5%) were recovered from the pools after used. Trichophyton Nentagrophytey was recovered in majority of the pools before and after use among the Molds while Candida Glabrata was isolated from Majority of the pools among the Yeasts before and after use. The presence of these fungi in the pools is a public health risk; therefore, such pools should be adequately tested to safeguard the health of the Swimmers.

Keywords: Fungal, Contamination, Public, outdoor, swimming pools.

Introduction

Water is a basic nutrient of the human body and is essential to living organisms, agricultural production industrial processes and domestic use for human [1]. The most foundation need is for water that is suitable for drinking, food preparation and personal hygiene and that poses no risk in any way to human health. A swimming pool is an artificially enclosed body of water intended for swimming or water-based recreation. Public pools are often as part of a larger leisure center or recreational complex and may belong to a hotel or holding resort as an amenity for the recreation of their guest [2]. The water supply to a pool is usually taken from the mains of a public supply. Swimming pools sanitation refers to both visual clarity and levels of micro flora such as bacteria, protozoan's, fungi and viruses in the swimming

pools. The goal of sanitation is to prevent the spread of diseases and pathogens between users [3]. The majority of people attend swimming facilities for recreational activities, rehabilitative treatment or sport. In recent years, these have been reported case of infectious diseases coursed by the inadvertent swallowing of swimming pool water that was contaminated with bacteria, fungi or protozoa cysta while swimming [4]. Swimming pools are often association with outbreak of water borne infections. The infection agents recovered from swimming pool water include a variety of pathogens embracing bacteria, viruses, protozoa and fungi including *trichophyton spp* [5-9]. Although modern swimming pools have a re-circulating system so that water can be filtered and disinfected, effectively, relevant studies shows that neither hi-tech systems nor disinfectants can prevent that colonization of a pool water with hazardous pathogens [10,11]. Contaminated swimming pool can lead to vanity of diseases including skin ulcer, diarrhea, conjunctivitis, traschoma, ear infection such as otitis media, cholera, dysentery, eczema [12]. Because of the fact that public swimming facilities are continuously contamniateted with potentially harmful microorganism from urinary and fecal accidents, various notice washings and other forum of contamination, public swimming facilities can be compared to iolite sewage if not properly maintained, filtered and disinfected [13].

Approximately 10-20% of the population worldwide is infected by dermatphyter which as a type of fungus that can live on the external skin, within hair and in skin and cause skin diseases. The factors that may contribute to the spread of skin diseases in the pools include the swimming water, water temperature, humility, PH, residual chlorine level as well as the age, profession, life, conditions and hygiene status of the bathers before entering the swimming pools. These factors can endanger swimmers health and case invectives diseases including *Candida Vaginitis* [14]. Microbiology evaluation has for many years been the most significant method for sanitary and quality control of swimming pools. For effective quality control, a test for indicator bacteria is usually of primary importance. The most common indicators are the total coli forms, faecal coliforms, intestinal Enterocoic and *clostridium perfringens* [15-16] while the most common microbiology test for assessing the sanity quality of recreational are the heterotrophic plate counts, total coliform and faecal coliform test.

Several fungi know to be pathogenic humans have been isolated from swimming pools in Nigeria. They include *Penicillium sp, Rhizopus sp, Aspafillus sp, Fusarium sp, Mucor sp*, and *Trichophyton Mentagrophytes* [17]. A good knowledge of the mycological quantity of swimming pools is imperative to guide their suitability for use by bathers and curb the spread of infectious diseases, thus in this work, mycological studies of some public outdoor swimming pools in Awka, Anambra state were carried out. The result of this work will establish the baseline data of the mycological quality of the swimming pools and enlighten on the need for the sanitary conditions of such pools and their environments.

Material and Methods

Samples collection: seventy –five water samples was collected from fifteen Public outdoor swimming state. The samples were collected between September and December, 2016. Information regarding the average number of bathers per day, the source of the pools water and the mode and frequency of the pools treatment were obtained from the pools manager voluntarily. Sterile sampling bottles of 500ml capacity each containing four drop of 0.01%

sodium thiosuphate were used. The samples were labeled a-o and analysed with 24 hours of collection.

Total yeasts count: Sabouraud dextrose gar was used as the culture medium. It was prepared according to the manufacturer's instructions and introduced into Petri dishes containing 0.05mll of chloramphenicol added to inhibit bacterial growth and One milliliter of each water sample. The dishes were mixed properly by gentle swirling. The medium was allowed to solidify and incubated at 37^{o}c for hours, after while the yeast colonies that grew were counted and the number expressed as colony forming unit per milliliter.

Total moulds count: The pour plate method was used with Sabouraud dextrose agar as the growth medium. The medium was sample and 0.5mgll of gently swirled and the medium allowed to solidify. Incubation was in an inverted position at 37^{o}c for seven days after while the moulds that developed were counted and expressed as colony forming unit per milliliter.

Characterization and identification of the isolate: The isolates were individual subcultured repeatedly in sterile SDA plates to obtain pure cultures. The pure culture was stored in Bijou bottles. The tests carried out to identify the yeasts were direct mount, lactophenst cotton blue test, gram stain, germ tube test, urease test and sugars, (glucose, lactose, sucrose, raffinose, maltose, saccharose and mannit) assimilation test while and slide cultures were used to identify the moulds. The tests were performed as done by Onuorah et al; [18]

Analysis of Data: The results obtained were subjected to statistical analysis using t-distribution to determine if there is a correlation between the counts during both seasons or not.

Results

The average fungal count of the swimming pools before and after use is presented in Table 1.

Table 1. Average fungal count of the swimming pools before and after use.

Pools	Fungal count before use (cfu/mol)	Fungal counter after use (cfu/mol)
A	1	4
B	3	5
C	ND	1
D	4	6
E	1	4
F	5	7
G	5	7
H	3	5
I	5	7
J	2	7
K	8	15
L	4	8
M	7	10
N	3	5
O	5	7
WHO Standard	0	0

ND = Not detected

The counts before use ranged from 0cful/mol to 8cfumol while the counts after use were 1cfu/mol to 15cfu/mol. Pool A had the lost count of 0cfu/mol and 1cfu/mol before and use respectively while pool K had the highest count of 8cfu/mol and 15cfu/mol before and after

use respectively. The colonial and microscope characteristics of the moulds from the swimming pools before and after use are shown in Table 2.

Table 2. Colonial and Microscopic Characteristics of the moulds from the swimming pools before and after use

Isolates	Colonial Characteristics	Microscopic Characteristics	identity
1	Colonies use slightly raised, white and dolony. The reverse side was yellow in colour	Slender microconida use present. Macroenidia use absent	Trichophyton rebrum
2	Colonies use slow growing and white in colour. The reverse side Was brown in colour	Hyphae use branched, irregular, segmented and septate.	Trichophyton Concentricum
3	Colonies were flat, white in colour, with a powdery surface and downy area. The reverse side was reddish-broom .	Numerous single-celled micronidia and multi-celled macroconidia were seen.	Trichophyton mentagraphytes
4	Colonies were flat, spreading and white in colour with a dense cottony surface. The reverse side was golden yellows in colour	Macroconida were spindle-shaped with 5-15 cells and thick –walled	Microsporium canis
5	Colonies were flat growing, downy and white in colour with yellow-green conidia heeds.	Conidiophoros were smooth walled, and bore terminal vesicles of metulne, each bearing phialides.	Penicillium marneffei
6	Colonies were slow growing with a flat syede like surface.the mycelia and reverse pigment were deep apricot-orange in colour	The hyphae showed right angle branching	Trichophyton soudanense
7	Colonies were slow growing, waxy, heaped, folded and deep violet in Colour	Hyphae were broad, branched and distorted. No conida were present	Trichophyton violaceum

The moulds were identified as *Trichophyton rubrum, Trichophyton, Concentricum, Trichophyton soudanense* and *Trichophyton violoceum.* The occurrence of the moulds in the swimming pools before and after use is shown in Table 3.

Table 3. Colonial, Microscopic and biochemical Characteristics of the Yeast from the swimming pools

Isolate	**Gram stain**	**Colour**	**Shape**	**Gert ube test**	**Urease test**	**Glucose fermentation**	**Lactose fermentation**	**Sucrose fermentation**	**Maltose fermentation**	**Raffinose fermentation**	**Identity**
1.	**+**	**Creamy**	**Round**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**Candida Albicans**
2.	**+**	**Creamy**	**Round**	**-**	**+**	**+**	**-**	**-**	**-**	**-**	**Candida Glabrata**
3.	**+**	**Creamy**	**Round**	**-**	**+**	**+**	**-**	**+**	**+**	**+**	**Rhodotorua Mucilag**

											inosa
4.	**+**	**Creamy**	**Round**	**-**	**+**	**+**	**-**	**-**	**-**	**-**	**Cryptococcus Neoformans**
5.	**+**	**Pink**	**Round**	**-**	**+**	**+**	**-**	**+**	**+**	**-**	**Rhodotorula Glutinis**

+ = Positive
— = Negative

Trichophyton rubrum, Trichophyton, Concentricum, Trichophyton mentagrophytes and *microsporium canis* were isolates from the pools before *Trichophyton mentagrophytes, microsporium canis, Penicillium marneffei, Trichophyton soudanese* and *Trichophyton violaceu* were isolated from the pools after use by bathers. The colonial,microscopic and biochemical characteristics of the yeasts from the swimming pools are presented in Table 4.

Table 4. Occurrence of the moulde in the swimming pools before and after use

Isolates	Occurrence before use	Occurrence after use
Trichophyton rubrum	**+**	**+**
Trichophyton concentricum	**+**	**+**
Trichophyton mentagrophytes	**+**	**+**
Microsporium canis	**+**	**+**
Penicillium marneffei	**-**	**+**
Trichophyton soudanense	**-**	**+**
Trichophyton violaceum	**-**	**+**

+ = Detected
_= Not detected

The yeasts were *Candida Albicans, Candida glabrata, Crytococcus neoformans, Rhodotorula glutinis* and *Rhodotorula mucilaginesa*. The occurrence of the yeasts in the swimming pools before and after use is shown in Table 5.

Table 5. Occurrence of Yeast in the swimming pools before and after use

Isolates	Occurrence before use	Occurrence after use
Candida albicans	**+**	**+**
Candida glabrata	**+**	**+**
Rhodotorula mucilaginosa	**+**	**+**
Cryptococcus neoformans	**+**	**+**
Rhodotorula glutinis	**-**	**+**

+ = Detected
_= Not detected

Carnida albicans, Candida glabrata, Cryptococcus neoformans and *Rhoditorula mucitaginesa* isolated from the pools before use while *candida albicans, Candida glabrata, Cryptococcus neoformans, Rhodotorula glutinis* and *Rhodotorula mucilaginesa* isolated from the pools after use. The number of swimming pools with the moulds before and after use is shown in table 6.

Table 6. Number of swimming pools with the moulds before and after use

Isolates	Number with the isolate before use (%)	Number with the isolate after use (%)
Trichophyton rubrum	5 (33.3)	8 (53.3)
Trichophyton concentricum	4 (26.7)	6 (40.0)
Trichophyton mentagrophytes	8 (53.3)	12 (80.0)
Microsporium canis	3 (20.0)	5 (33.3)
Penicillium marneffei	ND	2 (13.3)
Trichophyton soudanense	ND	3 (20.0)
Trichophyton violaceum	ND	2 (13.3)

ND = Not detected

Trichohyton mentagraphytes was isolated in majority of the pools before and after use (53.3% and 80.0% respectively) while *pencillium marneffei* and *Trichophyton violaceum* were isolated in 2(13.3%) of the pools assessed each. The number of swimming pools with the yeast isolation before and after use is presented in table 7.

Table 7. Number of swimming pools with the yeast before and after use

Isolates	Number with the isolate before use (%)	Number with the isolate after use (%)
Candida albicans	4 (26.7)	6 (40.0)
Candida glabrata	6 (40.0)	10 (66.7)
Rhodotorula mucilaginosa	3 (20.0)	5 (33.3)
Cryptococcus neoformans	ND	1 (6.7)
Rhodotorula glutinis	1 (6.7)	3 (20.0)

ND = Not detected

Candida glabrata was isolated from majority of the pools assessed before and after use while *Cryptococcus neoformans* was isolated in one pool (6.7%) only. The frequency of isolation of the moulds in the swimming pools before and after use is presented in Table 8.

Table 8. Frequency of Isolation of the moulds from the swimming pools before and after use

Moulds	**Frequency of isolation (n) before use (%)**	**Frequency of isolation (n) after use (%)**
Trichophyton rubrum	**9 (28.1)**	**12(20.7)**
Trichophyton concentricum	**6 (18.8)**	**10 (17.2)**
Trichophyton mentagrophytes	**12 (37.5)**	**18 (31.1)**
Microsporium canis	**5 (15.6)**	**8 (13.8)**
Penicillium marneffei	**ND**	**2 (3.4)**
Trichophyton soudanense	**ND**	**5 (8.6)**
Trichophyton violaceum	**ND**	**3 (5.2)**
Total	**32 (100.0)**	**58 (100.0)**

n = number of colonies
ND = Not detected

Trichophyton mentagrophytes had the highest frequency of 37.5% and 31.1% before and after use respectively while *penicillium marneffei* had the lowest frequency of 0.0% and 3.4% before and after use respectively by bathers. The frequency of isolation of the yeasts from the swimming pools is shown in table 9.

Table 9. Frequency of Isolation of the moulds from the swimming pools before and after use

Yeasts	Frequency of isolation (n) before use (%)	Frequency of isolation (n) after use (%)
Candida albicans	7 (29.2)	10(25.0)
Candida glabrata	10 (41.7)	14 (35.0)
Rhodotorula mucilaginosa	5 (20.8)	8 (20.0)
Cryptococcus neoformans	ND	3 (7.5)
Rhodotorula glutinis	2 (8.3)	5 (12.5)
Total	24 (100.0)	40 (100.0)

n = number of colonies
ND = Not detected

Cryptococcus neoformans had the lowest frequency of 0.0% and 7.5% before and after use respectively while *Candida glabrata* had the highest frequency of isolation of 41.7% and 35.0% before and after use respectively.

Discussion

The pools assessed were filed with an average number of users ranging between 2 and 30 individuals per day, comprising males and females. The source of the pools water was the hotels borehole facilities. While most of the pools were treated with chlorine occasionally before use, other pools were not treated at all before use by bathers. The average fungal count was 0cfu/ml to 8 cfu/ml before use and 1cfu/ml to 15cfu/ml after use (Table 1). Fungi were isolated from all the pools assessed. This could be attributed to poor disinfection, lack of treatment, human activities and contaminated pools environment. There was an increase in the average fungal count after use by bather, which could be as result of increased human activities and increased temperature that encouraged the growth of the fungi. Itah and Ekpombok [17] however obtained a fungal count of $5x10^6$ Cfu/ml – $3x10^7$ Cfu/ml for the swimming pools they assessed in Calabar, South-South Nigeria. The variation may come from the medium used, the number of samples used; the number of bathers, the Sanitary condition of the pools environment, the hygiene status of the bathers before using the pools and the source of the pools water. The fungal isolated from the swimming pools before and after use are *Trichophyton rubrum, Trichophyton concentricum, Trichophyton mentagrophytes, Microsporium canis, Penicillium marneffei, Trichophyton soudanense, Trichophyton violaceum, Candida albicans, Candida glabrata, Rhodotorula mucilaginosa, Cryptococcus neoformans, Rhodotorula glutinis* (Table 2 and 3).

Sima et al [19] assessed the microbial contamination of public simming pools in Kashon, Iran and isolated *Penicillium sp.* Papdoulou at el [20] studied the microbiological quality of swimming pool water in Northwastern Greece and recovered *Candida albicans, Penicillium spp* and *Trichophyton spp*. Itah and Ekpombok [17] assessed the pollution status of swimming pools in Awka Ibom and Cross River state, Nigeria and isolated *Penicillicum spp*. *Candida albicans and Trichophyton mentagrophytes.* Mbata et al [21] isolated *Penicillium spp*, *Trichophyton rubrum* and *Tichophyton mentagrophytes* in the swimming

pools they studied in Enugu, Nigeria while Rafiei and Amirrajab also [22] isolated *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Penicillium spp* in indoor public swimming pools in Ahwaz, South-west of Iran. Most of the moulds isolated from the pools were deatophytes that are known to cause superficial, fungal infections of the hair, finger, nail and skin. More fungi were isolated from the polls after use by bathers (Tables 4 and 5). The organism may have come from the swimmers bodies particularly their skins, hairs, fingers and nails. More moulds were also isolated from the pools than yeasts before and after use. The dermatophyte *Trichophyton mentagrophytes* were isolated most frequent 37.5% and 31.1% before and after use respectively while the filamentious fungus *Penicillium marneffei* had the lowest frequency of isolated of 0.0% and 3.4% before and after use respectively Table 8. However, Mbata et al [21] isolated *Penicillicum spp* (38%) and *Trichophyton rubrum*.

Majority of the pools assessed had Trichophyton mentagrophytes before and after use while Penicillium marneffei and Trichophyton violaceum were each isolated in only 2 (13.3%) of the pools (Table 6). This may be attributed to the cosmopolitan nature of the dermatophyte. *Candida glabrate* was isolated is majority of the pools assessed (40.0% and 66.7%) before and after use respectively while *Cryptococcus neformans* was isolated in only one (6.7%) of the pools (Table 7). (3%) from the pools they assessed in Enugu Nigeria. In addition, the yeast *Candida glabrata* had the highest frequency of isolation of 41.7% and 35.0% before and after use while *Cryptococcus neoformans* had the lowest frequency of 0.0% and 7.5% before and after use respectively by swimmers (Table 9).

Conclusion

This work showed that the swimming pools assessed were contaminated with yeast and moulds which have been reported to cause infections in humans. Adequate treatment of such pools must be carried out to avert a public health hazard. The Sanitary condition of the floors surrounding the pools should be improved while individuals with infections should be denied entry into the pools. Regular mycological analysis of the pools as well as frequent changing of the pools water are also recommended.

Conflicts of Interest

There is no conflict of interest

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