Research Square

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The Best Commercial Chemical Disinfectant for Swimming Pools Environments Contaminated With Fungi

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Abstract

Background: Swimming pools are contaminated with different microorganisms. This study aimed to investigate the fungal contamination of the swimming pools and detect the susceptibility of the isolated fungi to routinely used disinfectants.

Methods: Surface and water samples were collected from different parts of 13 indoor swimming pools. Isolated fungal species were identified based on respective colony morphology, microscopic examination, and RFLP-PCR. Susceptibilities of fungal species to common disinfectants used in swimming pools were investigated, according to M38-A and M27A3 protocols.

Results: Of the 795 collected specimens, 2211 isolates from 35 fungal species were identified. The most isolated fungi were filamentous hyaline hyphomycetes (especially *Aspergillus* spp.), and dematiaceous (especially *Cladosporium* spp.), followed by Mucorales, *Candida* species and dermatophyte species. The most contaminated places were shoes changing and dressing rooms. Sodium hypochlorite (bleach) and chloroxylenol (Dettol) were found to be effective against all isolated fungi after 2.5 and 5 minutes, respectively. Isopropyl alcohol (Afrooz disinfectant solution) was not an effect on *Aspergillus* spp., dematiaceous fungi, and *Candida* spp. after 10 minutes' exposure. The efficacy of hydrochloric acid (liqueur de Javel) showed the most fungicidal activity against dermatophytes at all times.

Conclusions: For efficient cleaning, the disinfectant must remain on the surfaces for an appropriate period. Sodium hypochlorite was efficient antifungal activity in a shorter time for killing all species of fungi. Given the different sensitivity and resistance profile of fungi to disinfectants, regular assessment of the disinfectants used for cleaning the pools is suggested.

Background

Swimming in the pools has benefits for health by relaxing, recreating, practicing on water-related sports activities, and socializing. However, there are many chemical compound (chlorine), physical features of the water (temperature), and microbiological agents can produce some health risks associated with the swimming pools (1–3). Numerous infectious agents in the water of pools and spa (bacteria, fungi, viruses, and protozoa) may be harmful to public health (3). Mold fungi are ubiquitous and widespread in our environment and proliferated by spores. Exposure to fungi can cause systemic fungal infections, especially in immunocompromised patients, and dermatophytosis (4–8). Sufficient moisture is an essential factor for the growth of fungi and environmental contamination by such microorganisms.

Cleaning the pools (removal of all foreign materials) and surfaces is very effective in reducing the load of microorganisms present on contaminated equipment (9). Disinfectants play an important role in the control of contamination, particularly in the prevention of micro-organisms transmission. As there are reports about resistant fungi (10), the selection of appropriate disinfectants is important because the response of pathogens is varying.

Disinfection is defined as a process that eliminates many or all pathogenic microorganisms, with the exception of corresponding spores. There are many different types of disinfectants with different spectra of activity and modes of action (11). The efficacy of disinfection is influenced by a number of factors, like previous cleaning and the existing organic load, the type of microbial contamination, the concentration and exposure time of the germicide, and the temperature and pH of the disinfection process (9). The aim of this study was to investigate the prevalence of fungi isolated from different parts of public indoor swimming pools and evaluate their susceptibility to commonly used disinfectants.

Methods

Specimen collection and fungi isolation

The present cross-sectional study was carried out in 13 public indoor swimming pools. Samples were collected from different surfaces of the swimming pools such as shoes changing rooms, showers, dressing rooms, pools surround, Sauna, and Jacuzzi during spring and summer of 2018. After decontamination of swimming pools in the morning and before using, surface sampling was done by carpet sterile pieces (approximately 4×4 cm^2). All the samples were cultured on sabouraud dextrose agar (Merck, Germany) with chloramphenicol at 25–30 °C for 7–14 days and examined at frequent intervals. Isolated fungi were characterized by their colony morphology and microscopic examination with slide culture method using lactophenol cotton blue staining. Also, dermatophytes and yeast were identified by RFLP PCR (12) (13). Data about swimming pools including the type and exposure times of disinfectants used and the manner of washing surfaces were collected from swimming pool managers through interviews and recorded on special forms.

Susceptibility Of Fungi To Disinfectants

Susceptibilities of frequent fungal species were investigated to common disinfectants used in the swimming pools. The isolated fungi selected randomly from different parts of swimming pools for susceptibility test comprised 30 species of *Aspergillus (A)* (*A.flavus*, *A.fumigatus*, and *A.niger*), 10 species of dematiaceous (*Cladosporium*, *Epicoccum*, *Aureubasidium*, *Alternaria* and *Stemphylium*), 10 species of dermatophytes (*Epidermophyton (E) floccosum*, *Trichophyton (T) tonsurans, T. mentagrophyte*, *T. verrucosum*), 10 species of *Mucorales*, and 10 *Candida (C)* species (*C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei*). Four common disinfectants used in the pools were included in this study; sodium hypochlorite solution (Golrang, Bleach & disinfectant, Iran), hypochloridric acid (Golrang, Iran), Isopropyl alcohol antiseptic (Afrooz, Iran) and chloroxylenol (Dettole, Alyasamin, multi-action cleaner, Egypt). Final concentrations of the disinfectants were prepared, according to the corresponding manufacturers' recommendations.

The inoculums were prepared by inserting sterile distilled water on mature isolated slants medium and gently scraping the surface. Then, they were vortexed for 2–3 min and permitted to settle for 5 min. An

inoculum concentration of $0.4–5 \times 10^4$ conidia ml^{-1} was prepared and adjusted spectrophotometrically to the optical density for each species, according to M38-A and M27A3 protocols references (14), (15). In some isolates where fungi did not produce conidia, the small portion of the mycelial growth was harvested and gently homogenized in sterile distilled water using the ultrasonic device (Hielscher Ultrasonics, USA). Final fungal suspensions were cultured on sabouraud dextrose agar and incubated at 30 °C. Visible colonies were counted after 3 to 7 days of growth to check the viability of the fungi.

For each isolated fungus, four sterile test tubes containing 200 µl of suspension and 200 µl of each disinfectant were prepared, and incubated at room temperature for 2.5, 5, 7.5, and 10 minutes. After each exposure time, 200 µl of European neutralizing solution (1% v/v phosphate buffer, 0.5% w/v sodium thiosulfate, 0.1% w/v L-histidine, 0.3% w/v lecithin, and 10% v/v Tween-80 [refined soybean oil]) was added to the tubes to inactivate the disinfectants. After 5 minutes, suspensions were spun in 3000 rpm for 3 minutes using the micro centrifuge. The supernatants removed. The sediments washed three times with 200 µl PBS buffer. Final sediment suspended in 200 µl of sterile distilled water. 50 µl of each tube was cultured triply.

Statistics

Number of fungal colonies on sabouraud dextrose agar media was interpreted as the survival of the fungi and resistance to disinfectants at exposure time. The mean colony counts of plates were recorded as mean viable count (MVC). Mean of three plates and those with colony count less than 5 colonies were considered significant for the assay. Data were analyzed by SPSS 16.

The ethics committee of Prof. Alborzi Clinical Microbiology Research Center approved this research (Grant number: 93 – 21).

Results

According to the data extracted from the forms, 13 indoor swimming pools were cleaned daily with disinfectants. From 795 collected specimens, 2211 isolates were identified from 35 fungal species. Many species were isolated from all the pools. The most isolated fungi were filamentous hyaline hyphomycete, dematiaceous, followed by *Mucorales*, *Candida* spp. and dermatophyte spp. (Fig. 1). By PCR-RFLP identification, *C. albicans*, *C. tropicalis*, *C. glabrata, C. krusei*, and *T. mentagrophyte, T. tonsurans, T. verrucosum*, *Microsporum canis* and *E. floccosum* were identified. The most contaminated places in swimming pools were changing shoes areas, dressing rooms, and showers (Fig. 2).

According to Table 1 and Fig. 3, the effect of sodium hypochlorite at 2.5 min on all isolated fungi was high and the MVC/ml for *Aspergillus* spp., *Mucorales* were 3 and 0.2, respectively. Dematiaceous fungi, *Candida* spp. and dermatophyte spp. did not grow in 2.5 minutes' exposure time. This disinfectant was very effective in short time length for cleaning the environment of the pools (Fig. 3). Isopropyl alcohol was not a suitable disinfectant, after 10 minutes' exposure to *Aspergillus* spp., dematiaceous fungi, and

Candida spp. with the means MVC/ml being 78.23, 6.3, and 5.5, respectively (Fig. 4). Chloroxylenol was found to be an effective antifungal agent against all isolated fungi after 5 minutes (mean MVC/ml < 5). It is the most effective on dermatophytes, after 2.5 minutes all of them were killed. To clean the environment, 5 minutes' exposure time was essential (Fig. 5). Hydrochloric acid exhibited (Fig. 6) the most fungicidal activity for dermatophytes at all times. The time lengths needed to kill *Aspergillus spp.*, dematiaceous, *Candida* spp., and Mucorales fungi isolated from the pools were 10 min (mean MVC/ml = 3.13), 7.5 minutes (mean MVC/ml = 3.8) and 5 minutes (mean MVC/ml = 2.6) and 5 minutes (mean MVC/ml = 0.6), respectively.

Table 1
The mean count of viable colony of the isolated fungi after different exposure times with disinfectant

Fungi Species	Disinfectants	Fungal growth (colony count)			
		2.5 Minute	**5 Minute**	**7.5 Minute**	**10 Minute**
Aspergillus **spp**.	Sodium hypochlorite	Mean: 3.0 Std Dev*: 5.98	Mean: 1.73 Std Dev: 2.65	Mean: 1.70 Std Dev: 5.05	Mean: 1.53 Std Dev: 4.92
	Afrooz solution	Mean: 105.43 Std Dev: 145.21	Mean: 97.0 Std Dev: 131.53	Mean: 94.23 Std Dev: 153.41	Mean: 78.23 Std Dev: 112.59
	Chloroxylenol	Mean: 3.57 Std Dev: 6.500	Mean: 0.70 Std Dev: 1.291	Mean: 0.30 Std Dev: 0.651	Mean:0.13 Std Dev: 0.434
	Choloridric Acid	Mean: 23.06 Std Dev: 45.62	Mean: 6.43 Std Dev: 11.59	Mean: 6.700 Std Dev: 13.09	Mean: 3.13 Std Dev: 6.08
Dematiaceous Mold	Sodium hypochlorite	Mean:0.0 Std Dev: 0.0	Mean: 0.0 Std Dev:0.0	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0
	Afrooz solution	Mean: 17.20 Std Dev: 15.14	Mean: 15.90 Std Dev: 15.86	Mean: 11.50 Std Dev: 12.94	Mean: 6.30 Std Dev: 6.27
	Dettol	Mean: 9.80 Std Dev: 24.77	Mean: 3.40 Std Dev: 8.36	Mean: 1.80 Std Dev: 4.68	Mean: 1.20 Std Dev: 3.12
	Choloridric Acid	Mean: 8.70 Std Dev: 21.00	Mean: 7.50 Std Dev: 19.24	Mean: 3.80 Std Dev: 9.69	Mean: 0.70 Std Dev: 1.88
Dermatophytes fungi	Sodium hypochlorite	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0
	Afrooz Solution	Mean: 1.30 Std Dev: 4.11	Mean: 1.00 Std Dev: 3.16	Mean: 0.80 Std Dev: 2.52	Mean: 0.6000 Std Dev: 1.89

***** Standard deviation: Std Dev

	Chloroxylenol	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0
	Choloridric Acid	Mean: 0.10 Std Dev: 0.31	Mean:0.10 Std Dev: 0.31	Mean: 0.10 Std Dev: 0.31	Mean: 0.10 Std Dev: 0.31
Candida **spp.**	Sodium hypochlorite	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0	Mean: 0. 0 Std Dev: 0.0
	Afrooz Solution	Mean: 60.50 Std Dev: 80.73	Mean: 13.50 Std Dev: 18.68	Mean: 7.20 Std Dev: 15.18	Mean: 5.50 Std Dev: 11.65
	Chloroxylenol	Mean: 5.00 Std Dev: 5.538	Mean: 2.40 Std Dev: 4.088	Mean: 1.00 Std Dev: 1.333	Mean: 0.80 Std Dev: 1.033
	Choloridric Acid	Mean: 18.60 Std Dev: 21.08	Mean: 2.60 Std Dev: 2.36	Mean: 1.00 Std Dev: 1.63	Mean: 0.60 Std Dev: 0.84
Mucorales	Sodium hypochlorite	Mean: 0.20 Std Dev: 0.63	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0
	Afrooz Solution	Mean: 17.20 Std Dev: 17.43	Mean: 1.40 Std Dev: 2.06	Mean: 0.20 Std Dev: 0.42	Mean: 0.00 Std Dev: 0.0
	Chloroxylenol	Mean: 13.80 Std Dev: 23.05	Mean: 5.0 Std Dev: 10.54	Mean: 3.40 Std Dev: 7.16	Mean: 1.20 Std Dev: 2.53
	Choloridric Acid	Mean: 10.30 Std Dev: 15.42	Mean: 0.60 Std Dev: 0.84	Mean: 0.0 Std Dev: 0.0	Mean: 0.0 Std Dev: 0.0

***** Standard deviation: Std Dev

Discussion

In the present study, many fungi were isolated from indoor swimming pools. Sensitivity of the isolates to disinfectants varied depending on the type of disinfectant and exposure times. Accordingly, just washing the pools with a disinfectant is not a suitable method for cleaning. For efficient cleaning, the disinfectant

must remain on the surfaces for an appropriate period. Clean and safe environment of swimming pools is relaxing for swimmers. The absence of effective disinfectant in swimming pools makes the pools an important source of microorganisms including fungi (1). There are reported data about bacterial and fungal contamination of public indoor swimming facilities due to sufficient moisture for fungal growth (2), (7), (16). In this study, sampling was done after the sterilization program and isolation of the fungi in different parts of the pools revealed the inappropriate cleaning.

In the present study, 35 various species of fungi like dematiaceous, hyaline hyphomycete, yeast, and dermatophyte were isolated from the pools. In epidemiologic studies of fungi in Italy and Poland on indoor swimming facilities, the similar genus of filamentous and yeasts were detected (7), (16). In two cities of Iran, researchers reported various kinds of fungal species including *Aspergillus* spp., *Penicillium* spp., dematiaceous fungi, *Rodotorolla*, *Fusarium*, and dermatophytes (1), (17). The most contaminated places were the dressing rooms, changing shoe areas, and showers. In the literature, also showers, dressing rooms, the bottoms of the swimming pools were reported as the most contaminated places in the pools (1), (17). Our study revealed a high prevalence of fungal species in the indoor swimming pool facilities, consistent with previously published studies.

Hyaline hyphomycetes in public swimming pools and/or baths are identified as predisposing factors for many infections like otomycoses. From all the monitored facilities, *Aspergillus* spp., and *Penicillium* spp., were constantly isolated. Significantly, allergic bronchopulmonary aspergillosis, asthmatic and fungal sinusitis are some characteristic manifestations of hypersensitivity to fungi, particularly due to *Aspergillus* species. Isolation of such airborne microorganisms raises concerns for possible onset of adverse health effects among patrons and employees (16). Bush et al. affirmed that although fungi such as *Aspergillus* and *Penicillium*, might be found in normal indoor environments at high levels, it is specifically a health risk for those allergic to the mold (18).

Disinfectants are chemical agents that play an important role in controlling microorganisms in the environments by reducing their count. The specific time and temperature needed to kill the microorganisms must be considered as per the respective manufacturers'. Sodium hypochlorite solutions (NaOCL), commonly known as bleach, is a chemical compound with a pale greenish-yellow dilute solution and stable in the refrigerator. It is widely used as a disinfectant and bleaching in the house. Hydrochloric acid (H_2O: HCl, liqueur de Javel) is the aqueous solution of hydrogen chloride. It is a colorless fluid with a pungent smell. It is used on a large scale like in agriculture, food and glass industries, lime, and waste disposal industries. Hydrochloric acid can be used to disinfect water and prevent algae and shellfish growth and disinfection of the house, pools, odor removal, surface purification, bleaching, and water disinfection. Isopropyl alcohol is isomer of 1-propanol and ethyl methyl ether with strong odor and use as an antiseptic. Chloroxylenol (Dettol) is an antiseptic used for skin disinfection and surgical instruments cleaning. It is also used within several household disinfectants and wound cleaners. Depending on the type of fungus, the antifungal activities of these disinfectants were different. There are limited data about the effect of disinfectants on filamentous fungi. The response of micro-organisms to disinfectants varies and filamentous fungi are more resistant to disinfectants than

yeasts and non-sporulating bacteria (19). Time and concentration are the two influential factors in increasing of antifungal activity of disinfectants (20). Limited studies have addressed the antifungal activity of disinfectants.

Mattei and co-worker reported that Chlorhexidine- cetrimide, benzalkonium chloride, and a chlorophenol derivative presented effective property against all *Aspergillus* isolate, except for the *A. flavus* strain (21). Sodium hypochlorite in Mattei et al. was ineffective against three *A. fumigatus*, three *A. flavus*, and one *A. niger* isolate. In the present study, after 2.5 minutes, the mean MVC/ml of isolated *Aspergillus* sp. was 3 colonies (21). In a study carried out by Nowrozi and co-workers, in the low dilution (2.5%) of Chloroxylenol at 15 min contact time, all isolates were resistant but with the increase of contact time, the resistant dcresed (20). In our study, with an exposure time of 2.5 minutes, Sodium hypochlorite and Chloroxylenol were acceptable for killing these fungi and after 5 minutes Hydrocholoridric acid was also found effective. Isopropyl alcohol was not suitable for killing the isolated *Aspergillus* spp.

In the present study, dermatophytes were isolated from the surface of pool environments. Some of the isolates are responsible for human dermatophytosis like *T. rubrum* which is the etiologic agent of the athlete's foot (16). In the current study, with 2.5 minutes' incubation period, Chloroxylenol and Sodium hypochlorite were the most effective on dermatophytes. Other disinfectants exhibited antifungal effects on dermatophyte fungi, too. Dematiaceous fungi due to melanin and sporopollenin in cell components might be involved in cellular resistance to physical and chemical agents (9),(11). They can cause invasive and systemic infections in immunocompromised patients. Our results showed Sodium hypochlorite was effective against these fungi at all times. Chloroxylenol was effective after 5 minutes while other disinfectants were effective after 10 minutes. In Nowrozi et al, Chloroxylenol® 2.5% solution was reported as an effective disinfectant against *Cladosporium* spp. (20). The findings of Sandle et al. showed the MIC90 value of Benzalkonium chloride against *Alternaria* spp, was higher than that for other hyaline fungi (11). In the present study, Sodium hypochlorite was an effective disinfectant after 2.5 minutes on Mucorales. The MVC/ml of Mucorales after 5 minutes of incubation time with other disinfectants was reliable. In one study, Chloroxylenol® 2.5% solution was the most effective disinfectant against *Cunninghamella* and *Mucor* spp. than Betadine®, Benzalkonium chloride, and Chlorhexamed® (20). *Candida* species can cause infection on cutaneous and mucosal sites of human bodies. Sodium hypochlorite was an effective disinfectant after 2.5 minutes on *Candida* spp. After 5 minutes, Chloroxylenol and Choloridric acid were effective. Isopropyl alcohol was not a suitable disinfectant against *Candida* spp.

Limitation

For surface sampling, we encountered some technical difficulties such as growing colonies overlaps due to numerous contaminating spores, and performing sensitivity tests for all isolates.

Conclusion

Given the different sensitivity and resistance profiles of fungi to disinfectants, regular assessment of the disinfectants used to clean the pools is suggested. The rapid cleaning is not suitable and the disinfectant must remain on the surfaces for an appropriate period. According to the results, Sodium hypochlorite as an antifungal agent was more efficient in shorter time length and useful for killing all species of fungi. For 5 minutes' exposure times, Chloroxylenole and Choloridric acid were suitable for cleaning the indoor swimming pools. Strict adherence to current disinfection and sterilization guidelines is essential to prevent infections and exposures to infectious agents.

List Of Abbreviations

Epidermophyton (E), *Trichophyton (T)*, *Candida (C).*

Declarations

Ethics approval:

The manuscript was approved by the "Local Research Ethics Committee" of Prof. Alborzi Clinical Microbiology Research Center with ID EC93-21.

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Conflict of interest:

The authors declare that they have no conflict of interest.

Authors' contributions:

P.B: Designed study, Analyzed Data, Wrote the paper, S.R: Data collection, F.Z: Data collection, F.G: Performed research, Analyzed Data. P.B, S.R, F.Z, F.G: Critical revision of the manuscript for important intellectual content and approved the final manuscript.

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Figures

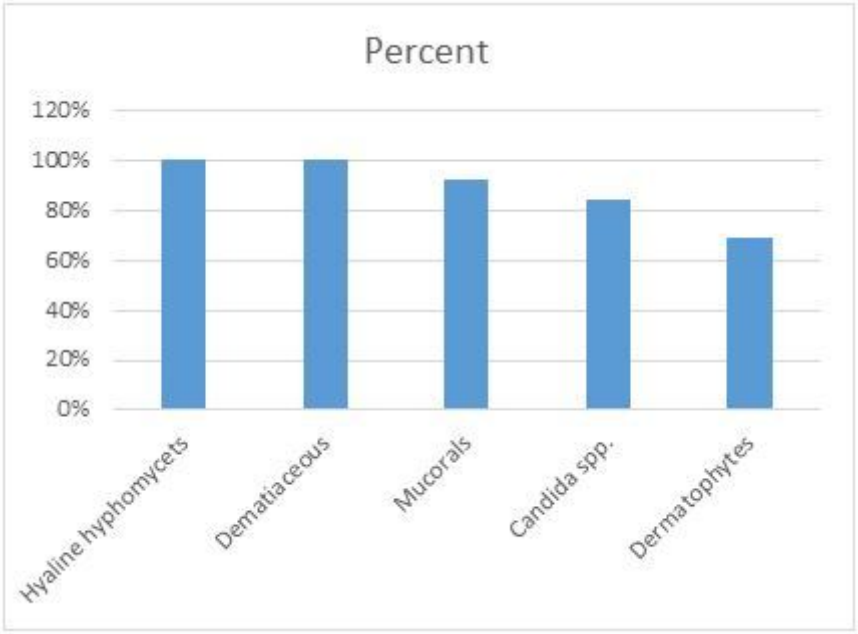

Figure 1

The percent of isolated fungi from 13 swimming pools Hyaline hyphomycetes: Aspergillus spp. (flavus, niger, fumigatus, terreus, glaucus), Penicillium spp. (marneffei and unknown), Chaetomium spp., Verticillium spp., Trichothecium spp., Fusarium spp., Scopulariopsis spp., Paecilomyces spp., Monilia spp. and Gliocladium spp., Dematiaceous fungi: Alternaria spp., Stemphylium spp., Cladosporium spp., Phoma spp., Aureobasidium spp., Epicoccum spp., Drechslera spp., Chaetomium spp. And unknown spp., Mucorales: Mucor spp. and Rhizopus spp., Candida spp.: (albicans, tropicalis, glabrata and krusei), and

Rodotorolla spp., Dermatophyte: Trichophyton spp. (mentagrophyte, tonsurans, verrucosum), Microsporum canis and Epidermophyton floccosum.

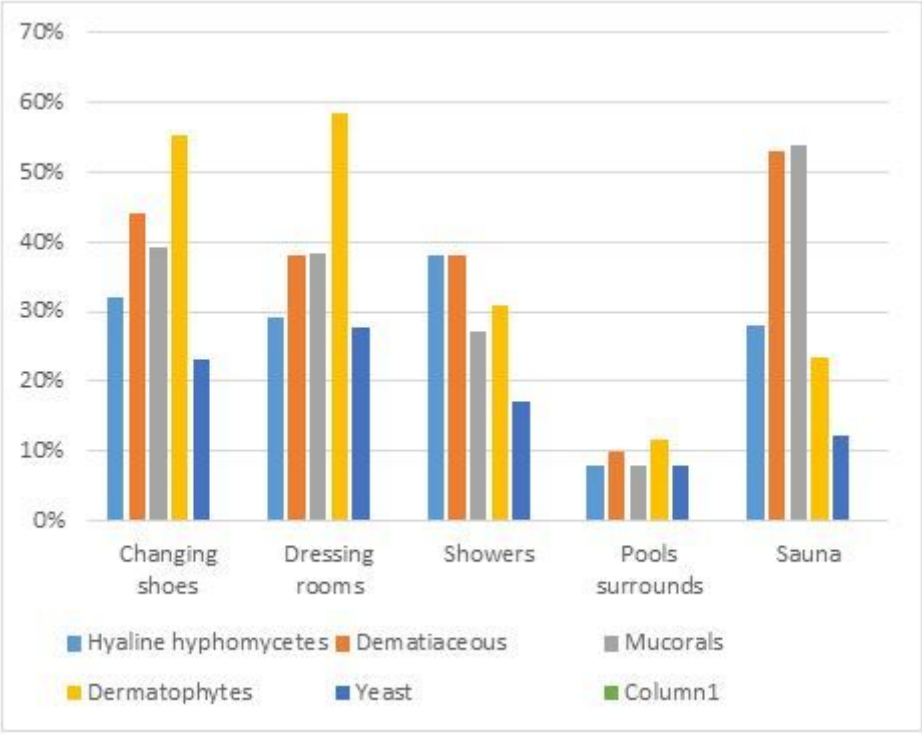

Figure 2

Percent of fungi isolated from different places in 13 indoor pools

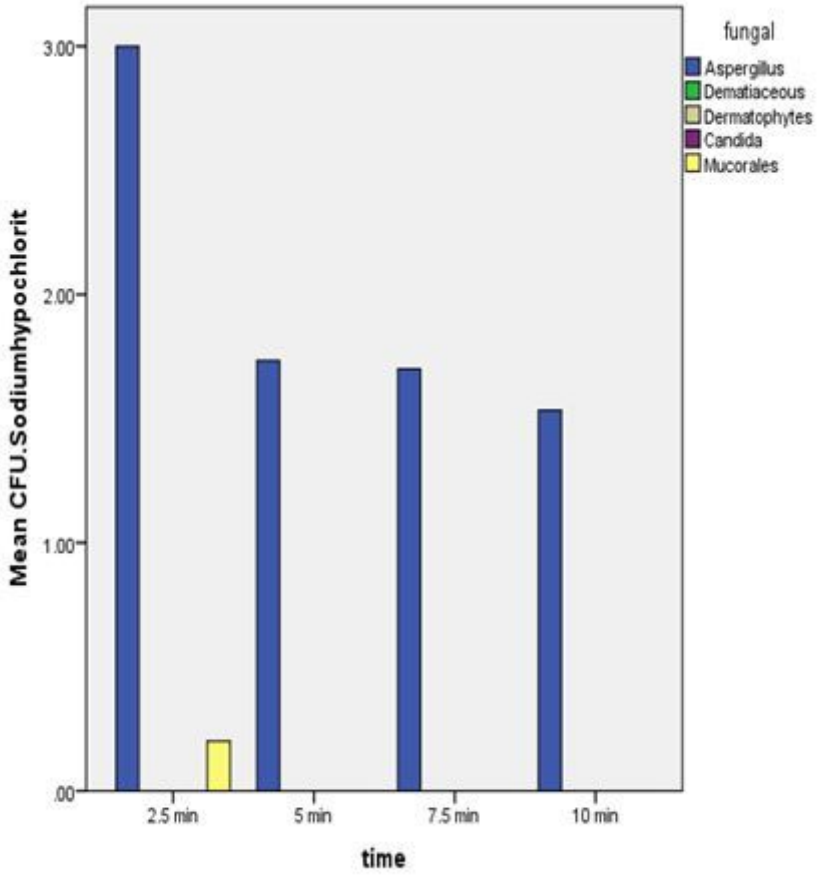

Figure 3

The efficacy of Sodium hypochlorides of isolated fungi from indoor swimming pools.

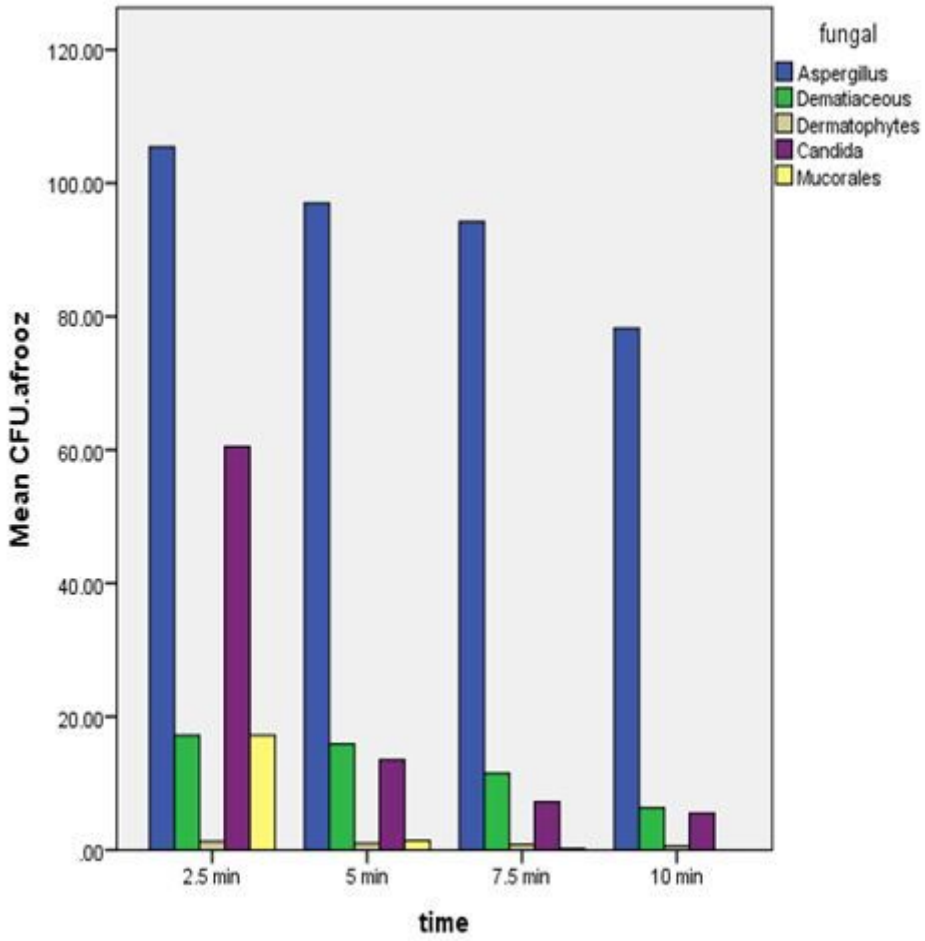

Figure 4

The efficacy of Afrooz of isolated fungi from indoor swimming pools.

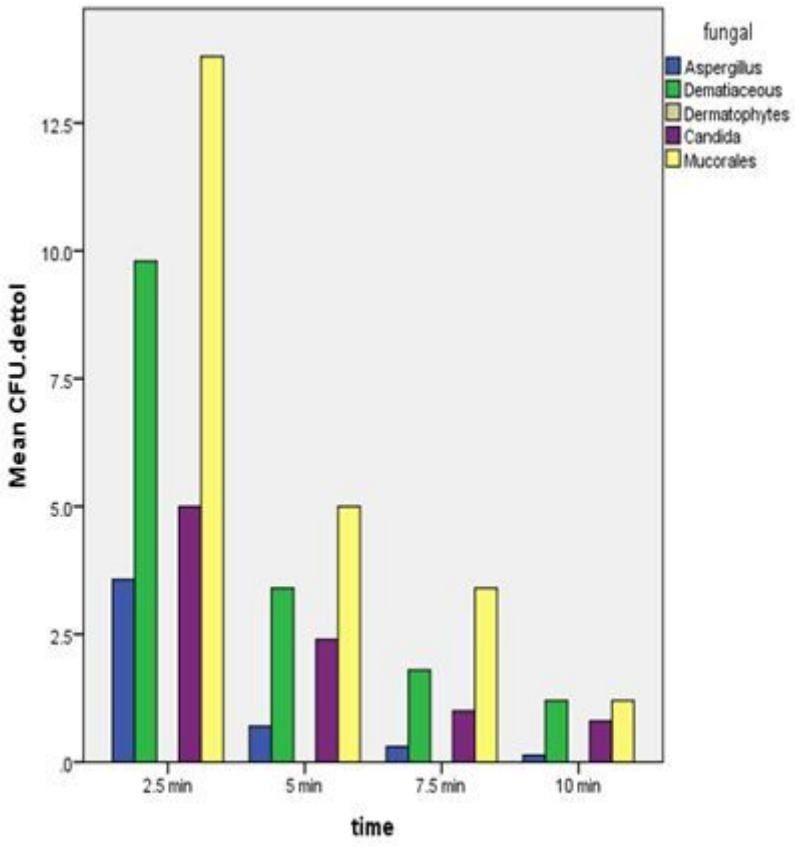

Figure 5

The efficacy of Chloroxylenol (Dettol)of isolated fungi from indoor swimming pools.

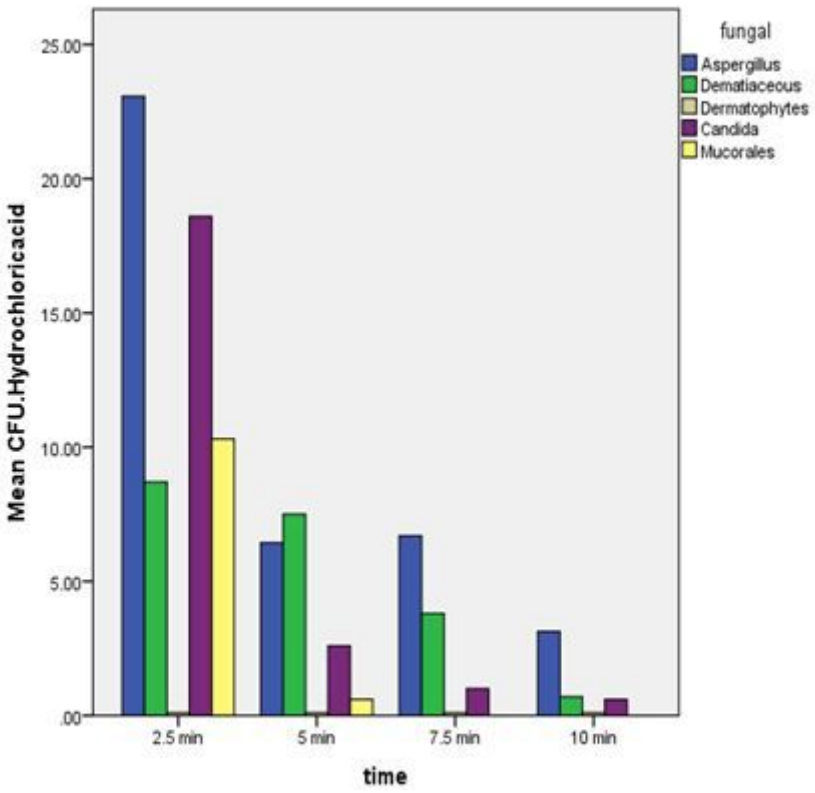

Figure 6

The efficacy of hydrochloric acid of isolated fungi from indoor swimming pools.