ENVIRONMENTAL HYGIENE

Fungal contamination of indoor public swimming pools and their dominant physical and chemical properties

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Keyword

Disinfection systems • Iran • Parasitic and fungal contamination • Physical and chemical characteristics • Swimming pool.

Summary

Introduction. *Considering the existence of both parasitic and fungal pathogens in the indoor public swimming pools and non-utilization of suitable filtration and disinfection systems in these places, this research aimed to determine the relationship between the indoor public swimming pools and possible pollution with parasitic and fungal agents, as well as physical and chemical characteristics of these pools and compare the results with national standards.*

Methods. *In this study, 11 active indoor swimming pools of Zahedan city were sampled, using plastic pump techniques, from the middle of winter to the late summer season. A total of 88 water samples (eight water samples from each pool) were examined to determine the residual chlorine, contamination with parasitic and fungal agents, using culture media and slide culture techniques. Results were analyzed with SPSS software (V16) and, Microsoft Excel (V2010).*

Results. *The findings revealed fungal contamination with Cladosporium, Penicillium, Aspergillus flavus and Aspergillus fumigatus, etc. and the physicochemical factors comply with the minimum standards, which indicates the need for continuous monitoring and control of water filtration and disinfection of water in the pools.*

Conclusion. *The results show reasonable derangement of physicochemical and microbial factors of the evaluated pools. Efforts shall be made by the concerned authorities to provide health education to users, quality water at the pools and to maintain the safety and quality of the water through proper and adequate chlorination.*

Introduction

Swimming pools are among the recreation and most attractive sport fields. Swimming has beneficial effects on human physical and mental health. Maintaining the quality and hygiene of the pools' water helps to protect swimmers from acquisition and dissemination of transmittable diseases, such as eye and skin diseases [1]. Water in recreation centers and public swimming pools are, therefore, a potential source of biological contamination, transmission and outbreaks of diseases if not properly treated [2, 3]. The study of opportunistic fungal pathogens found in these places and the establishment of their sources can effectively help to reduce contamination of swimming pools [4-6]. Pools and other water pollutions can result from non-compliance with health policies, leading to the development of skin, aural, and ocular diseases particular fungal, viral, and parasitic infections among swimmers [7-9]. Water disinfection affects biological factors such as skin and fat deposits, ammonia, urine, sweat, dirt, gum, and participatory food; environmental factors such as acid rain, air pollution, algae, fungi, gas, air moisture content, sunlight, ambient temperature, and evaporation [10]. The quality of pool's water depends on the number of swimmers per unit of time and the effect of disinfectants [11]. The study of potential pathogens and their survival in this environment can be very effective in developing control methods to eliminate contamination. And thus prevent possible infections [12, 13]. Today, swimming pools have been proven to be agents of transmission of infectious diseases in the world [14]. Considering the beneficial effects of swimming on human physical and mental health, disease treatment and personal hygiene, establishing and maintaining standards in swimming pools is important, and microbiological tests to determine the quality of water in swimming pools becomes essential [1]. It is known that the water quality depends on the type of pool (men's, women's and men's, or women's only), and therefore, the water treatment and

disinfection system is different. The optimal parameters for residual chlorine, temperature, and microbial load of the pool water must be under routine monitoring in order to meet minimum standards. Specifically, the undesirable levels of physico-chemical parameters including water temperature, pH, turbidity, T- sulfate, and residual chlorine, and biological parameters such as microbial fungi, parasites, and *Pseudomonas aeruginosa* results from an inability to meet up to the standards, and this leads to various diseases among swimmers. In connection with the evaluation of microbiological and chlorination of swimming pools, numerous studies have been conducted in other parts of the world [15, 16]. However, such studies have not been conducted in Zahedan. This study aimed to determine the types of parasitic and fungal contamination, as well as chemical and physical characteristics of most indoor public swimming pools in the city of Zahedan in 2017, with a view to healthcare, disease prevention, and control.

Materials and Methods

Study area and sample collection

This cross-sectional study was conducted at the Department of Medical Parasitology, Zahedan University of Medical Sciences, Iran. In this study 11, indoor pools (7 public and 4 private) were selected and sampled under practically the same condition. Due to the small number of swimming pools in Zahedan city and also according to similar studies [17], a convenient sampling was done and the sample size was determined to be 88 water samples.

Sampling strategy

A total of 88 water samples (eight water samples from each pool) were examined to determine the residual chlorine, contamination with parasitic and fungal agents, using culture media and slide culture techniques. Sampling was done during the middle of winter to the late summer season period. Generally, utilization of indoor swimming pools is throughout the year, however, utilization is at peak during summer and therefore sampling was done more during the summer in order not to miss the important aspects of parameters. Standard procedure was followed for collecting samples using a manual plastic pump, in a 500 mL sterilized bottle. Eight water samples were collected from each pool. During each sampling, 500 ml of water was collected at a different depth from the floor of the pool to a depth of 1.5 m, and between 2.5 m and 5 m (twice for each depth), in sterile glass bottles. Thus, total of 8 samples were taken from each pool. The samples were transferred to the laboratory within 2 hours in ice [17, 18].

Chemical and Microbiological tools and techniques

The residual chlorine, pH, and temperature were examined on site and then the samples were transported to the laboratory in a cool box and turbidity was measured. A thermometer was used to measure the temperature and turbidity was determined by (HACH Co, 2100 P turbidimeter; USA). The pH was assessed using a DPD kit (N, N-Diethyl-p-Phenylenediamine kit) and phenol red. To measure residual chlorine level in this study, Palin kit manufactured by US DPD was used. According to authorities, 1-3 ppm is considered as the acceptable level of residual chlorine in swimming pools. The standards of the physicochemical parameters of water in Iran are; turbidity less than 0.5 nephelometric turbidity units (N.T.U) and residual chlorine 1-3 ppm, pH 7.2-8, temperature 27-29°C [13, 19, 20]. For the purposes of diagnosing parasite cysts or eggs, and fungi spores, 150 mL of the samples were filtrated through Millipore filters with a pore size of 0.45 micrometers. Then, the filter paper was washed with 2 mL of sterile physiological serum and the residual water was centrifuged at 3000 rpm for 8 minutes. Immediately after that, the sediment was examined to see whether there were any trophozoites, protozoa cysts, or worm eggs [21]. Another 100 mL of the swimming pool water was passed through this filter. This filter was inoculated upside down over the Bactoagar medium enrichment with *E-coli*. Following the closing of the plates with Parafilm, they were kept in an incubator at 30°C for one week [22]. Then the plates were examined under the microscope to check for the presence of free-living ameba colonies and the results were recorded in a checklist. Fungal identification were based on macroscopic, microscopic characteristic and complementary tests when necessary. To detect fungal contamination, the sterile swabs were placed in the residual filtrated water; surface and deep samples, were then cultured in Sabouraud dextrose agar (S) and Sabouraud dextrose agar + chloramphenicol + cycloheximide (SCC). The plates were incubated at room temperature for two weeks and then the level of fungal growth was determined by routine laboratory methods. Dermatophyte confirmation was done using complementary tests such as hair penetration, corn meal agar medium containing 2% dextrose (CMA), and urea medium where necessary. In cases where there were signs of colonial fungal growth it was designated positive, and then it was examined by the teased mount method or slide culture [23].

Statistical analysis

Finally, one-way ANOVA test to examine the relationship between parasitic and fungal contamination in indoor public swimming pools along their physical and chemical properties were performed using the software SPSS 16. P-value of less than 0.05 was considered to be statistically significant. This study was approved by the Ethics committee at the Zahedan University of Medical Science [24].

Results

The results of one-way ANOVA showed no significant relationship between the components of fungal

Tab. I. One-way ANOVA test result of the relationship between the fungal contaminants and physicochemical parameters of the pools water.

Variable	Sig. p- value
Depth	1.000
	Not significant
T- sulfate	1.000
	Not significant
pH	0.000
	Significant
In terms of turbidity ntu	0.000
	Significant
Residual chlorine in ppm	0.000
	Significant
Temperatures in ° C	0.000
	Significant

contamination and physicochemical factors of swimming pools, such as depth and t-solfate and showed a significant relationship with pH, turbidity, residual chlorine, temperature, and communication (Tab. I).

Mean physicochemical properties of the pool's water studied were shown in Table II.

The results showed a total of 34 water samples were contaminated with fungal elements. The prevalence of fungal contamination was 38.7 %. Out of 11 swimming pools, 7 were old and crowded. The prevalence of fungal contamination in old and newer pools were 76.5 % and 23.5 %, respectively. The findings of this study indicated that the water of the pools had a relatively little amount of fungal contamination. In terms of the residual chlorine content, which is about 3 ppm, and within the standard range. Parasitic elements such as eggs of worms, trophozoites or intestinal cysts of pathogenic and non-pathogenic parasites were not seen in this study. Table III, showed the average residual chlorine component in pools' water that did not have fungal contamination was about 2.6852.

The level of fungal contamination in surface and deep water of the pools were, 48% and 29%, respectively. In this study, different types of saprophytic opportunistic fungi were isolated from 88 samples of surface and deep pools.

The most common isolated fungi belong to *Aspergillus* genus (35.3%) especially, *Aspergillus flavus, Aspergillus fumigatus,* and *Aspergillus Niger*. These fungi were most often isolated during the summer in the pool water. Only one case of *Trichophyton* spp was seen in the studied water sample (Tab. IV), (Fig. 1).

Discussion

Several studies were conducted to investigate the physical, chemical and microbiological elements of the pools' water in the world. The results indicate the presence of various chemical and infectious agents in these pools, which may under certain circumstances pose a serious health threat to those coming in contact with the pools' water. Since the indoor pool attracts people all year round, the safety and hygiene of the water are of particular importance [5, 21, 25-31]. We used Palin UDP kit (USA) to measure residual chlorine level in this study. According to authorities, 1-3 ppm is considered as the acceptable level of residual chlorine in swimming pools [32, 33]. In our results (Figure 2) approximately 12 samples studied, the residual chlorine level in the

Tab. II. Mean physicochemical properties of the pool's water studied.

Variable Swimming pool	Mean pH	The average in terms of turbidity ntu	Mean residual chlorine in ppm	Average temperatures in ° C
A	7.6500	0.2275	0.0500	29.5000
B	7.7700	0.2400	1.5000	28.5000
C	7.8500	0.1725	0.0375	30.0000
D	7.8000	0.3650	1.7000	29.0000
E	8.2000	0.4050	2.5000	30.5250
F	8.2000	0.3325	3.0000	29.0000
G	8.2000	0.4300	3.0000	26.5000
H	7.6000	0.3150	2.2500	29.5000
J	7.8000	0.3250	3.0000	28.0000
K	7.8000	0.3625	0.0000	30.5000
L	8.2000	0.4125	5.0000	29.5000
Sum	7.9155	0.3261	2.0034	29.1386

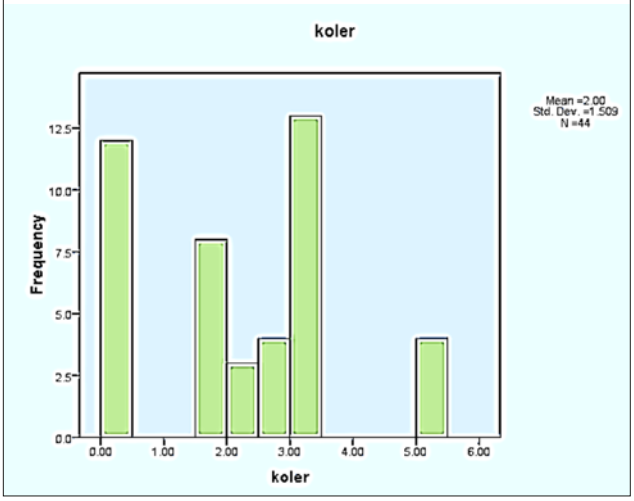
Tab. III. Comparison of mean and standard deviation for evaluation of fungal contamination and residual chlorine component, water-based milligrams per liter.

Variable	Fungal contamination	Number	Mean	Standard deviation	Deviation, Standard Error
Chlorine, water	had not	54	2.6852	1.42874	0.27496
	has it	34	0.9206	0.88106	0.21369

Tab. IV. Frequency of fungal contamination in eleven pool.

	Type of Fungi			
Valid	Frequency	Percent	Valid Percent	Cumulative Percent
	54	61.3	61.3	61.3
Alternaria spp	2	2.28	2.28	63.58
Aspergillus Flavus	7	7.96	7.96	71.54
Aspergillus Fumigatus	4	4.55	4.55	76.09
Aspergillus Niger	1	1.14	1.14	77.23
Cladosporium spp	4	4.55	4.55	81.78
Geotrichum	2	2.28	2.28	84.06
Mucor spp	1	1.14	1.14	85.2
Rhizopus spp	3	3.41	3.41	88.61
Phialophora spp	3	3.41	3.41	92.02
Trichophyton spp	1	1.14	1.14	93.16
Sterile mycelium	3	3.41	3.41	96.57
Penicillium spp	1	1.14	1.14	97.71
Unknown	2	2.28	2.28	100.0
Total	88	100.0	100.0	

Fig. 1. Frequency of residual chlorine levels in milligrams per liter in eleven indoor pool.



studied pools' water was within the acceptable standard range. Only 3 of the studied pools had a residual chlorine level (about 5 ppm) above the acceptable standard range. The level of physical, chemical, and microbial quality of chlorinated pools can be influenced by several factors. In the first place, the level of knowledge, experience, and understanding of the importance of water quality and the efforts to improve and maintain the quality of the water (including proper chlorination) by the administrative staff and the overseers of the swimming pool facilities are very essential. Secondly, the stringency of quality control and environmental health officials, including several other stakeholders can be a crucial factor that influences the microbial quality of the pool water. In addition, personal hygiene (e.g. shower before swimming) and health status of the swimmers can greatly affect the water quality of the swimming pools [3, 7, 34]. Human skin infections have dramatically increased in recent years and one of the reasons is the rise in recreational facilities and frequent contact among people polluted indoor water environments [35, 36]. Fungal skin infection is found worldwide, irrespective of age or sex. According to the results of the study, the frequency of fungal contamination of indoor swimming pools in Zahedan was found to be higher in the locker room showers than in other pools. Higher frequency of contamination was seen more in highly crowded pools and also in old pools that lack modern construction design. While the newer pool with modern construction that comply with minimal international standards showed the least pollution. Therefore, the less number of people who use pool facilities and the better design of pool building, the lower the level of pools' contamination. Maghazy et al. have studied two swimming pools in Assiut, Egypt and demonstrated contamination of the pools with various types of fungi including three dermatophyte species [37]. Another study conducted by Kraus et al, succeeded in getting a number of pathogens including *T. verrucosum* from pool's water with a free chlorine content of less than 0.35 mg/l (DPD 1) [38].

Conclusion

Based on our findings we can recommend authorities concerned with the pool management to be giving special health education to people attending and using facilities especially those identified with skin infection, tinea infections or vaginal infections. Water disinfectants shall be added and replaced regularly. After each use of the pool, dressing room environment shall be cleaned with disinfectants. If the aforementioned recommendation shall be observed fungal and microbial contamination of the water and environment around it will be a significantly decreased [39]. It is hoped that a way to solve such problems in order to improve the situation

arise. It is also suggested that future studies shall engage the proper and effective methods for the treatment and disinfection of swimming pool water [20].

Acknowledgement

We acknowledge the financial support of Zahedan University of Medical Sciences to this study.
Funding: the Research Council of Zahedan University of Medical Science supported the work; the project funding number is 1395. 75.

Conflict of interest

Authors and coauthors declare that they have no conflict of interest that affects this study.

Author's contribution

Study concept and design: NR, MJM, SD. Collecting samples and preparing for experiment: ED, NR and FP. Analysis and interpretation of data: SD, MJM, MY, AS, VR. Drafting of the manuscript: OR, MG, VR, BA. Critical revision of the manuscript for important intellectual content: OR, MG, S SH, SA. Statistical analysis: MJM, SD, OR.

Ethical statement

The study was approved by the joint Ethical Committees of Zahedan University of Medical Sciences ethic no. ir.zaums.rec.1395. 75.

Availability of data and materials

In this study, all data and materials are included. If more information is needed, please contact the author for data requests.

Consent for publication

Not applicable because this manuscript does not contain any individual personal data.

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Received on May 19, 2020. Accepted on September 23, 2021.

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How to cite this article: Rezaei N, Dabiri S, Getso MI, Damani E, Modrek MJ, Parandin F, Raissi V, Yarahmadi M, Shamsaei S, Soleimani A, Amiri S, Arghavan B, Raiesi O. Fungal contamination of indoor public swimming pools and their dominant physical and chemical properties. J Prev Med Hyg 2021;62:E879-E884. https://doi.org/10.15167/2421-4248/jpmh2021.62.4.1594