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Investigation of the Physical, Chemical Characteristics and Microbial Contamination of the Indoor Swimming Pools

Kapalı Yüzme Havuzlarının Fiziksel, Kimyasal Özelliklerinin ve Mikrobiyal Kontaminasyonlarının Araştırılması

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ABSTRACT

Objective: The aim of this study was to investigate the physical, chemical and microbiological contamination of indoor swimming pools.

Methods: Pool water specimens were collected using a plastic polypropylene sterilized bottle. The physical and chemical qualities of the waters were analyzed in terms of temperature, turbidity, pH, and free residual chlorine, with the standard methods for the examination of water. Bacteriological (routine methods) and parasitological (molecular methods) tests were carried out on pools water.

Results: The mean temperature, pH, and residual chlorine of the indoor pools were 31.2 °C, 7.6 and 1.5 mg/L, respectively. Turbidity was not observed in any of the pools. The pH and temperature values were in standard ranges in 92.3% and 15.4% of the waters of swimming pools, respectively. The prevalence rates of bacterial and amoebic contaminations of the water in the swimming pools were 53.8% and 46.2%, respectively. One pool (7.7%) was contaminated with both bacteria and amoeba. *Streptococcus viridans*, *Staphylococcus epidermidis*, *Pseudomonas stutzeri*, *Cryptosporidium* and *Bacillus* spp. were isolated from the pool waters.

Conclusion: In this study, some microorganisms were identified from the water pools. Effective management of swimming pools and proper control of the physical, chemical and microbiological property of water pools can produce the healthy recreational activity.

Keywords: Swimming pools, *pseudomona*, *cryptosporidium*, chlorine

ÖZ

Amaç: Bu çalışmanın amacı kapalı yüzme havuzlarının fiziksel, kimyasal ve mikrobiyolojik kirlenmelerini araştırmaktır.

Yöntemler: Havuz suyu örnekleri plastik polipropilen sterilize edilmiş bir şişe kullanılarak toplandı. Suların fiziksel ve kimyasal özellikleri, suyun incelenmesinde kullanılan standart yöntemler ile sıcaklık, bulanıklık, pH ve serbest artık klor açısından analiz edildi. Havuz sularında bakteriyolojik (rutin yöntemler) ve parazitolojik (moleküler yöntemler) çalışmalar yapılmıştır.

Bulgular: Kapalı havuzların sıcaklık, pH ve artık klor ortalamaları sırasıyla 31,2 °C, 7,6 ve 1,5 mg/L idi. Havuzların hiçbirinde bulanıklık gözlenmedi. PH ve sıcaklık değerleri yüzme havuzlarının sırasıyla %92,3 ve %15,4'ünde istenilen aralıktaydı. Yüzme havuzlarında suyun bakteriyel ve amipli kirlenme sıklığı sırasıyla %53,8 ve %46,2'dir. Bir havuz (%7,7) hem bakteri hem de amip ile kirlenmiştir. *Streptococcus viridans*, *Staphylococcus epidermidis*, *Pseudomonas stutzeri*, *Cryptosporidium* spp. ve *Bacillus* spp. havuz sularından izole edilmiştir.

Sonuç: Bu çalışmada, su havuzlarından bazı mikroorganizmalar tanımlanmıştır. Yüzme havuzlarının etkin yönetimi ve su havuzlarının fiziksel, kimyasal ve mikrobiyolojik özelliklerinin uygun şekilde kontrolü sağlıklı rekreasyon aktivitesini sağlayabilir.

Anahtar Kelimeler: Yüzme havuzları, *pseudomonas*, *cryptosporidium*, klor

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

People attend swimming facilities for practice on water-related sports and recreational activities, rehabilitative treatment, and relaxant sports. Microbial contamination and variation on physical quality were reported in the literature (1-3). Various diseases may be transmitted to the swimmers by exposure to physical, chemical and microbiological agents of the pools. Such diseases may be related to the hygiene in pool environment and water, temperature, pH, and residual chlorine levels.

Microbial contamination of the pool water is resulted by fecal contamination of the pool environment and water by bathers and swimmers, the release of an accidental diarrheic stool or direct animal contamination like rodents. In recent years, systemic microbial infections including parasitic or bacterial diarrhea, typhoid, hepatitis and cholera caused by swallowing the contaminated water have been reported especially in immunocompromised patients (4-6). Contamination of swimming pools with microbiological parameters including *Pseudomonas aeruginosa, Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Escherichia coli* have also been reported in the literature (1,2). Other pathogenic microorganisms such as protozoa may also be presented in recreational waters, which include *Naegleria fowleri, Giardia lamblia*, *Trichomonas vaginalis, Plasmodium, Acanthamoeba*, and *Cryptosporidium* spp. (7,8). In the patients with weakened or depressed immune systems and those taking certain immunosuppressant, swimming in the public pools can cause severe diseases. To provide healthy swimming pools and prevent swimmers from communicable infectious diseases, making a safe swimming environment free from microorganisms is necessary. The aim of this study was to investigate the physical and chemical parameters, and parasitic and bacterial contamination in indoor swimming pools waters. The findings were compared with local and international guidelines.

METHODS

Sampling

This cross-sectional study was conducted in 13 public recreation indoor swimming pools during spring and summer of 2016. Pool water specimens were collected in the morning before starting the swimming and after decontamination of the pools. Water samples (8 liters) were collected using a manual plastic polypropylene sterilized bottle, at approximately 1 meter below the surface of the pool water and transferred to the microbiological laboratory. The physical and chemical qualities of the waters were analyzed in terms of temperature, turbidity, pH and free residual chlorine, according to the standard methods for the examination of water (9). The samples were transferred to the laboratory in a cool box and microbiological analysis was performed within 2 hours. The information about the pool sanitation and usage of disinfectants was collected from the chief of the pools. The temperature was measured using a digital thermometer, and chlorine residual and pH were evaluated onsite by Chlorine-pH kit (Pool Tester, Poison Centers Berlin, Germany). The turbidity of the waters was evaluated by spectrophotometer (Biowave WPA, UK).

The water samples were filtrated by filter paper with 47 mm diameter (0.2 µm-pore-size) and vacuum motor (Sartorius Stedim, Biotech GmbH, Germany), immediately following the collection (Sartorius Stedim Biotech GmbH, Germany). The filter papers were divided into two parts; one for bacteriological and the other for parasitological studies (DNA extraction).

Bacteriological Study

In the first step, filter papers were cultured in brain heart infusion broth and incubated at 35±2 °C for 24-48 hours. After an incubation period, 100 µL of the medium was cultured on each of following microbiological media including blood agar, MacConkey agar, and eosin methylene blue agar. The plates were incubated at 35±2 °C and evaluated for bacterial growth every day until one week. For final identification of isolates, the supplementary morphological and chemical tests were used. All of the media were purchased from Merck Company (Darmstadt, Germany).

Parasitological Study

This part of the study was carried out using molecular methods. Filter papers were washed with 2 mL of phosphate-buffered saline by mild shaking. The residual fluid was centrifuged at 3000 rpm for 5 minutes. DNA was extracted from sediment cells using the Commercial kit (Invisorb Spin DNA Mini Kit- Stratec, Germany), according to the manufacturer's instructions. Polymerase chain reacton (PCR) tests for *Acanthamoeba* spp. (10), *T. vaginalis* (11), *Entamoeba histolytica* (12), *G. lamblia* (13), and *Cryptosporidium* spp. (14) were performed on pool water samples. In Table 1, the primers and related references were presented, and the PCR amplifications were performed, accordingly.

Table 1. The primers used in this study for identification of Amoeba in 13 swimming pools

***Giardia lamblia* (13)**	
AS1	CGACCGGGAGACACGCCC
AS2	AGGACTGCATATCACGGC
SG3	AGAGCAGCCGATCCCCCG
SG4	AATTGGAGGCTGACTGTG
***Entamoeba histolytica* (12)**	
E1	TAAGATGCACGAGAGCGAAA
E2	GTACAAAGGGCAGGGACGTA
EH1	AAGCATTGTTTCTAGATCTGAG
EH2	AAGAGGTCTAACCGAAATTAG
***Acanthamoeba* spp. (10)**	
JDP1	GGCCCAGATCGTTTACCGTGAA
JDP2	TCTCACAAGCTGCTAGGGGAGTCA
A1	AACGATGCCGACCAGCGATTA
***Trichomonas vaginalis* (11)**	
OP1	GTGAAAATCTCATTGGGGTATTAACTT
OP2	GTTTTATTTATCACTGGAAAATAACGCTT
IP1	AACATCCCCAACATCTT
IP2	CCATTCTTTTAGACCCTT
***Cryptosporidium* spp. (14)**	
SSU- F2	TTCTAGAGCTAATACATGCG
SSU- R2	CCCATTTCCTTCGAAACAGGA
SSU- F3	GGAAGGGTTGTATTTATTAGATAAAG
SSU- R3	AAGGAGTAAGGAACAACCTCCA

Table 2. The physicochemical and microbiologic property of 13 swimming pools

Swimming pools number	Temperature °C	pH	Residual chlorine mg/L	*Bacteria species*	*Parasite species*
1	33.0	7.8	1.0	*Streptococcus viridans*, *Staphylococcus epidermidis*	-
2	31.0	7.8	2.0	*Staphylococcus epidermidis*	-
3	33.0	7.6	2.0	*Bacillus* spp.	-
4	33.0	7.2	2.0	*Bacillus* spp.	-
5	34.0	7.6	0.1	*Pseudomonas stutzeri*	-
6	32.0	7.6	0.1	*Pseudomonas stutzeri* *Bacillus* spp.	*Cryptosporidium* spp.
7	30.0	7.8	3.0	*Staphylococcus epidermidis*	-
8	32.0	7.6	1.5	-	*Cryptosporidium* spp.
9	30.0	8.2	3.0	-	*Cryptosporidium* spp.
10	30.0	7.6	1.0	-	*Cryptosporidium* spp.
11	29.0	7.6	1.0	-	-
12	29.0	7.8	1.0	-	*Cryptosporidium* spp.
13	30.0	7.6	3.0	-	*Cryptosporidium* spp.

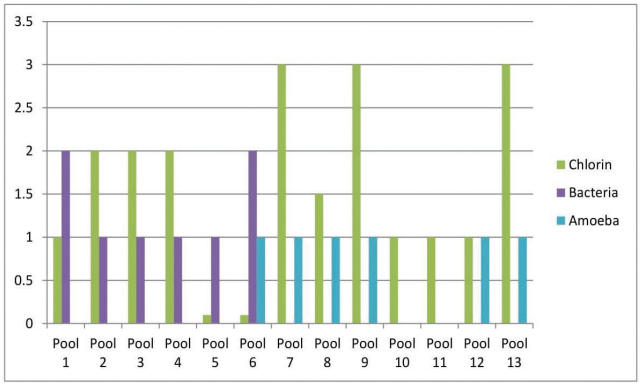

Figure 1. The relationship between residual chlorine and microbiology identification in 13 swimming pools

Statistical analysis

This study was descriptive and statistical analysis was not applicable**.**

RESULTS

In this study, all of the pools were disinfected manually. The mean of temperature, pH, and residual chlorine of the 13 indoor pools were 31.2 °C (range=29-34 °C), 7.6 (range=7.2-7.6) and 1.5 (range=0.1-3) mg/L, respectively. Turbidity was not observed in any of the pools. Frequencies of the physicochemical parameters of the swimming pools are presented in Table 2. The pH values showed low fluctuations in all the investigated swimming pools, only one pool had a pH value equal to 8.2. The temperature was standard in 15.4% of the swimming pools. The prevalence rates of bacterial and amoebic contaminations of the water in the swimming pools were 7/13 (53.8%) and 6/13 (46.2%), respectively, and one pool (7.7%) was contaminated with *Pseudomonas stutrezi, Bacillus* spp., and *Cryptosporidium* spp. The contaminated isolates were *Streptococcus viridans, Staphylococcus epidermidis, P. stutzeri, Bacillus* and *Cryptosporidium* spp. *T. vaginalis*, *G. lamblia*, *E. histolytica*, and *Acanthamoeba* spp. were not detected in any of the water samples analyzed by PCR methods. According to Figure 1, in pools with the low concentration of free chlorine, a high count of microorganisms was isolated.

DISCUSSION

Swimming pools may be contaminated with microorganisms associated with swimmers like fecal contamination of the water, accidental fecal release or residual fecal material on bodies, and non-fecal shedding like vomit, mucus, saliva, skin, mouth, and upper respiratory tract contamination. Some may cause a variety of respiratory, dermal or central nervous system infections or diseases (4,5,15). Some studies have been investigated the quality of pool waters during the working days (4,5). Also, some researcher believed the highest number of swimmers during holidays and weekend are young children (16), therefore the water sampling would be better evaluated in these times. In order to monitor the environmental effect of physicochemical and microbial program, we examined the water pool conditions when the pools were disinfected and suitable for swimmers. In this time, the disinfectants cannot appropriately clean the water pools and microbial contamination was presented.

The mean pH value of the water pools in this study was 7.6 (7.2-8.2), the ranges recommended by the Iranian Ministry of Health and Egyptian standard range are 7.2-7.8 and 7.2-8 (17,18). The pH value of the pools water in Shahrekord city was reported 8.08±0.29 (2). The pH level and the residual chlorine measuring are important factors in the management of the pools because by increasing the pH, only a low percentage of residual chlorine changes into hypochlorous (4). Only one pool in this study had pH value equal to 8.2 and more than the standard range.

The standard turbidity of water in World Health Organization (WHO) is less than 0.5 Nephelometric Turbidity Units (NTU) (4), no turbidity of water pool was seen in this study. Water analysis in Lutz and Lee indicated low turbidity about 0.1-0.6 NTU (19). According to WHO standard breakpoints, the suitable water

temperatures for swimmers range from 26 to 30 °C, respectively (4). In this study, the mean temperature of water pools was 31.2 °C (29-34 °C). Swimming pools temperatures ranged from 25.9 -32.4 °C and hot tub temperature 38.7-39.3 °C were reported (18). As revealed, the temperature was not in standard range in 11/13 (84.6 %) swimming pools in the present study, and this condition is not comfortable for swimmers.

For the prevention of the contamination, water pools are treated with disinfectants such as chlorine compounds and/or ozone. Research studies showed that such disinfectants cannot prevent the pool water from the microorganisms (4,5). The mean residual chlorine in this study was 1.5 (0.1-3) mg/L, but in two pools the residual chlorine level was under the standard level. In El-Salam (20) 43.3% of the water samples were unacceptable for residual chlorine. The low concentration of residual chlorine was insufficient to eliminate all bacteria or free-living ameba within the swimming pools. Chlorine is considered the best disinfectant for swimming pool water. In the present study, most of the pools used chlorine gas and two pools used ozone combined with chlorine, according to Graph 1, in pools with the low concentration of free chlorine, a high count of microorganisms was isolated.

Diagnosis of microbial organisms with PCR assay was reported in the literature (21-23) and in the present study; we used this method to identify amoebic contaminations of the pools. In the current study, *T. vaginalis*, *G. lamblia*, *E. histolytica,* and *Acanthamoeba* spp. were not detected in any of the water samples, but *Cryptosporidium* spp. was detected in 6 out of the 13 pools. We did not find any study which examined swimming pools by PCR methods. According to standard protocols, swimming pools must be free from any protozoa. In the study by El-Salam (20), 3.3% (1/30) of the examined water samples had *Cryptosporidium* oocysts, and 6.6% (2/30 examined pools) had *G. lamblia* cysts. In Italian investigation of 10 chlorinated swimming pools, free chlorine levels were approximately 1 mg/L, and *Cryptosporidium* and *Giardia* were found in 3% of the pool water samples (24). *Acanthamoeba* can be found in all aquatic environments, including disinfected swimming pools. *Acanthamoeba* cysts are highly resistant to extremes of temperature, disinfection, and desiccation. The cysts will retain viability from -20 °C and 56 °C. In the untreated state, *Acanthamoeba* keratitis can lead to permanent blindness (7). *Giardia* cysts and *Cryptosporidium* oocysts can cause infection in humans and result in diarrhea with accompanying abdominal cramps and vomiting. *Giardia* cyst is very resistant to many disinfectants, including chlorine. *Cryptosporidium* is much more resistant to chlorine than *Giardia* cysts. *Cryptosporidium* requires chlorine concentrations of 30 mg/L for 240 min (at pH 7 and a temperature of 25 °C) for achieving a 99% reduction (4). Isolation of *Cryptosporidium* in this study can be due to resistant of its oocysts.

In the present study, the prevalence of bacterial contaminations in the water of the swimming pools was 7/13 (53.8%), and *S. viridans, S. epidermidis*, *P. stutzeri, and Bacillus* spp. were isolated from the pool waters. The isolated bacteria from the swimming pools in the literature include *Pseudomonas aeruginosa, Staphylococcus aureus*, *Legionella*, and *E. coli* (2), and *P. aeruginosa and Streptococcus* spp. (20). The isolation of bacteria was reported with respect to water temperature and free chlorine (1). The water pools may be contaminated with *Pseudomonas* by swimmers or natural contamination. *Pseudomonas* is resistant to sodium hypochlorite that used for disinfecting pools, and it is considered to be an opportunistic microorganism involved in the urinary tract, wound, sepsis and bed sores infections (17,25,26). That is able to grow in moist, warm conditions with low levels of organic nutrients. *P. aeruginosa* has intrinsic antimicrobial resistance due to low outer membrane permeability, an extensive efflux pump system and ability to spore production (27). *Staphylococcus* is a common skin organism which may be recovered from recreational waters. It may be considered as a risk indicator for skin, eye and ear diseases (2,28,29). Coagulase-positive *Staphylococcus* strains have been found in chlorinated swimming pools. In Italy, in a study on chlorinated pools, where the free chlorine level varied between 0.8 and 1.2 mg/L, *S. aureus* was not recovered from water samples (24). Fecal *Staphylococcus* was not detected in any of the examined samples in El-Salam (20). In the present study, 1/13 of the studied pools were contaminated with *P. stutzeri, Bacillus* and *Cryptosporidium* spp. The level of the chlorine in this pool was very low.

CONCLUSION

In this study, before opening and after cleaning the pools, the physical and chemical properties of some indoor swimming pools were not optimal and some microorganisms were identified from the water pools. Effective management of swimming pools and proper control of disinfectant levels, water temperature, turbidity, pH and microbiological property can produce the healthy water for people recreational activity.

* Ethics

Ethics Committee Approval: Parisa Badiee, was approved by the "Local Research Ethics committee" of Prof. Alborzi Clinical Microbiology Research Center with ID EC94-2.

Informed Consent: This study was not involving human or animal participants, and we only evaluate the environmental samples.

Peer-review: Externally and internally peer-reviewed.

* Authorship Contributions

Concept: P.B., F.G., Design: G.R.H., J.M., H.R.K., Data Collection or Processing: G.R.H., H.R.K., J.M., P.A., Analysis or Interpretation: P.B., G.R.H., J.M., H.R.K., Literature Search: P.A., F.Z., F.G., Writing: P.B., F.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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