

# **BACTERIOLOGICAL ASSESSMENT OF SWIMMING POOLS IN RESIDENTIAL AND COMMERCIAL SECTORS IN MUMBAI**

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## **ABSTRACT**

*This study documents the experimental findings of the bacteriological assessment of ten randomly selected swimming pools located in Mumbai and surrounding areas. This was conducted with an objective of analyzing the hygienic status of the pools. Twenty samples (n=20) from residential and commercial sector pools, categorized as chlorinated & dechlorinated were analyzed for their Total Heterotrophic Count (THC), Coliform counts and presence of pathogens viz., Pseudomonas aeruginosa. Amongst residential sector pools, THC for dechlorinated sample ranged from  $2.22 - 4.1 \times 10^5$  cfu/ml whereas amongst commercial sector pools, THC for dechlorinated sample ranged from  $0.16 - 8.01 \times 10^5$  cfu/ml, which were the highest THC values obtained and were far beyond the permissible limits of WHO standards. Amongst the various isolates obtained, Pseudomonas aeruginosa showed a greater frequency of occurrence (80%). This study thus brings to light the poor hygienic bacteriological status and impedes an urgent demand for monitoring pool management of public swimming pools to ensure its safety for public health in an urban city like Mumbai.*

**Keywords:** coliform count, commercial sector pools, residential sector pools, total heterotrophic count (THC).

## **Introduction**

Swimming is generally considered to be a healthy leisure activity for both the young and the old. Swimming pools are among the most popular centers that are increasingly being used by man for swimming and other recreational based activities such as canoe polo, underwater rugby, volleyball, and sports diving. They are also used for certain cultural/religious practices such as baptism, where the individual's head is submerged for a while as prayers are being conducted. Due to their increasing usage, swimming pools are now available at many recreation centers, hotels, schools, beaches, universities, wildlife parks, homes, and many other areas. Swimming is often recommended because of its potentially beneficial effects on general human health particularly bone joints. A large variety of people attend swimming pools for athletic, recreational or medical activities. Several types of opportunistic or pathogenic micro-organisms can be introduced to the water via direct or indirect human contamination, and can become prevalent to a density at which they may cause cutaneous, gastrointestinal or respiratory diseases in the bathers.

With an increase in the numbers of swimming pools, high maintenance is necessary to protect the users from any form of infection. Swimming pools are supplied by the water of environmental origin. The quality of swimming pool water is enhanced by frequently changing the water and the use of disinfectant, such as chlorine to highest possible concentration of about 1 part per million (ppm). Although it is known that swimming pool water should meet the standards set for safe and clean pool waters in most countries, such standards are not normally maintained in many countries (Joyce et al. 2017).

Pool water is exposed to contamination from body fat and waste material such as nasal secretions, saliva, sweat, fecal, urine, and body applicants like sunscreen lotions and creams. Moreover, hair and dust from the surrounding environment can also cause water contamination. The contamination in swimming pools is divided into three categories viz physical, chemical, and microbial contamination. Studies have shown that pool water can act as a medium to spread fungal diseases among the swimmers. Swimmers, who swim at contaminated pools, are prone to fungal infections, especially of the ears and toes etc. as these areas have a relatively high level of moisture. Previous studies have revealed that physico-chemical factors such as pH are very influential. If the water pH is alkaline, chlorine antiseptic performance will decrease. Moreover, microorganisms such as fecal based bacteria *Escherichia coli*, and *Pseudomonas aeruginosa*, fecal *Streptococcus*, are found to contaminate water in swimming pools. Defining hygiene standards to control water-based diseases is one of the most influential factors to ensure the safety of the pool water and the health of swimmers (Shirin et al. 2015).

Thus, investigating the hygienic criteria of the water in swimming pools seems necessary. Considering the new criteria, physical factors including the pH, remaining free chlorine, and temperature are among the most important factors to control the quality and quantity of microbial contamination.(Guida et al. 2009).

The current study aims at determining the total heterotrophic count, coliform count and detection of *Pseudomonas aeruginosa* in the pools located in residential and commercial sectors in Mumbai and surrounding areas

### **Materials and Methods**

The present study was carried out to study the bacterial load of swimming pool samples from 10 randomly selected pools which were categorized as follows: -

1. Residential sector pools (5 samples) and 2. Commercial sector pools (5 samples).

The residential pools were coded as P1, P2, P3, P4 and P5 and commercial pools were coded as P6, P7, P8, P9 and P10. Commercial pools included pools located at Health clubs, Tourist Homes and from Hotels, where swimming pools are becoming a major attraction.

### **Sample collection:**

From 10 residential and commercial swimming pools from Mumbai, 20 samples were collected for analysis at different periods of time over a period of six months.

Water samples were collected at random points from each pool. 250 ml sterile bottle was immersed from the top surface about 1 meter deep and then inverted to collect a water sample. Once the sample was collected, ample air space was left in the bottle (at least 2.5 cm) to facilitate mixing by shaking, before examination. Sample containing bottles were transported to the microbiological laboratory within two hours of collection. All the samples were analyzed on the day of collection. For bacteriological analysis, the samples were processed within 4 hours in the laboratory. Under unavoidable circumstances, where the samples could not be analyzed immediately resulting in a delay in investigation, they were refrigerated at 4°C for a maximum period of 18 hrs.

**Note:** For de-chlorination of water 0.2ml of 10% Sodium thiosulphate was added to the sample collection bottle of 250 ml capacity.

### **Estimation of Total Heterotrophic Count(THC):**

Comparison of the total heterotrophic count of chlorinated and dechlorinated water samples was determined in triplicates by using the Plate Count method in Sterile Glucose Yeast Extract Agar (GYEA). (Erin R. Sanders, 2012). Dilutions of the collected samples (both chlorinated and dechlorinated water samples) were carried out using sterile saline and  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  dilutions of each sample were used for estimating the total number of viable heterotrophic bacteria. From the respective dilution tubes, 0.1 ml of the diluted sample was inoculated by spread plate method on the St. GYEA plates in triplicates. Plates were incubated at 37°C for 24 hours.

### **Enumeration of coliforms:** MPN Test-(Most Probable number)

The Coliform count was carried out by using Sterile MacConkey's broth and the MPN of coliforms was estimated by referring to the Mc Crady's table (APHA, 1998). For enumeration of coliforms by the Most Probable Number method single strength and double strength MacConkey's broth was used. 10 ml, 1 ml and 0.1 ml of samples were inoculated into 10 ml each of Double strength medium (10 ml sample) and Single strength medium (1 ml and 0.1ml sample) in sets of fives and after incubation at 37°C for 24 hours, tested for the presence of fecal contamination. Each tube was checked for acid production (pink color development) and also the presence of gas in the inverted Durham's tube that was placed in each and every tube.

### **Detection of *Pseudomonas aeruginosa*:**

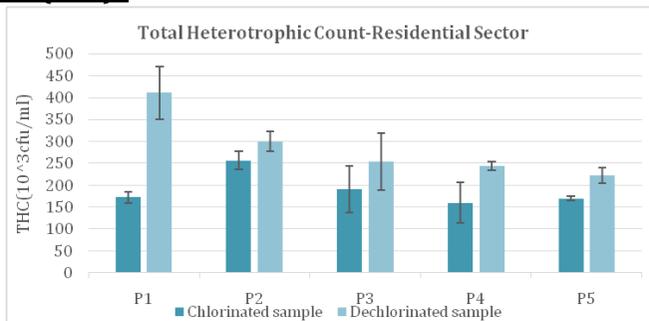
**A. Enrichment of *Pseudomonas aeruginosa*:** For enrichment 1 ml of sample was inoculated in 4 ml of St. *Pseudomonas* Asparagine broth (Himedia) and incubated at 37°C for 48 to 72 hours. Tubes were examined for growth in the form of turbidity and green fluorescence, typical of *P. aeruginosa*.

**B. Isolation of *Pseudomonas aeruginosa*:** A loopful of sample from the positive tubes showing growth and fluorescence was further streaked on St. Cetrinide agar (Himedia) and incubated at 37°C for 24 to 48 hours. Green pigmented growth observed was purified and maintained on St. Nutrient agar slants. Cetrinide agar is a highly selective medium for *P. aeruginosa* due to the presence of cetrinide which inhibits the growth of other bacteria. As it also contains  $MgCl_2$  and  $K_2SO_4$ , it facilitates the production of the characteristic green pigment of *Pseudomonas*.

**Identification of organisms:** Isolation of culturable bacteria from the water samples was carried out on Sterile Nutrient Agar and Sterile Trypticase Soya Agar. Purified representative colonies were named P-1-a,P-1-b,P-2-d,P-10-b and so on,and preserved and maintained on Nutrient agar slants. Isolates obtained were identified on the basis of morphological and biochemical characterization as per Bergey’s Manual (Eaton et al. 2005; Forbes et al., 1998; Holt et al,1995)

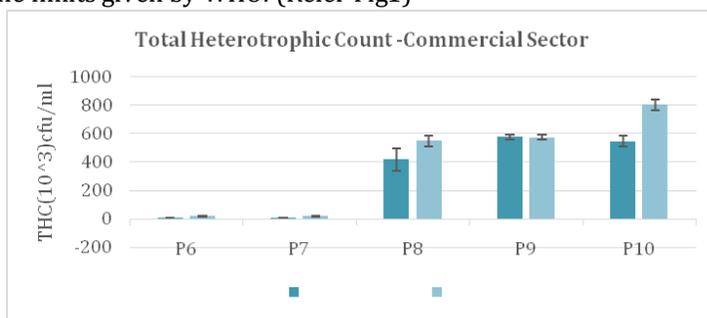
**RESULTS**

**Total Heterotrophic count (THC):**



**Figure 1- Total heterotrophic count for RESIDENTIAL SECTOR POOLS**

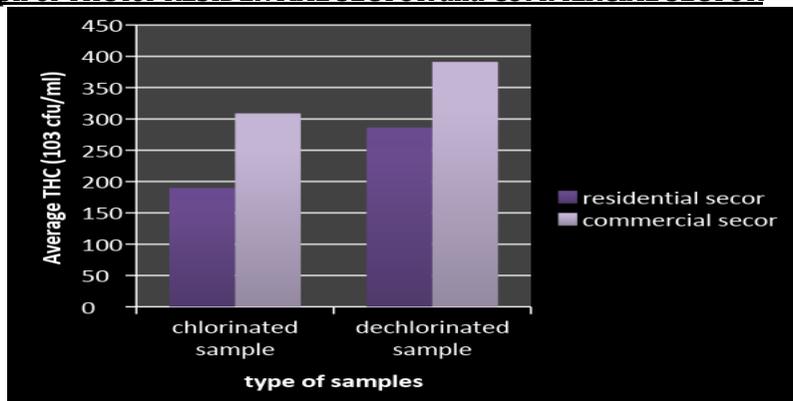
The findings of Total Heterotrophic Count of the samples(n=10) from 5 swimming pools (P1 to P5) in the residential sector revealed highest THC value for the chlorinated sample ( $2.56 \times 10^5$  cfu/ml) from pool P2 whereas the dechlorinated sample from pool P1 showed highest THC as  $4.10 \times 10^5$  cfu/ml. High values of THC obtained for both chlorinated and dechlorinated samples from all the pools (i.e. 100% of the tested samples) are beyond the limits given by WHO. (Refer Fig1)



**Figure 2-Total heterotrophic count for COMMERCIAL SECTOR POOLS**

Under commercial sector, amongst the 5 pools (P6 to P10) (n=10), chlorinated sample from pool P9 showed highest THC as  $5.73 \times 10^5$  cfu/ml and dechlorinated sample from pool P10 showed highest THC as  $8.01 \times 10^5$  cfu/ml. Pools P6 and P7 showed negligible values for both the samples, indicating good quality pool water. High THC values of chlorinated and dechlorinated samples for pool P8, P9 and P10 are beyond the standard limits given by WHO.(Refer Fig 2)

**Comparative Graph of THC for RESIDENTIAL SECTOR and COMMERCIAL SECTOR**



**Figure 3-Chlorinated and dechlorinated values of RESIDENTIAL AND COMMERCIAL sector POOLS**

A one-sample t-test was run to determine whether THC(Chlorinated)value in both residential and commercial sectors was different to permissible limit, defined as THC of  $\leq 100$ cfu/ml. Mean THC (Chlorinated) value ( $M = 249318$ cfu/ml,  $SD = 201149$ cfu/ml) was higher than the permitted THC value of 100.0cfu/ml, a statistically significant mean difference of 249218, 95% CI [105431.1118 to 393204.8882],  $t(9) = 3.918$ ,  $p = .0035$ .

Similarly, A one-sample t-test was run to determine whether THC(Dechlorinated)value in both residential and commercial sectors was different to permissible limit, defined as THC of  $\leq 100$ cfu/ml. Mean THC (Dechlorinated) value ( $M = 338690$ cfu/ml,  $SD = 247951$ cfu/ml) was higher than the permitted THC value of 100.0cfu/ml, a statistically significant mean difference of 338590, 95% CI [161316.5397 to 516063.4603],  $t(9) = 4.318$ ,  $p = .0019$ .

For both residential and commercial sector pools, THC of dechlorinated sample was higher than that of the chlorinated samples in 80% of the pools tested, indicating a greater presence of heterogeneous bacteria capable of causing pollution of pools as compared to the chlorinated sample. Thus, the study infers that chlorination might be effective in inhibiting bacteria, but is not sufficient to kill all of them. This also indicates that the bacterial flora in swimming pools have developed resistance against chlorine which is thus becoming ineffective as a disinfecting agent.

In both residential and commercial pools, the THC values obtained were higher than the WHO standards for pool waters in 80% of the pools tested, both for chlorinated as well as dechlorinated samples. Further as seen in Fig 3, the average THC in commercial sector pools is higher than residential sector pools. The possible reason for this could be inefficient inhibitory effect of chlorine as a disinfectant; higher bather load in commercial pools, being one of the reasons for more contamination of pool water; insufficient amount of disinfectant in the pool, probably less than what is required for maintaining good sanitation of pools.

**Most probable number (MPN) test-**MPN test for all swimming pool samples was carried out without dechlorinating the original samples, so as to enumerate the number of coliforms in the swimming pool sample. Following table includes the readings obtained for MPN tests. For residential sector pools i.e. P1 to P5, none of the pools showed positive MPN test. Thus, coliforms were found to be absent for residential sector pools.

#### **MPN for commercial sector pools-**

**Table 1 - MPN for commercial sector pools**

Pool code	Results obtained			Selected series	MPN index per 100 ml
	0.1 ml	1ml	10ml		
P6	0	0	0	0-0-0	-
P7	0	0	0	0-0-0	-
P8	5	3	1	5-3-1	110
P9	1	0	0	1-0-0	2
P10	0	0	0	0-0-0	-

**Note:** For residential sector pools i.e. P1 to P5, none of the pools showed positive MPN test, indicating absence of coliforms in residential sector pools.

In commercial sector pools i.e. P6 to P10, pool P8 and P9 showed positive MPN test. Pool P8 showed 110 cells/ 100ml and pool P9 showed 2 cells/100 ml. MPN values of pool P8 were very high as compared to P9, high MPN value of pool P8 indicates contamination of swimming pool water with fecal coliforms. Contamination of swimming pool water with fecal coliforms is an indication of enteric pathogens in pool water which can become possible reason for hazardous outbreaks of gastrointestinal disease in future.

#### **Detection of *Pseudomonas aeruginosa*:-**

For residential sector pools, all 5 pools i.e. P-1,P-2,P-3,P-4 and P-5 dechlorinated samples showed presence of *P. aeruginosa* with the green colored fluorescence indicative of a positive result, whereas for commercial sector pools i.e. dechlorinated samples of pool P-8, P-9 and P-10 showed presence of *P. aeruginosa*. However, dechlorinated samples of pool P-6 and P-7 did not show green colored fluorescence indicating absence of *P. aeruginosa* in the sample.

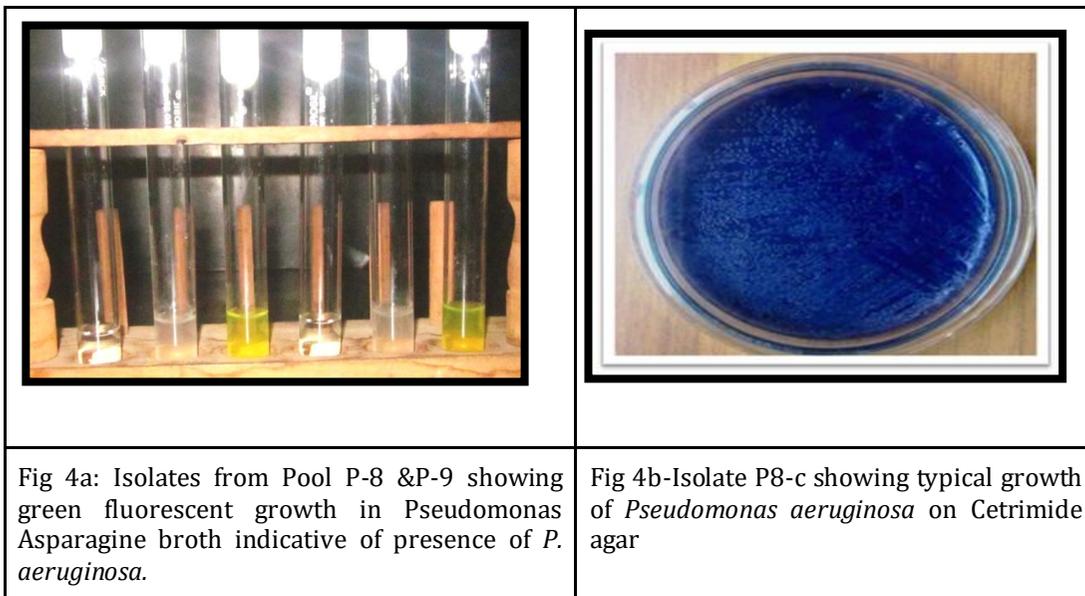


Fig 4a: Isolates from Pool P-8 &P-9 showing green fluorescent growth in Pseudomonas Asparagine broth indicative of presence of *P. aeruginosa*.

Fig 4b-Isolate P8-c showing typical growth of *Pseudomonas aeruginosa* on Cetrimide agar

**Fig 4** -Detection of *Pseudomonas aeruginosa* from Sampling locales

Isolation of *Pseudomonas aeruginosa*- Loopful of green fluorescent growth obtained on Pseudomonas Asparagine broth, was streaked on Cetrimide agar plate, and incubated at 37°C for 24-48 hours. Typical colonies of Pseudomonas, bluish green in color was obtained. (Fig 4b)

A total of 24 isolates were obtained from all the pools except P6 and P7, (3 isolates were obtained from P1, P2, P3 and P4 each; 1 isolate each was obtained from P5;4 from P8; 5 from P9 and 2 isolates from P10). The isolates were identified as *Pseudomonas aeruginosa*, *Esherichia coli*, *Serratia marcescens*, *Proteus vulgaris*, and *Klebsiellapnuemoniae*, on the basis of morphological, cultural and biochemical characterization. All the isolates obtained were found to be Gram negative. From the obtained morphological characteristics, following labeling was done: -

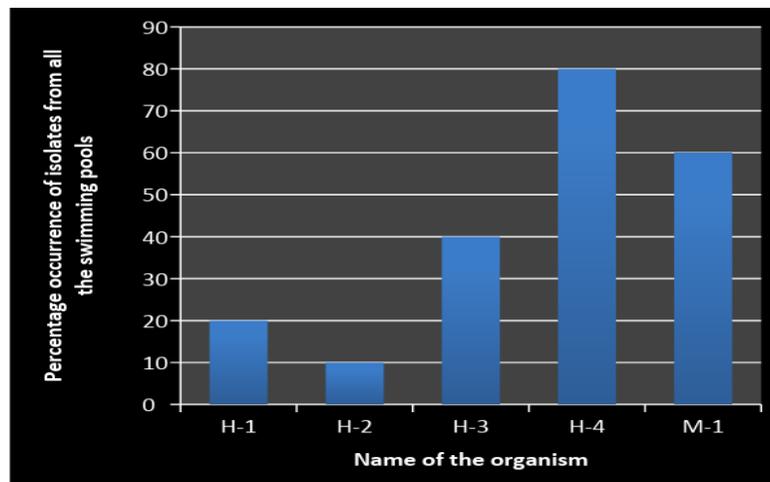
**Table 2** -Codes of obtained isolates

CODES of isolates obtained from respective pools								Isolate later identified biochemically as
P-8-a	P-9-a							<i>Proteus vulgaris</i>
P-8-b								<i>Klebsiella pnuemoniae</i>
P-1-a	P-2-a	P-3-a	P-4-a					<i>Serratia marcescens</i>
P-1-b	P-2-b	P-3-b	P-4-b	P-5-a	P-8-c	P-9-d	P-10-b	<i>Pseudomonas aeruginosa</i>
P-1-c	P-2-c	P-3-c	P-4-c	P-8-d	P-9-e			<i>Escherichia coli</i>

**Biochemical characterization**-Biochemical tests were carried out for the identification of all the isolates, as per Bergey’s manual of systematic Bacteriology.(Table 2). Results revealed their frequency of occurrence as percentage in all the pools tested as follows:

**Table 3-** Identification of bacterial pool isolates and their frequency of occurrence

Sr.no.	Culture code	Name of the organism	Percentage occurrence of the isolates in all the pools tested
1	H-1	<i>Proteus vulgaris</i>	20%
2	H-2	<i>Klebsiella pnuemoniae</i>	10%
3	H-3	<i>Serratia marcescens</i>	40%
4	H-4	<i>Pseudomonas aeruginosa</i>	80%
5	M-1	<i>Escherichia coli</i>	60%



**Fig 5-** Percentage occurrence of isolates from all the swimming pools

Thus, from all the swimming pools, it was seen that *P. aeruginosa* was dominant in all pools and it was found to be 80% in pools followed by *E. coli* which was 60 % in the pools, representing fecal contamination. Red pigmented *Serratia marcescens* also occurred at a higher percentage of 40%. The occurrence of *Proteus vulgaris* and *Klebsiella pneumoniae* was relatively less (20% and 10% respectively)

### **Discussion**

In the current study, 24 bacterial isolates were obtained from swimming pools of residential sector and commercial sector (n=10), which were further identified to be *Serratia marcescens*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. The swimming pool with the poorest bacteriological quality was Pool 10 with a THC value of  $8.01 \times 10^5$  cfu/ml.

Yedemeet *et al.*, (2017), in their study on the assessing the Physicochemical and Microbiological Quality of Public Swimming Pools in Addis Ababa, Ethiopia, have reported similar findings implying the lack of compliance to the WHO standards for swimming pools in most public pools and they have also stressed on the urgent requirement to improve the swimming pool water quality with public health as a primary concern.

In a similar study, the microbiological quality and susceptibility of bacterial isolates of swimming pool waters in indoor and outdoor swimming pools in Greece was investigated. All water samples were analyzed for the presence of bacteria, protozoa and fungi and susceptibility tests were performed for the bacterial isolates. Out of 107 bacterial isolates, 38 (35.5%) resistant strains were detected. Multi-resistant *Pseudomonas alcaligenes*, *Leuconostoc*, and *Staphylococcus aureus*, *Staphylococcus wernerii*, *Chryseobacterium indologenes* and *Ochrobactrum anthropi*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Aeromonas hydrophila*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *S. aureus* and *A. hydrophila* were detected. The swimming pool with the poorest microbiological quality (THC 500 cfu/ml in 12.1% of the samples, *P. aeruginosa* counts 1500 cfu/100 ml in 6% of the samples) and the highest prevalence of multi-resistant isolates (73.6%) was the hydrotherapy pool (Papadopoulou *et al.* 2008).

In another descriptive and analytical study, all indoor swimming pools of Yazd (12 pools) were evaluated during the spring and summer of 2013, in terms of bacterial contamination. The results showed that from 540 samples, bacterial contamination was observed in about 93 samples (17.22%); and was seen more in showers, edges of the pool and jacuzzis, and the slippers used in swimming pools. The most important isolated bacteria types were *E. coli*, *Actinobacteria*, *Pseudomonas alcaligenes*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Ehrampooshet *et al.* 2011).

### **Conclusion**

The current study included bacteriological analysis of residential and commercial sector pools in Mumbai and surrounding area. Only 20 % of the pools showed proper bacteriological status with respect to WHO standards while a majority of the pools, i.e. 80% indicated poor quality of pool waters. Thus, this indicates poor quality of pools and unhygienic status of all the residential sector pools (100%) and 60% of the commercial pools tested. These results highlight the need for an improvement in water quality at the pools examined, by setting the frequency and type of monitoring and disinfection/cleaning procedures in relation

to the type of pool and number of bathers. Attention should be focused on periodical cleaning of filtration systems in order to remove biofilm, and improving disinfection. To ensure a safer environment in these swimming pools, it is also necessary to increase users' knowledge and awareness of the risks in order to promote the correct behaviors. This study also highlights the increasing resistance of bacteria in pool waters to chlorine disinfectant and therefore emphasizes the need to search for alternative disinfection strategies.

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