

Microbiological and chemical indicators of water quality in indoor hotel swimming pools before and after training of swimming pool operators

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ABSTRACT

The present study was undertaken in order to determine the quality of indoor pool waters in hotels along the Croatian coast. We wanted to assess the risks of exposure to microbial and chemical contaminants and find out if training pool operators to use a quality assurance system, that we developed, influenced hygienic conditions and water quality in swimming pools or not. The samples were analysed for free chlorine, pH and several microbiological indicators according to standard laboratory methodologies. Of 1,329 samples tested, 276 were found to be unacceptable either by chemical (148) or microbiological parameters (128). After training, the proportion of unacceptable samples dropped by 23.5%, mostly according to the free chlorine values. According to our results, most of the microbiologically unacceptable samples had chlorine levels within the recommended range but their pH values were too high. A free chlorine level below 0.2 mg/L was found in 106 (82.8%) microbiologically unacceptable samples suggesting the need for maintaining the lower limit at least above 0.2 mg/L in order to reduce microbial risks to a more acceptable level. This measure combined with training of pool operators might result in reduced health risks in pool waters.

Key words | chemical indicators, health risks, microbiological indicators, swimming pools, swimming pool operators' training, water quality

INTRODUCTION

Recreational waters are monitored to protect bathers and swimmers from infections caused by pathogens in the water. Water has to be clean, clear and meet regulatory standards and/or WHO guidelines (2006). Health risks for bathers include physical, chemical and microbial, with the latter being the most common in public and semi-public pools. Poor swimming pool management can create conditions for developing infections such as gastrointestinal,

respiratory, eye (conjunctivae), outer and middle ear, intestinal and skin infections (especially fungal foot disease or athlete's foot and genital infections) (CDC 2000; Zmirou *et al.* 2003; WHO 2006; Wiedenmann *et al.* 2006).

Recreational waters are usually analysed for hygiene and microbial indicators, as higher levels of these indicators are associated with increased risk for bathers (Prus 1998; Castor & Beach 2004; Maida *et al.* 2008).

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Bathers are the greatest contributors to bacterial contamination of pool water. Some bacteria are transferred from wet shower benches, sanitary facilities, outlets and floor around the pool, which are ideal for bacterial growth. However, bacterial presence in pool water is directly associated with poor water management (Prus 1998).

Proper water management is key to preventing transmission of microbial infections and includes maintenance of optimal filtration, chlorine levels and pH, and use of flocculants (coagulants) to keep water clear. The choice of disinfectants is wide and depends on their safety profile, compatibility with water (fresh or sea, which is also related to pH), pool type and size, and, of course, on efficiency and rapid action. If the water is either acidic or alkaline, chlorine-based disinfectants will be less efficient (Esterman *et al.* 1984; Dadswell 1996; WHO 2006; Nikaeen *et al.* 2009). Added agents need to be evenly distributed throughout the pool to maintain health safety standards (WHO 2006). Free (residual) chlorine levels should be maintained within limits set by national, local regulations or WHO recommendations. A pool water environment with low free chlorine levels favours microbial growth, while excessive levels can irritate eyes, skin and the upper airways, and produce disinfection by-products such as trihalomethanes. Compared with microbial infections related to low chlorine levels, these by-products are less harmful (WHO 2006).

Infection prevention also depends on microbial resistance to certain agents, bathers' hygienic behaviour and hygiene maintenance around the pool (Esterman *et al.* 1984; Hajjartabar 2004; WHO 2006). In addition, the proper use of disinfectant and pH, and thus microbial contamination of pool water, depends on the pool maintenance staff having appropriate training (Rabi *et al.* 2008).

In minimising the risk of infection, it is necessary to regulate some specific parameters related to swimming pool operation. Croatia has not yet adopted a set of regulations at national level, but it has been left to local communities and counties to address this issue by setting their own regulations or by relying on the WHO (2006) Guidelines. Croatia is not unusual in this; a number of countries have still not regulated risk management for pool water (Zura *et al.* 1990; Wade *et al.* 2003; Zmirou *et al.* 2003; Soller *et al.* 2006).

The present study was focused on analysis of hotel swimming pools located in Opatija, a well known tourist

destination on the Croatian coast. The general aim of our study was to introduce professional hygienic and epidemiological monitoring of pools and to assess the risks of exposure to microbial and chemical contaminants. We also wanted to see whether training of employees to use the quality assurance system which we developed in order to maintain good hygienic conditions and water quality would influence these risks, by comparing chemical and microbiological parameters of water quality before and after training.

METHODS

Sample collection

Indoor pool water was collected for testing in sterile bottles twice a month over two years, unless hotels and/or pools were closed. Samples were taken on opposite sides of the pool, 5–30 cm below the water surface, put in thermos containers and transported immediately to the laboratory (within 4 h). Laboratory analysis began with 4–6 h after sampling. Samples were taken from 17 indoor pools in 15 hotels. Free chlorine levels, water and air temperatures were determined on-site, while other chemical and microbiological parameters were analysed in the laboratories of the Teaching Institute of Public Health of Primorsko-Goranska County.

Pool characteristics

The pools included in the study are indoor hotel pools with heated water (average temperature is approximately 27 °C). For all pools water is filtered continuously through sand filters of various sizes (depending on the pool volume). Filtration rates and filters cleaning are different and adjusted according to manufacturer's instructions (once or twice a week). Flocculation with aluminum salts is optional and is not used in all pools. Water circulation is adjusted to produce a daily supplement of total water volume per bather. All analysed pools use aqueous sodium hypochlorite as disinfectant, but with different methods of dosing (automatic, semiautomatic or manual). The same dosing methods are also used for other chemicals in water treatment (sulfuric acid for pH regulation and aluminium salts as flocculants).

Generally the analysed pools are in good technical conditions with proper maintenance.

Staff training

We noticed during the first series of sampling that the staff knowledge regarding safety issues was inadequate. Therefore, staff training was performed as series of short theoretical lectures combined with practical examples on-site during sampling. The main topics of training included pool-associated health risks, hygiene, maintenance and pool management.

Chemical and physical analysis

The analysis included free chlorine levels, pH, water temperature, colour, turbidity and ambient air temperature. We included in the study the results of free chlorine and pH determinations. Free chlorine was determined using a standard method (SM 21st Ed. 2005:4500-Cl (G)) with simple test kits based on the *N*, *N*-diethyl-*p*-phenylenediamine using tablet reagents and expressed as mg/L. We took the WHO value of 1.2 mg/L (WHO 2006) as a threshold. Samples above this threshold were not considered to meet safety criteria and were designated as unacceptable. The pH levels were determined by ISO 10523:2009 method. Samples with pH values within the range pH 7.2 and pH 7.8 were considered as acceptable.

Microbiological analysis

Microbiological analysis included detection of faecal coliforms, *Escherichia coli*, faecal streptococci, *Pseudomonas*

aeruginosa, *Staphylococcus aureus*, *Proteus* species and heterotrophic plate count at 37 °C. Colonies were counted after inoculation of agar media plates according to the method used. Table 1 lists the methods, volumes and reference values used for each parameter.

Statistical analysis

Statistical analysis was performed by commercial statistical software Statistica ver. 9.1 (StatSoft, Inc., Tulsa, USA). Differences in microbial findings between the two series of sampling, e.g. before and after pool operators' training were compared using the chi-square test (Fisher's exact test). Data regarding free chlorine and pH levels were expressed as arithmetic means \pm SD. The differences between analysed groups (microbiologically acceptable and unacceptable) were determined using Student's two-tailed *t*-test for independent samples. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

As previously mentioned legislation in Croatia regarding the swimming pool water standards is insufficient. Therefore, in routine practice WHO recommendations are used without lower limit determination of free chlorine. During the first year of our study, before pool operators' training, a total of 693 samples were analysed, while during the second year (i.e. after training), the number of samples was 636.

Table 2 presents results of chemical and microbiological analysis. The proportion of unacceptable samples was reduced from 23.5 to 17.9% after training ($p = 0.0144$),

Table 1 | Methods of determination of microbial pool water parameters and their threshold values

Microbiological parameter	Method	CFU/volume	Reference values
Faecal coliforms	SM 19th Ed. 1995:9222 (D)	CFU/100 mL	0
<i>Escherichia coli</i>	ISO 9308-1:2000	CFU/100 mL	0
Faecal streptococci	SM 19th Ed. 1995:9230 (C)	CFU/100 mL	0
<i>Pseudomonas aeruginosa</i>	EN 12780:2002 (E)	CFU/100 mL	0
<i>Staphylococcus aureus</i>	SM 19th Ed. 1995:9213	CFU/100 mL	<100
<i>Proteus</i> spp.	In-house membrane filtration method	CFU/100 mL	0
Heterotrophic plate count at 37 °C	EN ISO 6222:1999	CFU/1 mL	<200

Table 2 | Total number of indoor swimming pool samples, number of unacceptable samples according to residual chlorine levels and microbiological parameters

	Before education n (%)	After education n (%)	p
Samples collected	693 (100)	636 (100)	
Unacceptable samples	162 (23.4)	114 (17.9)	0.0144
Unacceptable samples with free Cl above threshold of 1.2 mg/L	98 (14.1)	50 (7.9)	0.0001
Unacceptable samples according to microbiological parameters	64 (9.2)	64 (10.1)	NS

NS: not significant.

which suggests an improvement in pool management. It is apparent that the difference is the result of a significantly greater proportion of samples containing free chlorine levels above threshold during the pre-training period ($p = 0.0001$). The proportion of microbiologically unacceptable samples did not differ significantly, although it increased slightly after training. The proportion of unacceptable samples due to free chlorine (above 1.2 mg/L) decreased from 14.1% of total samples before training to 7.9% after training (43.9% reduction). Excessive free chlorine levels are associated with the formation of disinfection by-products. These include trihalomethanes, chloroform, dichloromethane and bromodichloromethane; these substances can enter the human body through the airways, gastrointestinal tract or skin. Trihalomethane levels depend on the number of pool users, water temperature, pH and total organic content (Fantuzzi *et al.* 2001). However, excessive use of disinfectants seems to do less harm than microbial contamination occurring because of their under use (WHO 2006). According to Chu & Nieuwenhuijsen (2002), indoor pools tend to have higher trihalomethane levels than outdoor. The small increase in percentage of samples unacceptable by microbiological criteria may be a consequence of increased employees awareness after training on harmful effects of excessive residual chlorine levels on human health.

Detailed analysis of microbiologically unacceptable samples has shown that most of them have some common features. Most unacceptable samples were without free

chlorine or with free chlorine levels of up to 0.2 mg/L. This finding confirms the statement by Nikaeen *et al.* (2009). Table 3 shows that 26 of 38 (68.4%) samples without detectable free chlorine were microbiologically unacceptable before training, as well as 23 of 35 (65.7%) samples after training. Additionally, in samples with free chlorine levels of up to 0.2 mg/L, 29 out of 125 (23.2%) samples were microbiologically unacceptable before and 28 out of 148 (18.9%) samples were unacceptable after training. For free chlorine levels of 0.2–1.2 mg/L, only 9 of 432 (2.1%) samples were unacceptable before, and 13 of 403 (3.2%) samples were unacceptable after training. These findings suggest that vast majority of microbiologically unacceptable samples have a free chlorine level ≤ 0.2 mg/L. These results also suggest that in maintaining microbiological quality of water it is useful to keep free chlorine level above 0.2 mg/L. Unacceptable microbial findings in water samples with higher chlorine concentration (0.21–1.2 mg/L) may be the consequence of poor water mixing in pools, poorly maintained sand filters or the fact that samples were taken before complete chlorine efficiency was achieved. However, we must not ignore the dependence of disinfectant efficiency and pH value, which will be discussed below.

Table 4 presents indicator microorganisms isolated from pool water samples. Samples were considered unacceptable if they contained at least one parameter above the threshold (for thresholds see Table 1). The most frequently found cause of microbiological positivity is heterotrophic plate count at 37 °C above 200 CFU/ml. This indicator points to poor water maintenance. It does not reflect the immediate risk for human health, but speaks about the hygiene of the pool and its surroundings. The second most important

Table 3 | Relation between unacceptable microbial samples and free chlorine levels

Free Cl levels (mg/L)	Samples n	Before training n (%) ^a	Sample N	After training n (%) ^a
0	38	26 (3.7)	35	23 (3.6)
0.01–0.2	125	29 (4.2)	148	28 (4.4)
0.21–0.4	109	8 (1.2)	218	9 (1.4)
0.41–1.2	323	1 (0.1)	185	4 (0.6)
>1.2	98	0 (0)	50	0 (0)
Total	693	64 (9.2)	636	64 (10.1)

^aProportion of unacceptable samples within total number of examined samples.

Table 4 | Presence of microorganisms in samples according to free chlorine level

Free Cl levels (mg/L)		FC	EC	FS	PA	SA	P	HPC 37 °C	Total
0	B	13	11	8	17	2	-	19	70
	A	11	11	7	14	-	-	21	64
0.01–0.2	B	6	4	7	18	-	-	21	56
	A	16	13	11	16	1	1	22	80
0.21–0.4	B	-	-	1	2	-	-	6	9
	A	-	-	1	-	-	-	8	9
0.41–1.2	B	-	-	-	1	-	-	-	1
	A	-	-	-	-	-	-	4	4
>1.2	B	-	-	-	-	-	-	-	-
	A	-	-	-	-	-	-	-	-
Total no.		46	39	35	68	3	1	101	

FC: faecal coliforms; EC: *Escherichia coli*; FS: faecal streptococci; PA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*; P: *Proteus* spp.; HPC 37 °C: heterotrophic plate count at 37 °C; B: before training; A: after training.

cause of microbiological positivity is the presence of *P. aeruginosa* followed by faecal coliforms, *E. coli* and faecal streptococci. The presence of *P. aeruginosa* points to poor maintenance of free chlorine levels. As this bacterium is common in the environment (especially water and soil), the key to controlling it is to maintain residual disinfectant levels (such as free chlorine) and to wash and disinfect surrounding floor areas. The presence of *P. aeruginosa* or *S. aureus* in pool water indicates increased risk of infection, including *P. aeruginosa*-induced ear, urinary, respiratory, skin, wound and corneal infections, and *P. aeruginosa* and *S. aureus* skin and nasal mucosa infections (CDC 2000; Tate et al. 2003; Hajjartabar 2004; Papadopoulou et al. 2008; Nikaeen et al. 2009). As a general control measure, bathers should be encouraged to observe and obey the hygiene rules, e.g. use pre-swim showers and hyperchlorinated footbaths before entering the pool. If feasible, bather numbers should also be controlled as well as duration of their exposure in the water (Tate et al. 2003; Hajjartabar 2004).

The finding of faecal coliforms points to possible presence of pathogens but does not necessarily prove it (Itah & Ekpombok 2004). According to Wade et al. (2003), a more specific and reliable indicator of faecal contamination is *E. coli*. It is routinely analysed, as it is the most common source of gastrointestinal infection in recreational waters, and its presence suggests that pool water needs closer monitoring and disinfectants addition as needed

(Zmirou et al. 2003; Nikaeen et al. 2009). Faecal streptococci were confirmed in 35 samples in the study. Their presence suggests that either disinfectants were not adequately used or were ineffective.

In our study, *S. aureus* and *Proteus* spp. were isolated in a small number of samples. *S. aureus* is shed by bathers and can be reduced by hygienic measures in the dressing rooms and by use of showers and footbaths (Nikaeen et al. 2009).

Our results also show that most microbiological indicators were found in water samples with free chlorine levels ≤ 0.2 mg/L. The results also suggest that current guidelines regarding the chlorine level (between 0.2 and 1.2 mg/L) allow some microbial contamination. According to the results we speculate that by increasing minimal chlorine level to at least 0.21 mg/L most of microbial indicators will be affected.

The remaining contamination in samples with chlorine levels of 0.21 mg/L and greater consists mostly of heterotrophic bacteria grown at 37 °C (18 samples), while the *P. aeruginosa* and faecal streptococci were found in only three and two samples, respectively. It is known that the risk of infection can be minimised by optimal choice and dosing of disinfectants, by maintaining pH within recommended range, and by efficient filtration (Dadswell 1996). Risk management should take into account faecal coliforms and faecal streptococci when setting standards for recreational waters (Zmirou et al. 2003). In addition, Soller et al. (2006) underlined the importance of proper interpretation of faecal indicators in recreational waters and proposed more stringent limits than are current. All these are possible today, because there is a sophisticated swimming pool technology which can make water quality management easier. Poor water treatment may be responsible for the increased health risk associated with swimming pools. Incorrect water treatment influences indicators of water quality leading to an increase in numbers of unacceptable samples. If water is maintained properly, according to guidelines, and the analysis still shows unacceptable microbial levels, pool managers should take into account user frequency, as it correlates with the degree of contamination (Hajjartabar 2004), and adjust maintenance activities.

In addition, it is well known that chlorine disinfection efficiency depends on pH value. Namely, aqueous sodium

hypochlorite solution, which is added to the water, creates hypochlorous acid (HOCl) which dissociates into hypochlorite ion (OCl⁻). Hypochlorous acid and hypochlorite ion are in balance that is pH dependent. An increase in pH from 7.2 to 7.8 results in predominance of hypochlorite ion which is a much weaker disinfectant than hypochlorous acid. The usual tests for chlorine determination detect both components as free chlorine. Therefore, the prediction of chlorine disinfection is greatly dependent on pH value (WHO 2006).

Both chlorine and pH are easy to determine and adjust even several times daily to reach maximal disinfectant efficiency. Table 5 presents our data regarding free chlorine levels, microbial contamination and pH values of analysed water samples. In both series, before and after training, the proportion of correctly adjusted pH values was relatively

small (approximately 25%), with no statistically significant difference between two series. This finding suggests that pool operators have failed to recognise this issue as an important parameter that affects disinfectant efficiency. Most samples had pH values that exceed the level of 7.8 recommended by WHO (2006). The results also show that most microbiologically unacceptable samples belong to this group (101 of 128 unacceptable samples) regardless of free chlorine in the sample. According to our results we can reasonably speculate that adjusting pH to recommended values will help to minimise the number of microbiologically unacceptable samples. Additionally, if the free chlorine level is maintained between 0.21 and 1.2 mg/L, the proportion of microbiologically unacceptable samples will decrease even more, as well as the health risks for bathers.

As explained above, we noted that microbiologically acceptable and unacceptable samples differ in free chlorine levels and pH values. The statistical significance of the difference between those parameters in samples belonging to these two groups was tested. The results of the analysis are presented in Table 6. The analysis revealed that microbiologically unacceptable samples have significantly lower levels of free chlorine and higher pH values compared to microbiologically acceptable samples for both series, i.e. before and after staff training. The average value in microbiologically unacceptable samples in both series is below the lower limit that we propose (0.21 mg/L). On the other hand, microbiologically acceptable samples have an average of free chlorine within the range between 0.21 and 1.2 mg/L. Average values of pH are higher than upper limit recommended by WHO in both series with significantly lower values in microbiologically acceptable group. However, the values in the unacceptable group are significantly greater compared to the other group in both series.

Table 5 | Samples distribution according to free chlorine, pH values and microbiological findings

Free Cl levels (mg/L)		Total no. of samples (no. of unacceptable samples)		
		pH < 7.2	7.2 < pH < 7.8	pH > 7.8
0	B	–	8 (6)	30 (20)
	A	–	8 (8)	27 (15)
0.01–0.2	B	2	23 (5)	100 (24)
	A	4	33 (1)	111 (27)
0.21–0.4	B	4 (1)	22	83 (7)
	A	21 (1)	63 (2)	134 (6)
0.41–1.2	B	45	97 (1)	181
	A	26 (2)	51	108 (2)
>1.2	B	2	18	78
	A	–	12	38
Total	B	53 (1)	168 (12)	472 (51)
	A	51 (3)	167 (11)	418 (50)

B: before training.
A: after training.

Table 6 | Average free chlorine and pH values in analysed samples

		Microbiologically unacceptable	Microbiologically acceptable	p
Before education	Free Cl (mean ± SD)	0.12 ± 0.27	0.73 ± 0.58	0.0000
	pH (mean ± SD)	7.99 ± 0.27	7.84 ± 0.40	0.0035
	N	64	629	
After education	Free Cl (mean ± SD)	0.13 ± 0.51	0.53 ± 0.50	0.0000
	pH (mean ± SD)	8.01 ± 0.38	7.82 ± 0.41	0.0004
	N	64	572	

CONCLUSIONS

Maintaining good swimming pool water quality is an important issue in preventing health risks for bathers. Water quality should be regularly monitored in order to prevent these unwanted outcomes. Pool operators' training resulted in improving water quality. This improvement is mainly due to the reduction in the number of samples with excessive free chlorine level. Our findings confirm the existence of potential risks of microbial infections by some swimming pool operators failing to meet safety standards. Reasons for that may include poor user and staff training and/or poor water treatment. Continued chemical and microbiological water analysis give an objective insight into pool hygiene and help to prevent infection outbreaks among pool users. Free chlorine levels in pool water are directly related to microbial contamination. Currently, in Croatia, there are no standards that regulate swimming pool water quality. We routinely use in our practice WHO recommendations, without defining the lower limit of free chlorine. Our results suggest that setting the lower limit of free chlorine to >0.2 mg/L will reduce microbial contamination. Additionally, it is known that pH value directly affects the efficiency of chlorine disinfectants, and filtration is another fact for ensuring the swimmers safety. Therefore, pH should always be determined and kept at the recommended range. According to our results, this parameter is often disregarded by swimming pool operators. Continuous monitoring of both, free chlorine and pH, is important for minimising pool water associated health risks that include microbial contamination and exposure to excessive chlorine levels. Finally, we conclude that improvement in pool water safety can be achieved by training swimming pool operators in combination with regular water monitoring. However, we believe that both of these issues should be more clearly regulated by appropriate legislation in order to improve bathers' safety.

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