

Bacteriological quality of swimming pool and spa water in northern Greece during 2011–2016: is it time for *Pseudomonas aeruginosa* to be included in Greek regulation?

Ilias Tirodimos, Eleni P. Christoforidou, Sofia Nikolaidou and Malamatenia Arvanitidou

ABSTRACT

The risk of illness or infection associated with recreational water environments is mainly associated with faecal water contamination. Non-faecal human shedding into the pool water or surrounding area is also a potential source of pathogens such as *Pseudomonas aeruginosa*. The aim of the current study is to describe and evaluate the bacteriological quality of 2,844 swimming pool and jacuzzi/spa water samples in northern Greece during the 2011–2016 period. Bacteriological quality of recreational water includes heterotrophic bacteria, total coliforms and *Escherichia coli* according to Greek Hygienic Regulation. *P. aeruginosa* is a significant agent of opportunistic infection in aquatic environments and was also examined in the aforementioned samples. We aimed to indicate the importance of including *P. aeruginosa* as a recreational water quality indicator in the revised Hygienic Regulation. Data analysis was performed using SPSS 22.0. The quality of all types of swimming pools was evaluated as excellent since less than 5% was found not to meet the regulations. Considering the presence and concentration of *P. aeruginosa*, the number of samples that would not meet the standards would differ significantly (18.9%). *P. aeruginosa* was detected in a larger amount of samples (15.2%) than *E. coli* (0.6%), which indicates that *P. aeruginosa* should be assessed as an important factor.

Key words | bacteria, *Escherichia coli*, legislation, *Pseudomonas aeruginosa*, recreational water, swimming pool

Ilias Tirodimos (corresponding author)
Eleni P. Christoforidou
Sofia Nikolaidou
Malamatenia Arvanitidou
Department of Hygiene and Epidemiology, School
of Medicine,
Aristotle University of Thessaloniki,
54124 Thessaloniki,
Greece
E-mail: iltirodimos@yahoo.gr

INTRODUCTION

The risk of illness or infection associated with recreational water environments is mainly associated with faecal water contamination. Many of the outbreaks related to pools and similar environments have occurred because of unapplied or inadequate disinfection. *Pseudomonas aeruginosa* has been described as a tough and most frequently opportunistic pathogen bacterium in connection with the pool and spa environment, armed with a number of virulence factors and antibiotic resistance. *P. aeruginosa* is related to clinical

symptoms like swimmer's ear (otitis externa) and hot tub rash (folliculitis). Between 1999 and 2008 a total of 52 outbreaks of dermatological infection by *P. aeruginosa* were reported with 955 cases in the USA. *Cryptosporidium* and *P. aeruginosa* are the most prevalent pathogens in the pool and spa environment in the USA. Furthermore, *P. aeruginosa* is to blame for a clinically distinct, painful erythematous plantar skin eruption called hot foot syndrome in a pool with an abrasive flooring. *Pseudomonas* infection may negatively

affect corneal ulcers or wound infections, respiratory system diseases and urinary tract infections (Barna & Kádár 2012). It is also stated that various pool structures (linings, decks, drains, filters) and surrounding objects and fomites from the benches to towels and children's toys may favor the growth of *P. aeruginosa* that comes from faecal and non-faecal shedding from humans into the pool water or surrounding area (Barna & Kádár 2012; Giampaoli & Romano Spica 2014). In a CDC report it is stated that detection of *P. aeruginosa* points out the need for vigilant pool cleaning, scrubbing, and water quality maintenance (disinfectant level and pH) to ensure that concentrations do not reach levels that have a negative impact on swimmers' health (CDC 2013).

In the study by Schets et al. (2014) the presence of *P. aeruginosa* on inflatables and foam teaching aids in 24 public swimming pools in the Netherlands was investigated. A total of 230 samples were collected from 175 objects. Culture method and antibiotic resistance by disk diffusion were carried out. *P. aeruginosa* was detected in 63 samples, from 47 objects in 19 swimming pools. The study concludes that the presence of *P. aeruginosa* on pool objects requires the attention of pool managers and authorities. Infrequently used vinyl-canvas objects need to be strictly dried and properly cleaned.

Previously the review by Rice et al. (2012) aimed to identify the current understanding of risk factors associated with pool operation with regard to *P. aeruginosa*. A systematic risk assessment approach was applied to integrate existing data, with the view to improving pool management and safety. Sources of *P. aeruginosa*, types of infections, dose responses, routes of transmission, as well as the efficacy of current disinfectant treatments were taken into account. The review underscores the importance of applying quantitative risk management strategies concerning *P. aeruginosa* in order to develop a better understanding of the risk posed by *P. aeruginosa* in swimming pools.

The aim of the current study is to describe and evaluate the bacteriological quality of 2,844 swimming pool and jacuzzi/spa water samples in northern Greece during the 2011–2016 period. The bacteriological quality of recreational water includes heterotrophic bacteria, total coliforms and *Escherichia coli* according to national law (Greek Hygienic Regulation 1973) and the European

Directive (2006/7/EC). *P. aeruginosa* is a significant agent of opportunistic infection in aquatic environments and was also examined in the aforementioned samples (Pond 2005; WHO 2006). Identifying the prevalence of *P. aeruginosa* in the recreational water facilities under study and taking into account that *P. aeruginosa* is one of the most frequently identified agents associated with waterborne outbreaks of dermatitis, conjunctivitis, and otitis externa in recreational water (CDC 2001; Craun et al. 2005; Barna & Kádár 2012), we aimed to indicate the importance of including *P. aeruginosa* as a recreational water quality indicator in the revised version of the Greek Hygienic Regulation. Previous studies in Greece (Tirodimos et al. 2010; Blougoura et al. 2012) also suggest a revision of national and European law presenting findings on the prevalence of *P. aeruginosa* in various recreational water facilities.

METHODS

A total of 2,844 water samples were obtained from swimming pools ($n = 2,490$ samples), ice-plunge pools ($n = 61$ samples) and jacuzzis or spas ($n = 293$ samples) in northern Greece from 2011 to 2016. The majority (61%) of swimming pool samples came from athletic centers and the rest from hotel pools. Sampling was carried out by public health officers (41.5%) and pool operators (58.5%). Samples were taken in sterilized dark-coloured 1-litre bottles containing a chloride scavenger and were kept at a temperature of 4 °C before microbiological analyses. All information accompanying the samples such as disinfection or the type of pool was processed, as well.

From 2011 to 2016 total heterotrophic bacteria (THB) were counted on yeast extract plate count agar using the 1 mL infusion technique after incubation at 36 ± 2 °C for 48 h (ISO 6222: 1999). From 2011 to 2014 for the detection and enumeration of total coliforms the 100 mL membrane filtration technique was used, with mEndo Agar LES medium incubated at 35 ± 0.5 °C for 24 h (APHA 9222; APHA 2005). For the same period, for the detection and enumeration of *E. coli* the 100 mL membrane filtration technique was used, with MFC agar medium incubated at 44.5 ± 0.5 °C for 24 h (APHA 9222; APHA 2005). Due to compliance with the new ISO (ISO 9308-1: 2014) during

2015 for the detection and enumeration of total coliforms and *E. coli* the 100 mL membrane filtration technique was used, with TTC Tergitol 7 agar medium incubated at $36 \pm 2^\circ\text{C}$ for 24 h, and during 2016 the same technique was used, with Chromocult Coliform agar medium incubated at $36 \pm 2^\circ\text{C}$ for 24 h. In general, mEndo Agar LES and MFC agar were used for 79.4%, TTC for 10.2% and Chromocult Coliform agar for 10.2% of samples. From 2011 to 2016 for the detection and enumeration of *P. aeruginosa* the 100 mL membrane filtration technique was used, with *Pseudomonas* Agar Base medium incubated at $36 \pm 2^\circ\text{C}$ for 48 h (ISO 16266: 2006). All media used (apart from yeast extract agar) were selective, detecting typical colonies. All test methods were quantitative and the results were expressed as colony forming units (CFU) per 1 mL or 100 mL (cfu/1 mL or cfu/100 mL). All mediums were from Merck except for the *Pseudomonas* Agar Base, which was from Oxoid. Confirmation methods were also applied when necessary. Data analysis was performed using statistical package SPSS 22.0. The association between categorical variables was assessed with the chi-square test or Fisher's exact test, whichever was more appropriate, at the 5% significance level.

RESULTS AND DISCUSSION

The samples came mainly from public and private settings in Thessaloniki Prefecture (99%) by persons authorized by the

state (41.5%) or the private settings (58.5%). The samples came from indoor pools (62.4%) and outdoor pools (36.4%). The pools were characterized as 'Big pool' (77.4%), 'Small pool' (5.6%) and 'Children's pool' (17.1%). In particular, recreational water facilities were swimming pools (87.6%), jacuzzi/spa (10.3%) and ice-plunge pools (2.1%). Swimming pools could be either heated (70.8%) or non-heated (16.8%). Sampling was equally distributed among seasons (winter-spring 41.6%, summer-autumn 58.3%). Disinfection was systematic (99.8%) mostly with chlorine (97%) and bromine (2.7%). Only in 1.8% of samples was hyperchlorination observed. The distribution of sampling through the 2011–2016 period was as follows: 2011 12.1%, 2012 22%, 2013 22.3%, 2014 23%, 2015 10.2% and 2016 10.4%. Only 1.8% of the samples were not compliant with proper disinfection.

It was found that the presence of *P. aeruginosa* was associated with non-compliance of samples with microbiological standards (p -value < 0.001) while the presence of *E. coli* was not associated with the presence of *P. aeruginosa* (p -value = 0.738). However, *P. aeruginosa* was detected in a greater number of samples (15.2%) than *E. coli* (0.6%), which indicates that *P. aeruginosa* should be considered an important factor as well.

In Table 1 some important microbiological parameters and prevalence of *P. aeruginosa* in water samples from recreational water facilities in northern Greece and for the period 2011–2016 are shown. Compliance with disinfection

Table 1 | Some important microbiological parameters and prevalence of *P. aeruginosa* in water samples from recreational water facilities (northern Greece, 2011–2016)

Water category	Compliant with disinfection standard ^a No. (%)	Total heterotrophic bacteria (>200/mL) No. (%)	Total coliforms (>15/100 mL) No. (%)	<i>E. coli</i> (>0/100 mL) No. (%)	<i>P. aeruginosa</i> (>0/100 mL) No. (%)	Compliance with microbiological standards ^a No. (%)	Compliance with microbiological standards taking into account the presence of <i>P. aeruginosa</i> No. (%)
Swimming pools ($n = 2,490$)	2,437 (97.9)	35 (1.4)	24 (1.0)	15 (0.6)	362 (14.5)	2,375 (95.4)	2,034 (81.7)
Jacuzzis/spas ($n = 293$)	287 (98.0)	10 (3.4)	4 (1.4)	2 (0.7)	52 (17.7)	277 (94.5)	226 (77.1)
Ice-plunge pools ($n = 61$)	61 (100.0)	1 (1.6)	0 (0.0)	0 (0.0)	14 (23.0)	60 (98.4)	47 (77.0)
Total ($n = 2,844$)	2,785 (98.0)	46 (1.6)	28 (1.0)	17 (0.6)	433 (15.2)	2,712 (95.4)	2,307 (81.1)

n = no. of samples analysed.

^aGreek hygiene regulations (limit values): chlorination 0.4–0.8 ppm (>0.8 is considered as hyperchlorination); the limit for residual bromine is 8.0 ppm; total heterotrophic bacteria < 200/mL, total coliforms < 15/100 mL, *E. coli* 0/100 mL.

standards for all categories of pools was very high. Compliance with microbiological standards was for the total of samples 95.4% taking into account total heterotrophic bacteria, total coliforms and *E. coli* (according to the Greek Hygienic Regulation). When *P. aeruginosa* was taken into consideration compliance decreased to 81.1%. It is worth stating that in ice-plunge pools compliance of water samples was even more affected when *P. aeruginosa* was included.

In Table 2 colony-forming units of bacteria in water samples from recreational facilities (northern Greece, 2011–2016) are shown. Total heterotrophic bacteria were detected in 31.3% of samples in swimming pools, jacuzzis/spas and ice-plunge pools as well (2–300 cfu/mL). Total coliforms were detected in 87 out of 2,844 samples only in

swimming pools and jacuzzis/spas (2–60 cfu/100 mL). *E. coli* was detected in 11 out of 2,490 swimming pool samples and in one jacuzzi/spa sample (3–22 cfu/100 mL). *P. aeruginosa* was detected in 15.2% of samples (2–80 cfu/mL).

In Table 3 colony-forming units of bacteria in recreational water samples not in compliance with Greek Regulation (northern Greece, 2011–2016) are shown. During the 2011–2016 period the frequency of isolation of *P. aeruginosa* was remarkably reduced. It is worth noting that in 2011 *P. aeruginosa* was detected in 35.5% of samples, while in 2016 only 13.5% of samples were positive for the bacterium. The frequency of isolation of total heterotrophic bacteria, total coliforms and *E. coli* was rather stable except for 2013 when the highest frequencies were observed.

Table 2 | Colony-forming units of bacteria in water samples from recreational water facilities (northern Greece, 2011–2016)

Water category	Median no. of colonies (min–max) ^a /n (%)			
	Total heterotrophic bacteria	Total coliforms	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>
Swimming pools (<i>n</i> = 2,490)	34 (2–300)/763 (30.6)	9.5 (2–60)/78 (3.1)	11 (3–22)/15 (0.6)	10 (2–80)/324 (13.0)
Jacuzzis/spa (<i>n</i> = 293)	48 (2–300)/105 (35.8)	13 (2–38)/8 (2.7)	–/2 (0.7)	18 (2–46)/52 (17.7)
Ice-plunge pools (<i>n</i> = 61)	34 (2–240)/23 (37.7)	–	–	11 (3–40)/11 (18.0)
Total (<i>n</i> = 2,844)	36 (2–300)/891 (31.3)	10 (2–60)/87 (3.1)	10.5 (3–22)/17 (0.6)	12 (2–80)/390 (13.7)

n = no. of samples analysed.

^aMedian of CFUs is shown due to the statistically abnormal distribution of the samples.

Table 3 | Colony-forming units of bacteria in recreational water samples not in compliance with Greek Regulation^a (northern Greece, 2011–2016)

Bacteriological indicator		2011	2012	2013	2014	2015	2016
Total heterotrophic bacteria	<i>n</i>	7	7	15	12	3	2
	Median ^b	250	300	250	242	250	257
	Range	220–270	260–300	210–300	200–260	250–278	254–260
Total coliforms	<i>n</i>	2	5	9	2	6	4
	Median	20	40	23	24	28.5	22
	Range	16–24	17–54	15–60	20–28	20–40	15–58
<i>E. coli</i>	<i>n</i>	–	5	7	3	–	2
	Median	–	10	3	12	–	7.5
	Range	–	1–22	1–20	4–16	–	4–11
<i>Pseudomonas aeruginosa</i>	<i>n</i>	122	119	56	57	39	40
	Median	6	6	10	14	18	18
	Range	1–80	1–70	1–61	2–54	4–62	1–48

n = no. of samples analysed.

^aGreek hygiene regulations (limit values): total heterotrophic bacteria < 200/mL, total coliforms < 15/100 mL, *E. coli* 0/100 mL.

^bMedian of CFUs is shown due to the statistically abnormal distribution of the samples.

In Table 4 compliance with microbiological standards of Greek Regulation and compliance taking into account the presence of *P. aeruginosa* in water samples from recreational water facilities in northern Greece (2011–2016) are presented. Every year separately the compliance of water samples from swimming pools, jacuzzis/spas and ice-plunge pools was remarkably reduced when *P. aeruginosa* was taken into account. It is worth stating that 95.4% of samples from all types of recreational water facilities were compliant with microbiological standards of Greek Regulation and the EU, while only 81.1% were when the presence of *P. aeruginosa* was taken into consideration.

In Table 5 compliance with microbiological standards of Greek Regulation and compliance taking into account the presence of *P. aeruginosa* in water samples from recreational water facilities in northern Greece (2011–2016) between seasons are shown. Compliance of water samples in swimming pools was higher during summer and in ice-plunge pools during winter whether *P. aeruginosa* was taken into consideration or not. In jacuzzis/spas compliance of samples was steady through the seasons.

Pool type (indoor or outdoor), pool size (big, small or children's), pool temperature (heated, non-heated, jacuzzi/spa and ice-plunge), type of decontamination (chlorine, bromine, ozone) and type of sampling organization (public or private) do not affect compliance of samples whether *P. aeruginosa* is taken into consideration or not. More specifically, 63.4% of indoor pools complied with regulations and when *P. aeruginosa* was taken into consideration 64.2% of indoor pools also complied; 77.9% of big pools complied and when *P. aeruginosa* was taken into account 78.9% complied too; 71% of heated pools complied with standards and when *P. aeruginosa* was taken into consideration 73.8% complied; 96.9% of recreational water facilities that were decontaminated with chlorine complied with regulations and a similar 96.4% when *P. aeruginosa* was taken into account.

In the review by Barna & Kádár (2012) a range of factors such as the contact time, the biotype and virulence of the implicated strain and personal conditions of the bathers (time spent and repeated exposures in water, water temperature, history of earlier infections) are believed to have a heavy impact on the probability of getting ill from a

Table 4 | Compliance with microbiological Standards of Greek Regulation and compliance taking into account the presence of *P. aeruginosa* in water samples from recreational water facilities (northern Greece, 2011–2016)

Water category	Compliance with microbiological standards ^a , n (%)										Compliance with microbiological standards taking into account the presence of <i>P. aeruginosa</i> , n (%)				
	2011	2012	2013	2014	2015	2016	2011–2016	2011	2012	2013	2014	2015	2016	2011–2016	
Swimming pools (n = 2,490)	232 (9.3)	537 (21.6)	551 (22.1)	589 (23.7)	229 (9.1)	237 (9.5)	2,375 (95.4)	129 (5.1)	437 (17.5)	507 (20.4)	545 (21.9)	208 (8.3)	208 (8.3)	2,034 (81.7)	
Jacuzzis/spas (n = 293)	48 (16.3)	46 (15.7)	47 (16.0)	44 (15.0)	47 (16.0)	45 (15.3)	277 (94.5)	38 (12.9)	38 (12.9)	42 (14.3)	37 (12.6)	34 (11.6)	37 (12.6)	226 (77.1)	
Ice-plunge pools (n = 61)	20 (32.8)	16 (26.2)	5 (8.1)	7 (11.4)	4 (6.6)	8 (13.1)	60 (98.3)	16 (26.2)	9 (14.8)	5 (8.2)	7 (11.5)	3 (4.9)	7 (11.5)	47 (77.0)	
Total (n = 2,844)	300 (10.5)	599 (21.1)	603 (21.2)	640 (22.5)	280 (9.8)	290 (10.2)	2,712 (95.4)	183 (6.4)	484 (17.0)	554 (19.5)	589 (20.7)	245 (8.6)	252 (8.7)	2,307 (81.1)	

n = no. of samples analysed.

^aGreek hygiene regulations (limit values): chlorination 0.4–0.8 ppm (>0.8 is considered as hyperchlorination); the limit for residual bromine is 8.0 ppm; total heterotrophic bacteria < 200/mL, total coliforms < 15/100 mL, *E. coli* 0/100 mL.

Table 5 | Compliance with microbiological standards of Greek Regulation and compliance taking into account the presence of *P. aeruginosa* in water samples from recreational water facilities between seasons (Northern Greece, 2011–2016)

		Winter	Spring	Summer	Autumn	Total
Compliance with microbiological standards, <i>n</i> (%)	Swimming pools (<i>n</i> = 2,490)	419 (17.6)	548 (23.1)	743 (31.3)	665 (28.0)	2,375 (100.0)
	Jacuzzis/spas (<i>n</i> = 293)	64 (23.1)	67 (24.2)	72 (26.0)	74 (26.7)	277 (100.0)
	Ice-plunge pools (<i>n</i> = 61)	26 (43.3)	9 (15.0)	9 (15.0)	16 (26.7)	60 (100.0)
	Total	509 (18.8)	624 (23.0)	824 (30.4)	755 (27.8)	2,712 (100.0)
Compliance with microbiological standards including <i>P. aeruginosa</i> , <i>n</i> (%)	Swimming pools (<i>n</i> = 2,490)	372 (18.3)	452 (22.2)	624 (30.7)	586 (28.8)	2,034 (100.0)
	Jacuzzis/spas (<i>n</i> = 293)	52 (23.0)	54 (23.9)	61 (27.0)	59 (26.1)	226 (100.0)
	Ice-plunge pools (<i>n</i> = 61)	22 (46.8)	7 (14.9)	7 (14.9)	11 (23.4)	47 (100.0)
	Total	446 (19.3)	513 (22.2)	692 (30.0)	656 (28.4)	2,307 (100.0)

P. aeruginosa infection. However, the major factor is considered to be inadequate or completely missing disinfection. In the current study, only 1.8% of the samples were not compliant with proper disinfection and the type of decontamination seems not to affect the compliance of samples whether *P. aeruginosa* is taken into consideration or not.

In the study by [Szczygłowska et al. \(2012\)](#) water quality assessments were carried out using routine and extended microbiological and physicochemical analyses in an indoor recreational facility. In terms of routine microbiological indicators, water quality was satisfactory at all sampling sites except for the spa tubs and wading pools, where mesophilic bacteria alone were found to occur in excessive numbers (115 to 1,000 cfu/cm³). Only with extended microbiological analysis was it possible to detect the occurrence of bacteria of the *Pseudomonas* genus, including *P. aeruginosa*. The water samples examined in this mode also displayed lower redox potential values (507 to 582 mV) and lower free chlorine content (0 to 0.1 gCl₂/m³). The periodic occurrence of *Pseudomonas* bacteria could be attributed to the stagnation of water in some parts of the installation, where their growth rate was found to exceed 100 cfu/cm³, and where the presence of coagulase-positive staphylococci was detected. *Pseudomonas* bacteria were also present in the water samples collected from spray irrigators and rain showers. In contrast to the two previous studies, in our study only water samples from the facilities were available.

Previous studies in Greece ([Tirodimos et al. 2010](#); [Blougoura et al. 2012](#)) also presented findings on the prevalence of *P. aeruginosa* in various recreational water facilities. In the study by [Blougoura et al. \(2012\)](#) between 2008 and 2010, 60 swimming pools (75% of public swimming pools operating

in Greece, municipal and athletic, indoor and outdoor, heated and unheated) were investigated concerning bacterial pollution and physicochemical parameters such as pH, residual chlorine and temperature. Microbiological tests included heterotrophic plate count at 37 °C, total coliforms, *E. coli*, *S. aureus* and *P. aeruginosa*; 37% of the swimming pools were not compliant according to the requirements as far as total coliforms, faecal coliforms and HPC were concerned, while *P. aeruginosa* was detected in 76.6% of the samples. The study suggests that the 40-year old Greek Hygienic Regulation should be updated taking into account recent scientific and legislative data. Among others it is proposed that *P. aeruginosa* should be included as an imperative parameter as is suggested by the current study.

In the study by [Tirodimos et al. \(2010\)](#) 271 water samples were obtained from jacuzzis/spas and from swimming and hydrotherapy pools in northern Greece. *P. aeruginosa* was detected in a total of 16.6% (45/271) of the samples. Of the examined facilities, the most contaminated with *P. aeruginosa* were hydrotherapy pools (25% of samples positive) contrasting with the current study where *P. aeruginosa* was detected in 14.5% of swimming pools, 17.7% of jacuzzis/spas and 23% of ice-plunge pools. In [Tirodimos et al. \(2010\)](#) no significant differences were found between different facility categories in the rate of isolation of pseudomonads or the median number of colony-forming units of *P. aeruginosa* per 100 mL ($p > 0.05$). The prevalence of *P. aeruginosa* in the aforementioned samples in northern Greece was considered to be relatively low and compliant with established local microbiological standards.

In the study by [Thorolfsson & Marteinsson \(2013\)](#) a microbiological analysis in three popular but different

natural pools in Iceland was carried out. All samples were analyzed for heterotrophic plate counts, coliforms, *Enterococcus* spp. and pseudomonads. The results indicate higher fecal contamination in the geothermal pools where the geothermal water flow was low and bathing guest count was high during the day. The number of cultivated *Pseudomonas* spp. was high (13,000–40,000 cfu/100 mL) in the natural pools, and several strains were isolated and classified as opportunistic pathogens.

Similar studies have occurred very recently in Egypt and Iran. The study by Abd El-Salam (2012) aimed to assess the quality of water samples from some of Alexandria's swimming pools in order to determine its compliance with the Egyptian standards. Five swimming pools were selected randomly from different regions. A total of 30 samples were collected monthly over 6 months and examined with respect to physical, chemical, as well as biological parameters using standard analytical methods. Compliance of the pool water to the microbial parameters, residual chlorine, pH, and turbidity was low. Significant association between water contamination with microbial indicators and physico chemical aspects such as residual chlorine, temperature, turbidity, and load of swimmers was indicated. *P. aeruginosa* was included in the microbial parameters.

The very recent study by Aboufotouh Hashish *et al.* (2017) aimed to assess the bacteriological quality and the occurrence and antimicrobial resistance of *P. aeruginosa* in swimming pools in Egypt. A total of 120 samples from ten different swimming (competition and recreational) pools were collected. Approximately two-thirds of the examined pool water samples failed to meet the Egyptian standards of bacteriological indicators. *P. aeruginosa* was found in 21.7% of the samples. Indoor pools showed higher isolation rates than outdoor pools. Isolation of *P. aeruginosa* was positively correlated with pH, heterotrophic plate count, total coliforms and *E. coli*, while it was negatively correlated with chlorine. Three out of the 26 isolates were sensitive to all used antibiotics and nine (34.6%) were multidrug resistant *P. aeruginosa*. The findings indicate that *P. aeruginosa* strains in swimming pools can be multidrug resistant creating a hazard especially for individuals at high risk for infections.

In the study conducted by Fadaei & Amiri (2015) 459 samples from two main indoor swimming pools in a major city of Iran were collected and analyzed from swimming

pools, showers and dressing rooms for chemical, biological and physical quality assessment. Results indicated that the values of total faecal coliforms, *P. aeruginosa*, *Legionella*, *E. coli* and heterotrophic plate count for both swimming pools exceeded the guidelines, except for *Staphylococcus aureus*. The turbidity, free residual chlorine, and hardness of both pools were not compliant with standards.

The study carried out by Amagliani *et al.* (2012) aimed at the development of a new molecular assay for *P. aeruginosa* identification in recreational water. Bacterial cell concentration through membrane filtration, a brief culture enrichment, DNA extraction and its amplification through a real-time PCR assay constituted the four steps of the method applied. Forty-four samples of swimming pool water were used in order to compare the molecular method with the reference method (ISO 16266:2008). With the culture method, positivity rates were calculated equal to 6% in pool water and 74% in inlet water, and with the molecular method they were 23% and 74% respectively. Applying Cohen's Kappa index (0.6831) substantial agreement was indicated between the two approaches. Furthermore, RAPD typing of *P. aeruginosa* isolates showed identical fingerprint profiles, showing their epidemiological correlation. The developed protocol showed very high specificity and a detection limit of ten genomic units. Therefore, it was suggested that this technique, being reliable, quick and effective, could be applied to screen large numbers of environmental samples as part of a self-monitoring plan for swimming pool facilities.

It was made apparent from the majority of the studies that when a proper disinfection process of the swimming pools is conducted with sufficient efficacy, microorganism density is very low without posing health hazards to swimmers. Monitoring the water quality for various microorganisms systematically can prevent serious public health implications. Proper training of the operational personnel and increasing public awareness of the risks of potential swimming-related diseases are considered to be crucial.

CONCLUSIONS

The quality of all types of swimming pools in our study was evaluated as excellent as less than 5% was found not to meet the regulations throughout the 2011–2016 period. In the

small percentage of samples that did not meet the regulations, medians of the indicators were slightly higher than limits. Considering the presence and concentration of *P. aeruginosa*, the number of samples that would not meet the standards would differ significantly. *P. aeruginosa* was detected in a greater number of samples than *E. coli* which indicates that *P. aeruginosa* should be assessed as an important factor. At last, over time the frequency of isolation of *P. aeruginosa* was remarkably reduced perhaps because of staff vigilance (Barna & Kádár 2012; CDC 2013) and better compliance with the maintenance instructions concerning swimming pools.

Existing literature indicates the incidence and persistence of *P. aeruginosa* in various recreational water facilities. Taking into consideration the great impact on public health and the costly estimated health burden of swimming-related illnesses, major efforts should be directed toward improving both public health surveillance systems concerning recreational water facilities and related legislation. According to our results, in northern Greece the assessment of the pools would have been significantly different if the relevant Greek regulation included *P. aeruginosa* as a mandatory one. In this direction, we believe that *P. aeruginosa* should be included in the revised version of national laws and the European Directive as well.

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