

## Microbiological quality of indoor and outdoor swimming pools in Greece: Investigation of the antibiotic resistance of the bacterial isolates

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### Abstract

During 1997–2005, the microbiological quality and susceptibility of bacterial isolates of swimming pool waters were investigated. A total of 462 water samples were collected from three indoor swimming pools (a teaching pool, a competition public pool, a hydrotherapy pool) and two outdoor swimming pools (a hotel semi-public and a residential private pool) in Northwestern Greece. All water samples were analyzed for the presence of bacteria, protozoa and fungi and susceptibility tests were performed for the bacterial isolates. Sixty-seven percent of the examined water samples conformed to the microbiological standards and 32.9% exceeded at least one of the indicated limits. Out of 107 bacterial isolates, 38 (35.5%) resistant strains were detected. Multi-resistant *Pseudomonas alcaligenes*, *Leuconostoc*, and *Staphylococcus aureus* (isolated from the teaching pool), *Staphylococcus wernerii*, *Chryseobacterium indologenes* and *Ochrobactrum anthropi* (isolated from the competition pool), *Pseudomonas aeruginosa*, *P. fluorescens*, *Aeromonas hydrophila*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *S. aureus* (isolated from the hydrotherapy pool) and *A. hydrophila* (isolated from the hotel pool) were detected. The swimming pool with the poorest microbiological quality (THC  $\geq$  500 cfu/ml in 12.1% of the samples, *P. aeruginosa* counts  $\leq$  1500 cfu/100 ml in 6% of the samples) and the highest prevalence of multi-resistant isolates (73.6%) was the hydrotherapy pool. No *Cryptosporidium* or *Giardia* cysts and no Legionella, Mycobacteria and Salmonella were detected, but there were isolations of *Candida albicans*, *Aspergillus* spp., *Mucor* spp., *Alternaria* spp., *Rhizopus* spp., *Trichophyton* spp., and *Penicillium* spp.

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### Introduction

A variety of microorganisms can be found in swimming pools, which may be introduced in the pool

water in a number of ways. In many cases, the risk of illness or infection has been linked to faecal contamination of the water, due to faeces released by bathers or a contaminated source water or, in outdoor pools, may be the result of direct animal contamination (e.g. from birds and rodents) (WHO, 2006; Nichols, 2006). Faecal matter is introduced into the water when a person has an accidental faecal release (through the release of diarrhetic stool into the water) or when residual faecal material on swimmers' bodies is washed into the pool (CDC, 2001a). Non-faecal human shedding (e.g. from vomit, mucus, saliva or skin) in the swimming pool is also a potential source of pathogenic organisms. In addition, infected users can directly contaminate pool waters with pathogens (particularly viruses or fungi), which may lead to skin infections in other patrons, who come in contact with the contaminated water. 'Opportunistic pathogens' (mainly bacteria) can also be shed from users and transmitted via contaminated water. Some bacteria, most notably non-faecally derived bacteria may accumulate in biofilms and present an infection hazard. In addition, certain free-living aquatic bacteria and amoebae can grow in pool waters, in pool components or facilities (including heating, ventilation and air-conditioning systems) or on other wet surfaces within the facility to a point at which some of them may cause a variety of respiratory, dermal or central nervous system infections or diseases (WHO, 2006; Nichols, 2006).

Therefore, swimming pools are often associated with outbreaks or incidents of waterborne infections (Favero, 1984; Kee et al., 1994; CDC, 1998, 2000; Lee et al., 2002; Dziuban et al., 2006). The infectious agents recovered from swimming pool waters include a variety of pathogens embracing bacteria, viruses, protozoa and fungi (Ratnam et al., 1986; Turner et al., 1987; Lenaway et al., 1989; CDC, 1994; Kee et al., 1994; Hildebrand et al., 1996; Papapetropoulou and Vantarakis, 1998; Friedman et al., 1999; Benkel et al., 2000; Ali-Shtayeh et al., 2002; Tate et al., 2003; Somekh et al., 2003; Schets et al., 2004; Braue et al., 2005; WHO, 2006). In addition to infections due to acknowledged pathogens, infections due to opportunistic microorganisms (*M. fortuitum*, *M. chelonae*, *M. marinum*) are reported too, which are able to cause mild-to-severe disease in immunocompetent and immunodepressed subjects (Gbery et al., 1996; Falkinham, 2003). Many of the outbreaks related to swimming pools would have been prevented or reduced if the pool had been well managed.

A swimming pool is a body of water of limited size contained in a holding structure. This water is generally of potable quality and is treated with additional disinfectants (chlorine compounds, ozone). Although modern swimming pools have a re-circulating system so that the water can be filtered and disinfected effectively, relevant research studies, show that neither hi-tech systems nor disinfectants can prevent the colonization

of the pool water with hazardous pathogens (Fiorillo et al., 2001; Tate et al., 2003; CDC, 2004a,b; Lumb et al., 2004; Reali et al., 2004; Barben et al., 2005). In addition, the surviving chlorine tolerant bacteria might also be antibiotic resistant, a fact already documented for bacterial isolates from treated drinking water (Papapetropoulou et al., 1994; Papandreou et al., 2000; Cordoba et al., 2001) and purified sewage effluents (Morozzi et al., 1989; Koenraad et al., 1995; Kummerer, 2000). The antibiotic resistance of bacterial strains isolated from recreational waters, which belong to the natural aquatic environment (lake, river, coastal, natural warm-water springs) is an important issue of public health and there is a number of publications on this topic (Reali and Rosati, 1994; Arvanitidou et al., 1997; Butler and Ferson, 1997; Filali et al., 1997; Okeke and Edelman, 2001; Tejedor Junco et al., 2001). Although elevated microbial resistance to antiseptics and disinfectants has been reported from public swimming pools (Hingst et al., 1995), to our knowledge the antibiotic resistance of strains isolated from the artificial aquatic environment (swimming pools, spa, water parks) has been considered scarcely. The objectives of the present study were to investigate the microbiological quality of indoor and outdoor swimming pools, and the distribution of antibiotic resistant bacteria in the pool water.

## Materials and methods

### The swimming pools

The microbiological quality of the water from five different types of swimming pools (three indoor and two outdoor pools) located in NW Greece was investigated. The indoor swimming pools were heated public pools, operating throughout the year, and included: a children's teaching pool, a competition pool and a hospital hydrotherapy pool, while the outdoor swimming pools were unheated pools, operating only during the summer months (June–August) and included: a hotel semi-public pool and a domestic private swimming pool. All swimming pools were made of concrete covered with glazed tiles, had rectangular shapes and different sizes ranging from 18 m<sup>2</sup> (domestic pool) to 1250 m<sup>2</sup> (competition pool) and were supplied with fresh ground water. Flocculation prior to filtration with sand filters was used in all pools. No system of hazard control of critical points (HACCP) was applied to any of the investigated pools. Nevertheless, the microbiological quality of the water for the competition and the children's teaching pool was checked by means of periodic controls by the Health Authorities, while the rest pools had occasionally chemical (measurement of pH and free chlorine levels) and mechanical maintenance by the pool operators.

The pH was monitored automatically in the public pools (training, competition, hydrotherapy) and was checked manually in the private pools (weekly the hotel pool, twice a month the house pool). According to the Greek standards the suggested pH range for swimming pools is 7.2–7.8 (ideal range of 7.4–7.6). It has to be mentioned that in Greece, it is compulsory to shower before a swim in a public pool. Showering removes traces of sweat, urine, faecal matter, cosmetics and other potential water contaminants and keeps the pool water cleaner. The ventilation of the training and competition pool area was achieved by keeping the surrounding windows open (no air-condition system was installed), while the hydrotherapy pool ventilation was depending on the installed air-condition system. The heating of the indoor pools area was facilitated by radiators (central heating) for the competition and training pool and by the air-condition system for the hydrotherapy pool.

Of the indoor swimming pools, the children's teaching pool was used for training kids aged 3–12 yr, the water temperature was maintained at 30–33 °C and the water pH at 7.2–7.4. This pool was disinfected with chlorine by automated chlorine feeders with stabilized cyanurated chlorine throughout the day, the free chlorine levels were checked every day and according to the records, the maximum free chlorine level was 2.5 mg/l. The filter backwashing frequency was twice a week, the fresh water replacement was over 25% of pool water volume every week and the filtration rate was 20–25 m<sup>3</sup>/m<sup>2</sup>/h. The average number of bathers was 80 per day and 12 at any time.

The competition swimming pool was used for training athletes (aged 13–25 yr) and for the public (exercising adults over 18 yr); the water temperature was maintained at 26–28 °C and the water pH at 7.4–7.8. This pool was disinfected with chlorine by automated chlorine feeders with stabilized cyanurated chlorine throughout the day, the free chlorine levels were checked every day and according to the records, the maximum chlorine level was 3.6 mg/l. The filter backwashing frequency was twice a week, the fresh water replacement was over 25% of pool water volume every week and the filtration rate was 20–25 m<sup>3</sup>/m<sup>2</sup>. The access for the public was restricted to 4 weekdays only and for limited period (5 h per day). For the rest hours of these days and for the rest weekdays this pool, was used for training athletes of the local sports associations and for hosting athletic events. The average number of bathers was 120 per day and 20 at any time.

The hydrotherapy pool was used only by subjects (outpatients and inpatients) following a rehabilitation program (mainly from orthopedic, neurological and rheumatoid disorders). The age range of the majority of pool users was 50 to >60 years old. The average number of bathers was 40 per day and 10 at any time, and the pool was operating for a limited period of time

every day (9 am–13 pm). The pool was installed inside the premises of the University Hospital, the water temperature was retained at 34–36 °C, the water pH at 7.0–7.6. The filter backwashing frequency was once a week and sometimes once every 2 weeks, less than 10% of the pool water volume was replaced with fresh water every week and the filtration rate was 25–30 m<sup>3</sup>/m<sup>2</sup>/h. This pool was disinfected by ozonation according to the following operation process: coagulation–filtration–ozonation–activated carbon filtration–chlorination. Yet, at the onset of the present study the ozone feeder maintenance was poor, no chlorine or UV combination was used and no regular checks or records were kept. The female staff (physiotherapists, trainers), who were immersed into the water everyday instructing the patients, were complaining for urinary tract infections and their complaints initiated investigation of this pool.

Of the two outdoor swimming pools, the hotel pool was used by the hotel guests and visitors; the water temperature was at 24–28 °C and the water pH at 7.4. This pool was disinfected with chlorine by automated chlorine feeders, the free chlorine levels were checked every other day and according to the records, the maximum free chlorine level was 4.6 mg/l. The filter backwashing frequency was once a week, the fresh water replacement was over 10–20% of pool water volume every week and the filtration rate was 20–30 m<sup>3</sup>/m<sup>2</sup>. The average number of bathers was 30 per day and 5 at any time.

The domestic private pool was used by the house owners and family members; the water temperature was at 23–27 °C and the water pH was at 7.5. This pool was disinfected with chlorine by automated chlorine feeder, the free chlorine levels and pH values were checked twice a week and according to the records, the maximum free chlorine level was 4.8 mg/l. The filter backwashing frequency was once every two weeks, the fresh water replacement was over 10% of pool water volume every week and the filtration rate was 20–25 m<sup>3</sup>/m<sup>2</sup>. The average number of bathers was 6 per day and 2 at any time.

### Sampling and microbiological procedures

During 8 years (1997–2005), 462 water samples were collected, when no bathers were in the pools (access was denied during the maximum bather density). The samples were collected twice a month for the first 3 years and from then onwards once a month, with the exception of the two outdoor pools where sampling was limited to three months (summer period) only and the hydrotherapy pool where additional samplings were requested whenever there were health complaints from the bathers or staff (urinary tract infections, vaginitis, rashes). All samples were collected into sterile dark glass

bottles with capacities 250, 500, 1000 and 2000 ml. Sufficient (100 mg/l) sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ , Sodium thiosulfate pentahydrate, 106509, Merck KGaA, Darmstadt, Germany) was added to each bottle for dechlorination. The samples were collected from a depth of 30 cm, at a point about 40 cm away from the pool edge and they were transferred to the laboratory at 4 °C within 1–2 h from collection, using appropriate insulated coolers and they were processed immediately after arrival at the laboratory.

Bacteriological samples were analyzed by the membrane filter technique, using 0.47 mm diameter, 0.45 µm pore size filters (S-Pak sterile membrane Filter, HAWG047S1, Millipore, Billerica, USA) as specified in Standard Methods (APHA, 1998) to determine the following parameters: Total Heterotrophic Plate Counts (THC) per 1 ml at 20 and 36 °C (Plate Count Agar Cat. No. 1.05463, Merck KGaA, 64271, Darmstadt, Germany), total coliforms (TC) per 100 ml at 36 °C (m-ENDO Agar LES, Cat. No. 11.11277, Merck KGaA, 64271, Darmstadt, Germany), faecal coliforms (FC) per 100 ml at 44 °C (m-FC Agar, Cat. No. 1.11278, Merck KGaA, 64271, Darmstadt, Germany), Faecal Streptococci (FS) per 100 ml at 36 °C (D-Coccosel Bile Esculin Agar Selective isolation of enterococci N51 025, BioMerieux, Marcy – l' Etoile, France), *Staphylococcus aureus* (SA) per 100 ml at 36 °C (Chapman Agar. *Staphylococcus* Selective Agar, Cat. No. 1.05469, Merck KGaA, 64271, Darmstadt, Germany), *Pseudomonas aeruginosa* (PS) per 100 ml at 30 °C (Pseudomonas Selective Agar Base. Cetrimide Agar, Cat. No. 1.05284, Merck KGaA, 64271, Darmstadt, Germany).

*Mycobacterium* spp. was determined per 1000 ml, prior to filtration the samples were decontaminated with Cetylpyridinium Chloride (CPC, Serva, Germany), and subsequently they were cultured on Middlebrook 7H10 OADC agar (Difco Laboratories, Ditroit, USA) incubated at 35 °C in 5%  $\text{CO}_2$  atmosphere.

*Salmonella* was determined per 1000 ml, filtration was followed by pre-enrichment in Buffered Peptone Water (CM1049, Oxoid Ltd., Hampshire, England) followed by enrichment in Rappaport-Vassiliadis Broth (CM0669, Oxoid Ltd., Hampshire, England), and culture on Desoxycholate Citrate Agar (XLD) (CM0035, Oxoid Ltd., Hampshire, England), and Salmonella–Shigella Agar (SS) (CM0099, Oxoid Ltd., Hampshire, England).

*Legionella* spp. was determined per 2000 ml, using 0.22 µm filter membranes (S-Pak sterile membrane Filter, 0.22 µm, 47 mm, GSWG047S6, Millipore, Billerica, USA) according to the procedure described by Leoni et al. (2001).

Biochemical identification of bacterial species was performed using API STAPH, API 20E, API NE, API STREP (BioMerieux, S.a. Marcy l' Etoile, France) in addition to stains (Gram and Ziehl–Neelsen), motility,

oxidase and coagulase tests and colonial morphology. Also, *P. aeruginosa* strains were further O serotyped by slide agglutination (International Antigen Typing System, Sanofi Pasteur Diagnostics, Marnes-la-Coquette, France).

Yeasts and dermatophytes were determined per 500 ml, by filtration through 0.45 µm membranes (S-Pak sterile membrane Filter, HAWG047S1, Millipore, Billerica, USA), which were subsequently placed on Sabouraud Cycloheximide Chloramphenicol agar (c.n. 64634, Sanofi Diagnostics Pasteur, Marnes La Coquette, France). Plates were incubated at 37 °C for the first 18–24 h and from then onwards at 25 °C for 4 weeks. Identification of fungi was based on colonial morphology, microscopic examination of the mycelium and for *Candida* species the API CHAU (BioMerieux, S.a. Marcy l' Etoile, France) was used.

For the *Cryptosporidium* and *Giardia* sample collection, special equipment was used including filter housing, 25 cm long polypropylene filters with 1 µm porosity (Super Micro-Wynd Cuno filters), water meter, flow control valve (4–10 l/min), gasoline-powered pump and pipes and isothermal transfer boxes. A volume of 1000 l of water per sampling was filtered ad loc and the filters were transferred to the lab within an hour and were processed after arrival at the laboratory. Treatment of the collected samples was performed according to the method suggested by LeChevallier et al. (1991), as modified by Karanis et al. (1998). For the final identification the immunofluorescence test was performed using a commercially available kit (Merifluor *Cryptosporidium*/*Giardia* 250050, Meridian Diagnostics Inc., Cincinnati, OH, USA)

## Susceptibility tests

Susceptibility tests for Gram-positive cocci and Gram-negative bacteria (except *Pseudomonas*, *Chryseobacterium* and *Ochrobactrum* isolates) were performed using the disk diffusion method (Bauer et al., 1966), while *Pseudomonas* spp., *C. indologenes* and *O. anthropi* isolates were tested using MIC-E-tests (AB Biodisk, Solna, Sweden). The antibiotics used for the susceptibility tests and the disk contents are shown in Table 4. The antibiotic disks (Antibiotic Susceptibility Disks, Oxoid Ltd., England) were dispersed using a self-tamping dispenser onto the surface of Mueller–Hinton agar (Muller Hinton 2 c.n. 51075, BioMerieux S.a., Marcy l' Etoile, France) plates (15 × 150 mm). All plates were incubated overnight in ambient air incubators at  $36 \pm 1$  °C and the results were recorded by measuring the inhibition zone according to the Clinical and Laboratory Standards Institute (CLSI, former NCCLS) recommendations (NCCLS, 2002).

## Results

The microbiological quality of the investigated pools was considered acceptable/unacceptable according to the German standards DIN 19643 (DIN, 1984) and the British Standard PAS39:2003 (BSI, 2003), the relevant Greek Regulation is old and needs updating (GHR, 1973). The microbiological requirements according to the German, British and Greek standards are presented in Table 1.

Out of 462 water samples examined, 310 (67%) conformed to the microbiological standards specified in Table 1, and 152 (32.9%) exceeded at least one of the indicated limits. *Salmonella*, *Mycobacterium*, *Legionella*, *Cryptosporidium* and *Giardia* were not detected in any of the samples analyzed. Total Heterotrophic counts exceeded 500 cfu/ml at 36 °C and 200 cfu/ml at 20 °C, in samples collected from the hydrotherapy pool during the first two years of the study. From then onwards the THCs for the hydrotherapy pool from time to time were exceeding the limit of 100 cfu/ml, but never exceeded the limits of the Greek standards. *Escherichia coli*, TC, FC and *S. faecalis* exceeded 0 cfu/100 ml only in samples collected from the hydrotherapy pool, whereas *S. aureus* exceeded 0 cfu/100 ml in samples from the competition pool, the children's teaching pool and the hydrotherapy pool. The percentages of conformity to the standard limits for the tested microbiological parameters in relation to the pool type are summarized in Table 2.

*P. aeruginosa* was detected throughout the study in 3 out of the 5 pools investigated; notably no *Pseudomonas* species was ever isolated from the competition public pool and the household private pool.

*Candida* yeasts at concentrations 1–2 cfu/500 ml were detected in 15 samples (10.4%) from the hydrotherapy pool only. However, it has to be reported that *C. albicans* and *C. tropicalis* at concentrations exceeding 30 cfu/500 ml were isolated in two extra samples collected upon request of the hospital authorities and these findings coincided with female bather's complaints of having vaginitis-like symptoms after attending hydrotherapy courses. Also in these two samples *Aspergillus* spp., *Mucor* spp., *Alternaria* spp., *Rhizopus* spp., *Penicillium* spp. and *Trichophyton* spp. were detected. Due to this incident the pool was closed down and combined disinfection with chlorine (additional to ozone) was initiated from then onwards and finally the ozone disinfection was replaced by a chlorine only disinfection system. No Dermatophytes were isolated in any samples of the rest pools. Table 3 shows the range of microbial contamination of the investigated pools.

Among a total of 107 bacterial strains isolated from the five monitored swimming pools, 69 isolates were susceptible to all tested antibiotics and 38 isolates (35.5%) exhibited resistance to one or more antibiotics. Single resistance was observed in 3 strains (*S. wernerii* isolates from the competition pool) and multi-resistance in 35 strains (*Pseudomonas alcaligenes*, *Leuconostoc* and *Staphylococcus aureus* isolated from the teaching pool, *Chryseobacterium indologenes* and *Ochrobactrum anthropi* isolated from the competition pool, *P. aeruginosa*, *P. fluorescens*, *A. hydrophila*, *S. aureus*, *Enterobacter cloacae* and *Klebsiella pneumoniae* isolated from the hydrotherapy pool). Each pool type presented its own resistant bacterial flora, particularly the hydrotherapy pool, where all resistant isolates were common

**Table 1.** Microbiological requirements for swimming pool water according to German DIN 19643, BSI PAS 39:2003 and Greek Regulation 443/B/1974

Microbiological requirement	DIN 19643/1984	BSI PAS 39:2003	Greek Standards Regulation 443/B/1974.
Total heterotrophic counts at 20±2 °C	< 100 cfu/ml	–	500 cfu/ml
Total heterotrophic counts at 36±1 °C	< 100 cfu/ml	< 10 cfu/ml	< 200 cfu/ml
<i>Escherichia coli</i> at 36±1 °C	Not detectable/100 ml	Not detectable/100 ml	Not included in the regulation
Total coliforms	Not detectable/100 ml	≤ 10 cfu/100 ml	< 15 cfu/100 ml
Fecal coliforms	–	–	Absence/100 ml
<i>Pseudomonas aeruginosa</i> at 36±1 °C	Not detectable/100 ml	Not detectable/100 ml	0 cfu/100 ml
<i>Legionella pneumonophila</i> at 36±1 °C	Not detectable/100 ml	Not detectable/100 ml	Not included in the regulation
<i>Streptococcus faecalis</i>	–	–	Absence/100 ml
<i>Staphylococcus</i> spp.	–	–	Absence/100 ml
<i>Mycobacterium</i> spp.	–	–	Absence/1 l
Pathogenic fungi	–	–	Absence/100 ml

**Table 2.** Compliance to standards of microbiological parameters for pool water in relation to the pool type

Microbiological parameter	Complying samples/collected samples (% of complying samples)				
	Indoor pools			Outdoor pools	
	Childrens' teaching pool	Hydrotherapy hospital pool	Competition public pool	Hotel semi-public pool	Household private pool
Total heterotrophic counts at 20±2 °C	132/132 (100.0)	90/132 (68.1)	132/132 (100.0)	33/33 (100.0)	33/33 (100.0)
Total heterotrophic counts at 36±1 °C	132/132 (100.0)	100/132 (75.7)	132/132 (100.0)	33/33 (100.0)	33/33 (100.0)
<i>Escherichia coli</i> at 36±1 °C	132/132 (100.0)	97/132 (73.4)	132/132 (100.0)	33/33 (100.0)	33/33 (100.0)
Total coliforms	132/132 (100.0)	93/132 (70.4)	132/132 (100.0)	33/33 (100.0)	33/33 (100.0)
Fecal coliforms	132/132 (100.0)	89/132 (67.4)	132/132 (100.0)	33/33 (100.0)	33/33 (100.0)
<i>P. aeruginosa</i> at 36±1 °C	126/132 (95.4)	84/132 (63.6)	132/132 (100.0)	31/33 (93.9)	33/33 (100.0)
<i>L. pneumonophila</i> at 36±1 °C	132/132 (100.0)	132/132 (100.0)	132/132 (100.0)	33/33 (100.0)	33/33 (100.0)
<i>S. faecalis</i>	132/132 (100.0)	102/132 (77.2)	132/132 (100.0)	33/33 (100.0)	33/33 (100.0)
<i>S. aureus</i>	127/132 (96.2)	95/132 (71.9)	129/132 (97.7)	33/33 (100.0)	33/33 (100.0)
<i>Mycobacterium</i> spp.	132/132 (100.0)	132/132 (100.0)	132/132 (100.0)	33/33 (100.0)	33/33 (100.0)
Pathogenic fungi	132/132 (100.0)	110/132 (83.3)	132/132 (100.0)	33/33 (100.0)	33/33 (100.0)

**Table 3.** Range and mean values of microbial counts from pool water samples

Microbiological parameters	Range values (mean values)				
	Indoor pools			Outdoor pools	
	Children teaching pool	Hydrotherapy pool	Competition public pool	Hotel semi-public pool	Household private pool
Total heterotrophic counts at 20±2 °C (cfu/ml)	0–10 (0.2)	0–394 (20.4)	0–5 (0.5)	0–4 (0.02)	0–3 (0.01)
Total heterotrophic counts at 36±1 °C (cfu/ml)	0–12 (0.5)	0–504 (26.8)	0–4 (0.2)	0–2 (0.06)	0–2 (0.01)
<i>Escherichia coli</i> at 36±1 °C (cfu/100 ml)	0	0–55 (4.2)	0	0	0
Total coliforms (cfu/100 ml)	0	0–75 (3.0)	0	0	0
Fecal coliforms (cfu/100 ml)	0	0–46 (5.1)	0	0	0
<i>Pseudomonas</i> spp. (cfu/100 ml)	0–17 (0.2)	0–1500 (19.8)	0	0–80 (0.01)	0
<i>Streptococcus faecalis</i> (cfu/100 ml)	0	0–24 (2.4)	0	0	0
<i>Staphylococcus</i> spp. (cfu/100 ml)	0–80 (0.4)	0–100 (4.9)	0–4 (0.4)	0	0
<i>C. albicans</i> (cfu/500 ml)	0	0–40 (3.5)	0	0	0
Pathogenic fungi (cfu/500 ml)	0	0–10 (1.6)	0	0	0

pathogens involved in hospital infections and inhabiting nosocomial environments. The detailed results of the susceptibility tests are presented in Table 4.

The pool with the poorest microbiological quality and the highest incidence of multi-resistant bacterial isolates was the hydrotherapy pool (Tables 2–5). Out of the 132 total water samples collected from the hydrotherapy pool, 16 (12.1%) yielded THC over 500 cfu/ml and eight (6%) yielded *P. aeruginosa* counts 1500 cfu/100 ml. Also, 28 (73.6%) out of the 38 resistant strains were isolated

from the hydrotherapy pool water. According to the Clinical Microbiology and the Urology Department records, uro-genital and ear infections (otitis externa) were more common in bathers using the hydrotherapy pool, than the bathers using other pools. However, during the period of this study no evident differences or increased prevalence of related infections was reported between the bathers and non-bathers, or amongst the various bathing groups, particularly the hydrotherapy, children's teaching and competition pool users.

**Table 4.** Antibiotic susceptibility of the 38 resistant bacterial strains isolated from different type swimming pools

Antibiotic	Disk content	Children teaching pool			Competition pool			Hydrotherapy pool					Hotel pool	
		<i>P. alkaligenes</i> n = 2	<i>Leuconostoc</i> n = 1	<i>S. aureus</i> n = 1	<i>C. indologenes</i> n = 1	<i>O. anthropi</i> n = 1	<i>S. warnerii</i> n = 3	<i>P. aeruginosa</i> n = 7	<i>P. fluorescens</i> n = 2	<i>A. hydrophila</i> n = 3	<i>E. cloacae</i> n = 5	<i>K. pneumoniae</i> n = 3	<i>S. aureus</i> n = 8	<i>A. hydrophila</i> n = 1
Ticarcillin	75 µg	I	–	–	S	I	–	I	R	–	R	R	–	
Ticarcillin Clavulanic acid	75/10 µg	I	R	S	S	I	S	I	R	–	R	R	S	
Piper/Tazobactam	100/10 µg	S	R	S	S	I	S	S	I	S	S	R	S	
Imipenem	10 µg	S	R	S	S	R	S	R	I	S	S	S	S	
Aztreonam	30 µg	R	–	–	R	R	–	R	R	R	R	R	–	
Ceftazidime	30 µg	I	–	–	S	R	–	R	S	S	R	R	–	
Amikacin	30 µg	S	–	–	S	R	–	S	S	S	R	S	–	
Gentamicin	10 µg	S	S	S	S	S	S	R	S	R	S	S	S	
Tobramycin	10 µg	S	–	–	S	S	–	R	R	R	S	S	–	
Colistin	10 µg	R	–	–	R	R	–	S	S	S	–	–	–	
Penicillin	10 U	–	R	R	–	–	R	–	–	–	–	–	R	
Erythromycin	15 µg	–	R	S	–	–	S	–	–	–	–	–	I	
Clindamycin	2 µg	–	R	S	–	–	S	–	–	–	–	–	S	
Quinolones CIP/PEF/OFL	5/5/5 µg	I	S	S	S	S	S	S	S	S	S	S	S	
Tetracyclines	30 µg	–	S	R	–	–	S	–	–	S	–	–	R	
Cotrimoxazole	1.25/23.75 µg	R	S	S	R	R	S	R	R	–	R	R	S	
Rifampicin	5 µg	–	R	S	–	–	S	–	–	–	–	–	S	
Vancomycin <sup>a</sup>	30 µg	–	R	S	–	–	S	–	–	–	–	–	S	
Teicoplanin <sup>b</sup>	30 µg	–	R	S	–	–	S	–	–	–	–	–	S	
Oxacillin	5 µg	–	R	S	–	–	S	–	–	–	–	–	S	
Nitrofurantoin	300 µg	–	R	S	–	–	S	R	R	–	R	R	S	

R = resistant, S = susceptible, I = intermediate resistance.

<sup>a</sup>MIC<sub>VA</sub> = R > 4 µg/ml.<sup>b</sup>MIC<sub>TEIC</sub> = R ≥ 32 µg/ml.

**Table 5.** Relation of physical and chemical parameters to total heterotrophic counts and *Pseudomonas* counts per pool type

Pool type	Water temperature range (°C)	pH range	Maximum free chlorine level (mg/l)	Maximum combined chlorine level (mg/l)	Redox potential range (mV)	Pool water volume (m <sup>3</sup> )	Bathers per day	Bathers at a time	Water volume per bather (m <sup>3</sup> )	Maximum THC at 20 °C (cfu/ml)	Maximum THC at 36 °C (cfu/ml)	Maximum <i>Pseudomonas</i> spp. counts (cfu/100 ml)
<i>Indoor—public</i>												
Hydrotherapy	34–36	7.0–7.6	2.1	0.83	293–403	36	40	10	3.6	394	504	1500
Teaching	30–33	7.2–7.4	2.5	0.00	473–547	80	80	12	6.6	10	12	17
Competition	26–28	7.4–7.8	3.6	0.00	514–533	312.5	120	20	156.25	5	4	0
<i>Outdoor—private</i>												
Hotel	24–28	7.3–7.4	4.6	0.00	492–532	80	30	5	16	4	2	80
House	23–27	7.4–7.5	4.8	0.00	Not checked	36	6	2	18	3	2	0

## Discussion

Swimming pools have been increasingly popular and possible maintenance failures might cause public health problems. In Greece, Rigas et al. (1998) surveyed 11 outdoor and indoor pools in Athens, finding 45–91% microbiological compliance to the DIN 19643, and predominance of *S. aureus* and *P. aeruginosa*. In our study the compliance to the standards ranged from 63.6% to 100%, but the same pathogens were prevailing. Hadjichristodoulou et al. (2006) during the pre-Olympic and the Olympic year of the Athens 2004 Olympic Games, carried out 374 swimming pool inspections according to the WHO standards, reporting as unsatisfactory 16% of the water samples collected in 2003, 10.1% of the samples collected in January–May 2004, 5.2% of the samples collected in May–July 2004 and none unsatisfactory samples during the Olympic Games, in August 2004. Their results underpin the importance of continuous monitoring and good maintenance of the swimming pools.

Surveys reported from different countries have yielded variable results. In a study concerning 48 swimming pools in the West Bank of Palestine all samples were unacceptable according to the WHO standards, all pools were contaminated by at least one microbial indicator, 37% were contaminated with two or more indicators and only 34.1% had acceptable water (Al-Khatib and Salah, 2003). In our study four out of the five investigated pools were contaminated with at least one microbial indicator and two out of the five (40%) were contaminated with two or more indicators (Table 3). From 12 public swimming pools examined by Leoni et al. (1999) in Bologna, Italy, 34.2% did not conform to the recommended microbiological limits, 21.1% exceeded 200 cfu/ml for THC at 36 °C, 13.2% contained faecal indicators, 10.5% had Faecal Streptococci concentrations between 1 and 10 cfu/l, 2.6% contained *E. coli*, 10.5% and 7.9% were contaminated with *P. aeruginosa* and *A. hydrophila*, respectively, and *Candida* yeasts were isolated from 13.2% of the samples. In Iran, *P. aeruginosa* grew in 63.6% of the water samples from 11 public swimming pools, while 18.2% of the samples exhibited high rates of THC, TC and FC (Hajjartabar, 2004). In our study 32.9% of the samples did not conform to the recommended microbiological standards (Table 1), 12.1% and 0.8% were contaminated with *P. aeruginosa* and *A. hydrophila*, respectively, and *Candida* spp. were isolated from 4.7% of the water samples.

In South Australia, Esterman et al. (1984) surveyed 100 public and private swimming pools finding 18% of the pools with at least one unacceptable bacteriological result, but no significant difference between public and private pools was observed. In South America, Martins et al. (1995) examined 1345 water samples from 60

swimming pools, finding a frequency of positive results ranging from 2.0% (*P. aeruginosa* and *C. albicans*) up to 70.4% (THC) and positive relation among the levels of microorganisms, the bather load and the water temperature. We also observed that the hydrotherapy pool, which gave the higher microbial counts, had the greater bather load per water volume and the higher water temperature, while the private pools had better microbiological quality, smaller numbers of bathers per water volume and lower water temperatures (Table 5). In Ireland, *P. aeruginosa* was isolated from 30.8% of the hydrotherapy pools (<1000 cfu/ml), 72.5% of the Jacuzzis/spas (>1000 cfu/ml) and 38.2% of the rest swimming pools; the authors report that almost twice as many samples were positive in private pools compared to publicly operated facilities (Moore et al., 2002). In our study the highest frequency of acceptable bacteriological results was observed in the private and semi-private pools (Table 5) and *P. aeruginosa* (1500 cfu/ml) was isolated from the hydrotherapy pool only. *P. aeruginosa* is frequently present in small numbers in and around swimming pools and has been implicated in folliculitis outbreaks associated with swimming (Tate et al., 2003; Fiorillo et al., 2001; Schlech et al., 1986; Ratnam et al., 1986; Gustafson et al., 1983). However, there are no data available correlating the viable counts of *Pseudomonas* in the water with the risk of acquiring the infection.

In Spain, microbiological analysis of samples from sea and fresh water (chlorinated) pools, revealed contamination of the former with *S. aureus* and of the latter with *Mycobacterium* species, confirming the *Mycobacterium* resistance to chlorine (Martin Delgado et al., 1992). In Brazil, the microbiological examination of six chlorinated swimming pools resulted in isolation of 24 strains of *Mycobacterium* (non-*M. tuberculosis*), one strain EPEC 0127, one strain *Cryptococcus* and six strains *C. albicans* (Falcao et al., 1993). In Italy, in water samples from 12 indoor swimming pools, two were found positive for *Legionella* spp. (*L. micdadei* and *L. bozemanii*) (Leoni et al., 2001). During another monitoring program of 12 public pools in Italy, the positive rate for *Mycobacteria* was 59.4% (Reali et al., 2004). In our study no *Mycobacteria*, *Legionella*, *Salmonella* or *Shigella* species were isolated. However, some uncommon bacterial species (*S. warnerii*, *C. indologenes* and *O. anthropi*) were isolated from the competition pool, where users are healthy subjects. To our knowledge this is the first time *C. indologenes* and *O. anthropi* have been isolated from swimming pool water. *C. indologenes* is a frequent human isolate of minor clinical importance, but it has been documented to cause bacteraemia in hospitalized patients with severe underlying disease and it has been related to nosocomial infections linked to the use of indwelling devices (Schreckenberger et al., 2003). *O. anthropi* has been

previously recovered from environmental samples and tap water (Deliere et al., 2000) and is considered an emerging pathogen in immunosuppressed subjects, particularly in patients with indwelling catheters and central nervous catheter-related sepsis (Stiakaki et al., 2002).

In our study uncommon fungi (*Aspergillus* spp., *Mucor* spp., *Alternaria* spp., *Rhizopus* spp., *Penicillium* spp. and *Trichophyton* spp.) were isolated from two water samples from the hydrotherapy pool. These findings may be attributed to the moist environment of the hydrotherapy pool due to water evaporation, because of the high water temperature (34–36 °C), the poor ventilation (no open windows) and the air-condition system operating at >25 °C. In a study conducted in Nablus (Palestine) involving six swimming pools, 22 dermatophytes and other keratinophilic fungi were isolated, including *Trichophyton* spp., *Chrysosporium* spp., *Acremonium* spp., *Cladosporium* spp., *Aspergillus flavus*, *Alternaria* spp., *Fusarium* spp., and *Penicillium* spp. (Ali-Shtayeh et al., 2002). Similar fungal species (*C. albicans*, *Penicillium* spp., *Rhizopus* spp., *Aspergillus versicolor*, *Aspergillus niger*, *Fusarium* spp., *Trichophyton mentagrophytes*, *Mucor* spp., and *Absidia* spp.) have been reported from the water of swimming pools in a recent survey in Nigeria (Itah and Ekpombok, 2004). Although *Cryptosporidium* and *Giardia* are common findings in swimming pools often causing outbreaks (Joce et al., 1991; Bell et al., 1993; CDC, 1994; Lim et al., 2004; Schets et al., 2004) no cysts were detected from any sample during this survey. During a previous study concerning *Cryptosporidium* and *Giardia* infestation in drinking and recreational waters in NW Greece, low numbers (0.3/100 ml) of *Cryptosporidium* cysts were detected from pool water. (Karanis et al., 2002).

A considerable amount of literature is related to outbreaks due to use of contaminated swimming pools, spa and recreational waters. (Ratnam et al., 1986; Schlech et al., 1986; Turner et al., 1987; Lenaway et al., 1989; Brewster et al., 1994; CDC, 1994, 1998, 2000, 2004a,b; Kee et al., 1994; Hildebrand et al., 1996; Papapetropoulou and Vantarakis, 1998; Friedman et al., 1999; Benkel et al., 2000; Fiorillo et al., 2001; Tate et al., 2003; Barben et al., 2005). However, information on the antibiotic susceptibility of the bacterial isolates recovered during such outbreaks either from the water or the diseased bathers is scarce; also, relevant information for strains isolated during surveillance or monitoring studies is scarce. Enterococci strains resistant to penicillin (13.7%), tetracycline (13.7%), and piperacillin (4.5%) were isolated from environmental samples – including swimming pools – in Italy (Pinto et al., 1999) and resistant to  $\beta$ -lactams, thermophilic Gram-positive bacilli, have been reported from traditional baths in Morocco (Filali et al., 1997).

*Mycobacterium chelonae* strains resistant to imipenem, ciprofloxacin and norfloxacin have been isolated from environmental sources including swimming pools (Hernandez Garcia et al., 1995). Microbial resistance to biocides (antiseptics and disinfectants) of isolates from various aquatic biotopes including swimming pools has been reported in the region of Heidelberg, Germany (Hingst et al., 1995). In the present study multi-resistant strains of Gram-positive and Gram-negative bacteria were isolated (Table 4) from the pool waters. Among resistant strains, *S. aureus* was the most frequent isolate (23.6%), followed by *P. aeruginosa* (18.4%), *E. cloacae* (13.1%), *A. hydrophila* (10.5%), *S. warnerii* (7.8%), *K. pneumoniae* (7.8%), *P. alcaligenes* (5.2%), *P. fluorescens* (5.2%), *Leuconostoc* (2.6%), *C. indologenes* (2.6%), and *O. anthropi* (2.6%). *Chryseobacterium* species are supposed to have inherent resistance to many antimicrobial agents commonly used to treat infections caused by Gram-negative bacteria. *O. anthropi* is reported to be resistant to many antimicrobial agents including  $\beta$ -lactams and ceftazidime (Holmes et al., 1988).

The *P. aeruginosa* strains isolated from the hydrotherapy pool were serotypes O:6 and O:9; identical phenotypic resistance was observed between the *P. aeruginosa* isolates from the hydrotherapy pool and strains isolated from hospitalized patients. The isolation of important human pathogens, particularly multi-resistant *P. aeruginosa*, *K. pneumoniae*, *E. cloacae* strains from the hydrotherapy pool, indicates that they were inhabitants of the nosocomial environment. Whenever excessive counts were recorded in the hydrotherapy pool, additional samples from the inlet water were collected, but all microbiological indicators were at zero level, indicating that water was contaminated after entering the pool, either by the bathers and/or the hospital environment. The hydrotherapy pool generated the less satisfactory counts and the greater number of multi-resistant strains, findings which endorse the view that public warm-water therapeutic facilities provide an ideal setting for microbial growth and transmission amongst patients, nosocomial environment and staff, particularly when maintenance is poor as such is the case in this study. There is a number of publications reporting outbreaks or isolations of pathogens from various therapeutic warm-water facilities (Tredget et al., 1992; Richard et al., 1994; Embil et al., 2001; Moore et al., 2002; Chapuis et al., 2004; Angenent et al., 2005; Barben et al., 2005). Additionally to water temperature, other factors affecting the water quality of hydrotherapy pools include bather load per volume of water, the ability of microorganisms to withstand disinfectants and multiply rapidly in water and maintenance practices. All identified species are known to survive in the water and to tolerate well antimicrobial agents. Whether such resistant strains can contaminate bathers causing an

infection, which will be difficult to treat, raises concerns and needs further investigation. Certainly a waterborne infection depends on the total bacterial counts and the immune status of the subjects, the immunosuppressed or immunodeficient subjects been the most vulnerable. The results of the present study demonstrate the variability of the pool water quality and the need for continuous quality monitoring.

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