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ENVIRONMENTAL HEALTH | SHORT COMMUNICATION

Pseudomonas aeruginosa in swimming pools

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Abstract: Swimming pools, can pose a public health risk to users due to their bacterial contamination especially if they were drug resistant. This study aimed to assess the bacteriological quality and the occurrence and antimicrobial resistance of *Pseudomonas aeruginosa* in swimming pools. Approximately two thirds (66.7%) of the examined pool water samples in this study failed to meet the Egyptian standards of bacteriological indicators *P. aeruginosa* was found in 26 (21.7%) samples. Indoor pools showed higher isolation rates than outdoor pools (33.3 versus 16.7%, respectively). Isolation of *P. aeruginosa* was positively correlated with pH, HPC, TC, and *Escherichia coli*, while it was negatively correlated with chlorine. Nine (34.6%) *P. aeruginosa* isolates were multidrug resistant. The findings of this study indicate that *P. aeruginosa* strains in swimming pools can be multidrug resistant which creates a hazard especially for individuals at high risk for infections.

Subjects: Environmental Studies & Management; Sports and Leisure; Medicine, Dentistry, Nursing & Allied Health

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1. Introduction

Swimming pool water contamination with micro-organism can result from the environment as well as pool users (*Pool operators' handbook,* 2000; World Health Organization, 2003). Swimming pools remain a transmission vehicle for infectious diseases by several micro-organisms causing acute gastrointestinal, cutaneous, and respiratory illnesses (Hlavsa et al., 2011).

Pseudomonas aeruginosa is an aquaphilic bacterium that is isolated from water of all origins. It is also present in approximately 10% of normal stools and on the skin of some normal individuals (Mandell, Bennett, & Dolin, 2010). *P. aeruginosa* is passed into the pool water from colonized health humans, through broken pool pluming, or from dust and fecal matter getting into the pool.

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Amira Ezzat Khamis Amine graduated from Faculty of Medicine, Alexandria University. She obtained her PhD from the High Institute of Public Health, University of Alexandria, Egypt. She worked as a post doctoral researcher in Uppsala University, Sweden from 2009–2011. She is currently working as Associate Professor of Microbiology at the High Institute of Public Health, University of Alexandria, Egypt. Her major field of study is public health microbiology. She is specifically interested in the genotyping of epidemic and antimicrobial resistant bacterial strains. She has a 14-year experience in teaching and research.

PUBLIC INTEREST STATEMENT

Antimicrobial resistance is the ability of bacterial strains to resist and grow in the presence of antimicrobials. Resistance levels are on the increase globally at alarming rates. The public can be exposed to these resistant bacteria during using public facilities like swimming pools. Therefore, these bacteria should be detected and eliminated. This paper studies the presence of a particularly worrisome bacteria in swimming pools. *Pseudomonas aeruginosa* is capable of causing serious diseases and has the ability to become resistant to multiple antibiotics used in treatment.

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Unfortunately, a warm, moist environment, particularly is an ideal environment for the growth of *P. aeruginosa*. It can grow in pool water and on pool surfaces and filters. *P. aeruginosa* can attach itself to surfaces by forming a biofilm layer that makes it resistant to disinfecting chemicals including chlorine (Centers for Disease Control and Prevention (CDC), 2013; Mena & Gerba, 2009; World Health Organization, 2003).

Swimming pool water that harbors *P. aeruginosa* is a pathway for bacterial transmission and carries the risk of opportunistic infections occurring among pool users. Diseases occurring frequently among swimmers include dermatitis and folliculitis, hot-foot syndrome, otitis externa, and occular infections. Cases of pneumonia and urinary tract infections have also been reported following the use of contaminated pools (Kujundzic et al., 2012; Lutz & Lee, 2011; Yoder et al., 2004; Yu, Cheng, Wang, Dunne, & Bayliss, 2007).

P. aeruginosa is infamous for developing multidrug resistance. *P. aeruginosa* has intrinsic resistance mechanisms (low outer membrane permeability, as well as an extensive efflux pump system) (Mesaros et al., 2007; Schwartz et al., 2015). Acquired antimicrobial resistance can also emerge during therapy (Hirsch & Tam, 2010; Lister, Wolter, & Hanson, 2009). This study aimed to assess the occurrence and the antimicrobial resistance of *P. aeruginosa* in swimming facilities. Also, it aimed to assess their correlation with other indicators.

2. Methods and materials

The study was carried out during a two months period, from the beginning of June 2014 to the end of July 2014. A total of 120 water samples from 10 different swimming pools (Three indoor pools designated as A, B, and C and seven outdoor pools designated as D, E, F, G, H, I, and J). Five of these pools were large public pools used for training and competition; the rest were also used as public recreation pools. Twelve samples were collected from each swimming pool on weekly basis. Approval for sampling was obtained from swimming pool managers.

2.1. Sampling

Water samples were aseptically collected in 500 ml sterile bottles using standard methods (American Public Health Association, American Water Works Association, & Water Environment Federation, 2005). Physical and chemical parameters (temperature, residual chlorine, and pH) were done at swimming pool side during sample collection. All samples were transferred to the laboratory in an ice box within 1–2 h after collection

2.2. Bacteriological examination

All the collected swimming pool water samples were examined as follows: (American Public Health Association et al., 2005; Chigbu & Parveen, 2013)

(1) Enumeration of HPC using standard pour plate method using plate count agar (HiMedia India-Mumbai). It was done using the standard pour plate method. Water samples were subjected to serial 10-fold dilutions using sterile peptone water. Duplicate Petri dishes for each dilution were prepared. The plates were then and incubated at 37°C for 48 h. The plates showing 30–300 colonies were counted using Quebec colony counter and expressed as CFU/ml.

(2) Enumeration of TC, *Escherichia coli*, and *P. aeruginosa* by membrane filtration technique using chromogenic agar media using sterile cellulose acetate membrane filters (0.45 um pore size, 47 mm diameter). Membrane filters were placed directly onto Chromagar ECC: EF320 (CHROMagar, France-Paris) and incubated at 37°C for 24 h. Chromagar Pseudomonas (CHROMagar, France-Paris) plates were incubated at 30°C for 24 h. Blue *E. coli* colonies on Chromagar ECC were clearly differentiated from mauve total coliform colonies. Typical *E. coli* colonies were submitted to indole test at 44.5°C and citrate test for verification. Indole test positive results with no growth on citrate verified the colony as *E. coli*. Both were counted using Quebec counter and recorded as CFU/ml. Typical *Pseudomonas* colonies on chromagar

medium are blue green. All blue green colonies were subjected to Gram stain, oxidase test, and streaked over the surface of acetamide agar slant. Blue green colonies that microscopically appeared as Gram negative rods and were positive for oxidase, and produced alkaline reaction indicated by pink, red or purple color in acetamide agar slants were counted as *P. aeruginosa*.

(3) Antimicrobial susceptibility testing for *P. aeruginosa* isolates.

All *P. aeruginosa* isolates were subjected to antimicrobial susceptibility testing using the disc diffusion method described by Bauer, Kirby, Sherris, and Turck (1966). The inhibition zones were measured and susceptibility was recorded as susceptible (S), intermediate (I), and resistant (R) according to standard tables (Clinical & Laboratory Standards Institute, 2011). The following antibiotics were tested: piperacillin 100 µg, piperacillin-tazobactam 100/10 µg, ceftazidim 30 µg, cefepime 30 µg, aztreonam 30 µg, imipenem10 µg, colistin sulfate 10 µg, polymixin B 300 units, gentamycin 10 µg, and amikacin 30 µg.

Data were analyzed using IBM SPSS software package version 20.0.

3. Results

The present study was carried out on 120 swimming pool water samples collected from 36 indoor and 84 outdoor pools. According to the Egyptian standards, (*Egyptian fresh water swimming pool standards. Decree No. 418 for year 1995*, 1995) out of the 120 examined swimming pool water samples; 40 (33.3%) conformed to the bacteriological standards distributed as 9 (25.0%) in indoor pools and 31 (36.9%) in the outdoor pools. The corresponding figures for chemical standards were 15 (12.5%), 5 (13.9%), and 10 (11.9%), respectively. The compliance of both standards decreased to 6.7% (8). The percentages of total acceptable samples for indoor and outdoor pools were 5.6 and 7.1%.

Table 1 illustrates the compliance of each individual standard with Egyptian guidelines. *E. coli* was the most complying bacterial indicator followed by *P. aeruginosa*, TC then HPC. Compliance to chemical parameters was low where less than 50% of samples met the guidelines.

Most of the examined water samples (78.3%) were acceptable for *P. aeruginosa* (Table 2). The outdoor swimming pools showed higher acceptability percentage than the indoor ones 83.3 and 66.7%, respectively.

Residual chlorine was negatively correlated and pH was positively correlated with *P. aeruginosa*. $r = -0.319$, $p = <0.001$ for chlorine, and $r = 0.240$, $p = <0.008$ for pH (r_s: Spearman coefficient). Both correlations were statistically significant. Our results also showed that all water samples (indoor and outdoor) with residual chlorine level of <1 and >1.5, and pH level >7.8 did not comply with bacteriological standards (100%).

Table 1. Compliance of the 120 examined swimming pool water samples with Egyptian standards (*Egyptian fresh water swimming pool standards. Decree No. 418 for year 1995*, 1995)

Examined parameters	Complying samples		Non-complying samples		Egyptian standards
	No.	%	No.	%	
pH (120)	38	31.7	82	68.3	7.2–7.8
Chlorine (120)	51	42.5	69	57.5	1–1.5 ppm
HPC at 37°C (120)	50	41.0	70	58.3	<100 CFU/ml
TC (120)	65	54.2	55	45.8	Not detectable/100 ml
E. coli (120)	113	94.2	7	5.8	Not detectable/100 ml
P. aeruginosa (120)	94	78.3	26	21.7	Not detectable/100 ml

Table 2. Distribution of *P. aeruginosa* in the 120 examined swimming pool water samples

P. aeruginosa	Acceptable		Unacceptable	
	No.	%	No.	%
Swimming pool water samples (12 each)				
Indoor pools (36)				
A	8	66.7	4	33.3
B	11	91.7	1	8.3
C	5	41.7	7	58.3
Subtotal	24	66.7	12	33.3
Outdoor pools (84)				
D	10	83.3	2	16.7
E	12	100	0	0.0
F	10	83.3	2	16.7
G	12	100	0	0.0
H	6	50.0	6	50.0
I	10	83.3	2	16.7
J	10	83.3	2	16.7
Subtotal	70	83.3	14	16.7
Total	94	78.3	26	21.7

Table 3. Distribution of the examined swimming pool water samples according to their acceptability (in relation to *P. aeruginosa*)

P. aeruginosa	Acceptable		Unacceptable	
	No.	%	No.	%
Swimming pool samples				
Indoor pools (36)	24	66.7	12	33.3
Outdoor pools (84)	70	83.3	14	16.7
Total	94	78.3	26	21.7

As shown in Table 3, *P. aeruginosa* was found in 26 (21.7%) samples. Indoor pools showed higher isolation rates than outdoor pools. Isolation of *P. aeruginosa* was positively correlated with pH, HPC, TC, and *E. coli*, while it was negatively correlated with chlorine (Table 4).

Figure 1 shows that aztreonam showed the highest resistance rate (84.8%), while piperacillin-tazobactam, cefepime, imipenem, and gentamycin showed the least resistance rate (7.7% each). Only three *P. aeruginosa* isolates (11.6%) were sensitive to all the examined antimicrobial agents, while there were 9 (34.6%) multidrug resistant samples.

Table 4. Correlation between *P. aeruginosa* with the other parameters in the 120 examined swimming pool water samples

Parameters	*P. aeruginosa*	
	r	*p*
Chlorine	−0.319*	<0.001
pH	0.240*	0.008
HPC	0.474*	<0.001
TC	0.629*	<0.001
E. coli	0.232*	0.011

*Statistically significant at $p \leq 0.05$.

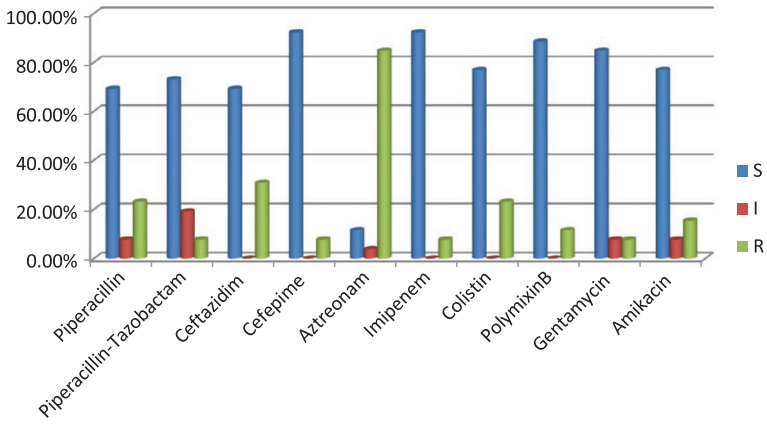

Figure 1. Antibiotic sensitivity testing of the 26 *P. aeruginosa* isolates from the 120 studied swimming pools water samples.

Notes: S: susceptible, I: Intermediate, R: Resistant.

4. Discussion

Quality of swimming pools can be measured by using fecal coliforms or other micro-organisms derived from the skin, mouth, and upper respiratory tract of bathers and in this study, we used both indicators.

TC along with other indicators were studied in this study to test water quality (American Public Health Association et al., 2005; Figueras & Borrego, 2010). In the present study, it was found that *E. coli* was the most complying bacterial indicator 113 (94.2%) followed by *P. aeruginosa* 94 (78.3%), TC 65 (54.2%), and HPC 50 (41.0%).

The percentage of the swimming pool acceptability regarding all examined parameters collectively was very low (6.7%). Approximately two thirds (66.7%) of the examined pool water samples in this study failed to meet the Egyptian standards of bacteriological indicators. In Alexandria, 2012, samples were more complying (56.7%) according to the same standards (Abd El-Salam, 2012). Lower figures were reported by Papadopoulou et al. who reported that 32.9% of swimming pools in Greece did not conform to their guidelines (Papadopoulou et al., 2008). In other developing countries similar results were reported. This was attributed to the lack of public awareness, and defects in the monitoring systems of water quality (Al-Khatib & Salah, 2003; Itah & Ekpombok, 2004).

Chlorine, in order to be effective, must be kept at 1–1.5 ppm as stated by the Egyptian standard (*Egyptian fresh water swimming pool standards. Decree No. 418 for year 1995*, 1995). Nearly half of the samples (57.5%), in this study, did not comply with the required chlorine standards. However, more pools had higher residual chlorine levels (more than 1.5 ppm) than lower (less than 1 ppm) standard chlorine levels (30.8 versus 26.7%). On the contrary, several studies showed all samples to have a chlorine level lower than acceptable (Hilles, Sarsour, Ramlawi, & Abed, 2014; Osei-Adjei, Sarpong, Laryea, & Tagoe, 2014; Rabi, Khader, Alkafajei, & Abu Aqoulah, 2008).

Water pH should be maintained at an optimum range to ensure the maximum activity of chlorine (*Egyptian fresh water swimming pool standards. Decree No. 418 for year 1995*, 1995; World Health Organization, 2003). pH of 47.50% of the studied samples was lower than 7.2. This can be an explanation to the higher detected chlorine levels. Around only 50% of swimming pools in the present study were equipped with pH testing equipment, and according to swimming pools operators during the study, pH was intentionally kept on acidic values for fear of chlorine inactivation. The problem with higher chlorine levels is that it can cause irritation of the skin and upper respiratory tract. Swimmers are also exposed to disinfection by-products such as THMs (World Health Organization, 2003). Also, lower pH on the other hand will lead to corrosion and erosion of different swimming pool's parts and affects the swimmer causing itching and dryness of the skin and eyes (Missouri Department of Health and Senior Services, n.d.).

Literature about outbreaks of *P. aeruginosa* in swimming pools is extensive but unfortunately not in Egypt (Barben, Hafen, & Schmid, 2005; Mena & Gerba, 2009; Tate, Mawer, & Newton, 2003). In this study, *P. aeruginosa* was isolated from 26 samples out of the 120 swimming pool water samples (21.7%). The percentage was slightly higher in indoor than in outdoor swimming pools (16.7 and 14.3%). There was a great variability within pools. Two pools had *P. aeruginosa* in more than 50% of their samples. This was attributed in one these pools to the poor maintenance as it had a non-functioning filtration system and pool operators resorted to fill and draw. In contrast, two outdoor pools were totally free from *P. aeruginosa*.

Similar high incidence was reported in several other countries (Hilles et al., 2014). In Italy, Guida high percentage was attributed to the presence of biofilm in the filtration systems, from which bacteria can be released in the water (Guida, Galle, Mattei, Anastasi, & Liguori, 2009). Lower Figures of *P. aeruginosa* were found by Papadopoulou et al. (2008) and Leoni, Legnani, Guberti, and Masotti (1999), where 12.1 and 10.5% of the samples in Greece and Italy, respectively, were contaminated with *P. aeruginosa*.

There was a positive correlation of *P. aeruginosa* with other parameters (pH, HPC, TC, and *E. coli*) and a negative correlation with residual chlorine. All these correlations were statistically significant. A positive correlation was also observed by Martins et al. (1995) among all examined microbial parameters in South America swimming pools.

We found that only 3 (11.6%) samples out of the 26 isolates were sensitive to all used antibiotics and that 9 samples (34.6%) were multidrug resistant *P. aeruginosa*. Fortunately, the incidence of antibiotic resistance of our isolates was lower compared with clinical isolates. In 2009 it was found that 19% of the samples obtained from Alexandria university students' hospital were multidrug resistant (Abaza, 2010). Compared to other swimming pools, we found different rates of antimicrobial resistance. For example, in Greece, no multidrug resistant *P. aeruginosa* strains were found (Tirodimos, Arvanitidou, Dardavessis, Bisiklis, & Alexiou-Daniil, 2010). On the other hand, 96% of *P. aeruginosa* isolates tested from swimming pools and hot tubs in USA were multidrug resistant (Lutz & Lee, 2011). This variability is probably related to the type of tested pool, the natural background resistance levels or shedding from colonized humans (Lister et al., 2009).

It can be concluded from this study that *P. aeruginosa* strains are present with unacceptable rates in Egyptian swimming pools according to Egyptian standards. There is also a high prevalence of multidrug resistant strains that poses a difficulty in treatment if individuals were infected.

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