Prevalence and antibiotic resistance of *Pseudomonas aeruginosa* isolated from swimming pools in northern Greece

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معدَّل مقاومة الزائفة الزنجارية المستفرَدَة من أحواض السباحة في شمال اليونان للمضادات الحيوية

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الخلاصـة: تعد الزائفة الزنجاريَّة من عوامل العدوى الانتهازية في البيئة المائية. واستهدف الباحثون تقيم معدَّل وقوع الزائفة الزنجارية في مياه أحواض السباحة في شمال اليونان، ومقاومتها للمضادات الحيوية. فقد حصل الباحثون على عينات من المياه من أحواض المعالجة بالمياه، وأحواض السباحة، والجاكوزي أو الأحواض المستخدمة في العلاج والترفيه، ووجدوا أن 16.6٪ (45 من أصل 271) عينة إيجابية للزائفة الزنجارية؛ ومن بين المواقع التي فحصها الباحثون، كانت أحواض المعالجة بالمياه هي الأكثر تلوثاً (25٪ من العينات إيجابية). وأظهرت نسبة مئوية ضئيلة من المستفردات (20٪) مقاومة للمضادات الحيوية. وتشير الدراسة إلى أنه معدَّل انتشار الزائفة الزنجارية يبدو منخفضاً نسبياً مقارنة بدراسات أخرى، في حين أن أنماط المقاومة للمضادات الحيوية لهذه المستفردات من المجتمع لم تكن مرتفعة.

ABSTRACT *Pseudomonas aeruginosa* is an important agent of opportunistic infection in aquatic environments. Our aim was to evaluate the occurrence and antimicrobial resistance of *P. aeruginosa* in the water of swimming pools in northern Greece. Water samples were obtained from hydrotherapy pools, jacuzzis/spas and swimming pools. A total of 16.6% (45/271) of the samples were positive for *P. aeruginosa*. Of the amenities examined, the most contaminated were hydrotherapy pools (25% of samples positive). A small percentage of isolates (20.0%) showed resistance to antibiotics. Compared with other studies, the prevalence of *P. aeruginosa* in swimming pools was relatively low, while the antibiotic resistance pattern of these community isolates was not high.

Prévalence et résistance antibiotique de *Pseudomonas aeruginosa* isolé dans des piscines du nord de la Grèce

RÉSUMÉ *Pseudomonas aeruginosa* est un agent d'infection opportuniste fréquent, qui prolifère dans les environnements aquatiques. Notre objectif était d'évaluer la présence et la résistance de *P. Aeruginosa* aux antimicrobiens dans l'eau de piscines situées dans le nord de la Grèce. Des échantillons d'eau provenant de bassins d'hydrothérapie, de jacuzzis/spas et de piscines ont été prélevés. Au total, 16,6 % des échantillons(45/271) étaient positifs à *P. Aeruginosa*. Les bassins d'hydrothérapie étaient les plus contaminés des équipements analysés, (25 % des échantillons étaient positifs). Un faible pourcentage d'isolats (20,0 %) a révélé une résistance aux antibiotiques. En comparaison avec d'autres études, la prévalence de *P. Aeruginosa* dans les piscines était relativement faible, et le profil d'antibiorésistance de ces isolats communautaires était peu élevé.

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Received: 05/11/08; accepted: 27/11/08

Introduction

Pseudomonas aeruginosa belongs to a vast genus of obligate aerobic, non-fermenting, saprophytic, Gram-negative bacilli widespread in nature, particularly in moist environments such as water, sewage, soil, plants and animals [1]. The organism is able to grow and multiply in a variety of water sources including river water, seawater, wastewater and bottled mineral water [2,3].

P. aeruginosa is an important agent of opportunistic infection in patients, particularly in those with respiratory complications and burns. According to Craun et al., *Pseudomonas* spp. was one of the most frequently identified agents associated with waterborne outbreaks of dermatitis (rash or folliculitis), as well as conjunctivitis, otitis externa and other symptoms, in recreational water in the United States of America (14%) [4]. Pseudomonads are well adapted to survival in whirlpools, hot tubs and indoor pools because of the warm water temperatures. These waters are especially prone to contamination during periods of high use when it is difficult to maintain adequate disinfection levels.

Although recreational water is a documented environmental source of *P. aeruginosa*, there are limited published data about the prevalence of this organism in swimming pools, saunas and hot tubs [5,6]. In this study, we aimed to identify the prevalence of *P. aeruginosa* in recreational water facilities in northern Greece and to examine the correlation of *P. aeruginosa* with standard faecal pollution indicator bacteria. We also used antibiograms as an epidemiological marker for our *P. aeruginosa* isolates in view of the fact that antibiotics are cheap and easily available without prescription to the Greek population, creating a risk of antibiotic-resistant strains emerging [7].

Methods

Sampling

The Laboratory of Hygiene is the government reference centre for assessing the chemical and bacteriological quality of potable and recreational waters for the area of northern Greece (Macedonia and Thrace). In the 1-year period 2005, 271 recreational water samples were sent from the local health authorities and other public services to our laboratory. The samples were obtained from 3 amenity categories, namely 4 hydrotherapy pools (n = 8 samples), 4 jacuzzis/spas (n = 49 samples) and 21 swimming pools (n = 214 samples). Samples were taken in sterilized dark-coloured 1-litre bottles containing chloride scavenger and were kept refrigerated at a temperature of 4 °C before microbiological analyses.

Microbiological analyses and antibiotic susceptibility testing

Total heterotrophic bacteria were counted on plate count agar using 1 mL infusion technique after incubation at 37 °C for 48 h. For total coliforms, the 100 mL membrane filtration technique was used, with m-Endo medium at 3 °C for 24 h. For *Escherichia coli*, the 100 mL membrane filtration technique was also used, with tryptone bile X-glucuronide agar at 44.5 °C for 24 h.

P. aeruginosa was confirmed by the Vitek 2 automated microbiology system (bioMérieux, Marcy l'Etoile, France). The minimum inhibitory concentrations (MIC) of antibiotics were determined by broth microdilution assay on the Vitek 2 system. MICs were interpreted according to the 2004 criteria of the National Committee on Clinical Laboratory Standards (NCCLS) [8]. *P. aeruginosa* (ATCC 27853) was used as the quality control strain. The antibiotics used were selected according to the 2004 NCCLS guidelines: amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin, ticarcillin + clavulanic acid and tobramycin. The MICs of an isolate resistant to carbapenems (imipenem and meropenem) were further confirmed by the epsilometer test (AB Biodisk, Solna, Sweden).

Statistical analysis

Data analysis was performed using the statistical package *SPSS* for Windows, version 14.0. The chi-squared test was used at 5% significance level.

Results

Measurements of some important health-related microbiological parameters and the prevalence of *P. aeruginosa* are given on Tables 1 and 2.

A total of 271 water samples from 3 amenity categories (n = 29) were obtained (Table 1). Of the samples examined 39/214 from swimming pools (18.2%), 4/49 from jacuzzis/spas (8.2%) and 2/8 from hydrotherapy pools (25%) were positive for *P. aeruginosa* (total 45/271, 16.6%). The highest isolation rate of *P. aeruginosa* (25.0%) was from hydrotherapy pools. However, no significant differences were found between different amenity categories in the rate of isolation of pseudomonads or the median number of colony-forming units of *P. aeruginosa* per 100 mL ($P > 0.05$) (Tables 1 and 2).

According to Greek regulations, the microbiological quality of the water samples from the different amenity categories of recreational water was reasonably good, with the exception of hydrotherapy pools (Table 1); 50% of specimens from hydrotherapy pools were not compliant with the Greek hygiene regulations. The median colony count of faecal coliforms in this amenity was also by far the largest at 30.5 per 100 mL (range 1–100) (Table 2). There was therefore a significant difference among the different categories of amenity in the rate of compliance with standards ($P < 0.05$).

Table 1 Some important microbiological parameters and prevalence of *Pseudomonas aeruginosa* in water samples from recreational water facilities in northern Greece

Water category	Compliant with chlorination standard[a]		Total heterotrophic bacteria (/mL)		Total coliforms (/100 mL)		*Escherichia coli* (/100 mL)		*Pseudomonas aeruginosa* (/100 mL)		Compliant with microbiological standards[a]	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Swimming pools (*n* = 214)	204	95.3	138	64.5	15	7.0	5	2.3	39	18.2	194	90.7
Jacuzzis/spas (*n* = 49)	47	95.9	38	77.6	7	14.3	2	4.1	4	8.2	42	85.7
Hydrotherapy pools (*n* = 8)	2	25.0	5	62.5	5	62.5	4	50.0	2	25.0	4	50.0
Total (*n* = 271)	253	93.4	181	66.8	27	10.0	11	4.1	45	16.6	240	88.6

[a]*Greek hygiene regulations (limit values): chlorination is 0.4–0.8 ppm (> 0.8 ppm is considered as hyperchlorination); total heterotrophic bacteria < 200 /mL, total coliforms < 5 /100 mL, E. coli 0 /100 mL.*
n = *no. of samples analysed.*

The rate of compliance of our samples with the Greek chlorination standard was 253/271 (93.4%) (Table 1). No significant differences were found between different amenities in the chlorination compliance with the standard ($P > 0.05$).

A total of 45 isolates of *P. aeruginosa* were examined for antibiotic susceptibility. The antimicrobial patterns exhibited by the isolates are shown in Table 3. There were 9 isolates (20.0%) that showed resistance to aztreonam, 9 (20.0%) to ticarcillin + clavulanic acid, 1 (2.2%) to imipenem and 1 (2.2%) to meropenem. No multi-resistant strains were found. The other antibiotics showed good activity with 100% susceptibility rates.

Discussion

In this study we found that the prevalence of *P. aeruginosa* in swimming pools and recreational waters in northern Greece was 16.6%. So far, only a few studies have examined the prevalence of *P. aeruginosa* in recreational waters. Our results are close to these obtained from a survey in the Athens area (17% prevalence) [9]. However, a study from Ireland reported a very high prevalence of *P. aeruginosa* in 38% of swimming pools and 73% of jacuzzis and spas, while another study from Switzerland showed an overall prevalence of 4% [5,6]. As Barben et al. suggested, the explanation for the widely varying rates of *P. aeruginosa* that have been identified in these studies reflects different approaches to the maintenance of recreational waters [6]. The level of free chlorine, the density of use, poor operation, construction and maintenance of these pools as well as the presence of large plastic inflatables in the pool may affect the prevalence of *Pseudomonas* spp. [10].

Waterborne outbreaks of conjunctivitis, otitis externa and dermatitis (rash or folliculitis) caused by *P. aeruginosa* have been reported [10,11]. Pseudomonads are well adapted to survival in pools because of the warm water temperatures, something quite common in Greece. Outbreaks of pseudomonas dermatitis are preventable if water is maintained at a pH of 2.0–5.0 with free chlorine levels in the range of 2.0–5.0 mg/L [12]. Close attention to bather overcrowding, as well as frequent monitoring of disinfectant levels and maintenance of adequate treatment can help prevent these outbreaks.

The rate of compliance of our samples with the Greek chlorination standard was 93.4%. One factor which should be kept in mind is that most pathogens of concern in water recreation and sports are more resistant than coliforms to chlorine. For instance, *Staphylococcus* spp. and *Pseudomonas* spp. were found to be many times more resistant to chlorine than coliforms [13]. It is therefore not surprising to recover *Pseudomonas* spp. in samples from swimming pools in which no coliforms were found.

The 1973 Greek hygiene regulations [14] determining various factors to ensure good water quality in swimming pools of all types are considered obsolete. Standards which are more complete and deal with modern processes such as ozonation include the German standard DIN 19643 *Treatment and disinfection of swimming pool and bathing pool water* and DIN 19605 *Filters for water treatment*, as well as the British Blue Book *Treatment and quality of swimming pool water* [15–17]. Thus, if we use the Greek regulations, 31/271 samples (11.4%) were non-compliant with sources. However, if we use DIN 19605 which demands absence of *P. aeruginosa* in the microbiological parameters, another 35 samples (12.9%, a total of 24.4%) would be non-compliant with standards ($P = 0.02$).

Half of the samples from hydrotherapy pools were non-compliant with Greek hygiene regulations and were also found with a high load of *E. coli*. Hydrotherapy pools in Greece are popular facilities used mainly by the elderly and

Table 2 Colony-forming units of bacteria in water samples from recreational water facilities in northern Greece

Water category	Median no. of colonies (min–max)[a]			
	Total heterotrophic bacteria	Total coliforms	*Escherichia coli*	*Pseudomonas aeruginosa*
Swimming pools (*n* = 214)	20 (2–3000)	4 (1–50)	2 (1–16)	14 (0–100)
Jacuzzis/spas (*n* = 49)	20 (2–3000)	4 (2–40)	4 (1–7)	10 (1–80)
Hydrotherapy pools (*n* = 8)	20 (10–48)	2 (1–120)	30.5 (1–100)	11.5 (3–20)
Total (*n* = 271)	20 (2–3000)	4 (1–120)	2 (1–100)	12 (0–100)

[a]*Median is shown due to the statistically abnormal distribution of the sample.*
n = *no. of samples analysed.*

sick people. These people have a low level of hygiene education and are very prone to faecal accidents. When these characteristics are combined with overcrowding, contamination of pools is very likely. Training of hydrotherapy pool managers, operators and staff should include information about the transmission of waterborne illnesses and the critical role of treatment, operation/maintenance and monitoring in preventing these illnesses.

P. aeruginosa is naturally resistant to many antibiotics because of their relatively impermeable membrane, constitutively expressed and inducible efflux systems and a chromosomally encoded inducible β-lactamase. These antibiotics include penicillin G, aminopenicillins—even when combined with β-lactamase inhibitors—and first- and second-generation cephalosporins; *P. aeruginosa* is also naturally resistant to macrolides, chloramphenicol, cotrimoxazole, rifampin, kanamycin and first-generation fluoroquinolones, such as norfloxacin [18]. At this point we must mention that the study of antibiotic resistance in environmental strains is not common and may produce poor results in relation to clinical isolates. In principle, all hospital and household wastewater should be treated before release into the environment. However, sometimes the uncontrolled disposing of antibiotics and chemicals into the environment may create a selective pressure on these drugs. Furthermore, the members of a particular environmental clonal complex may be very successful and widespread in natural as well as in clinical environments, having developed the ability to quickly adapt to noxious substances (antimicrobials, detergents, pesticides, heavy metals) entering their environment [19].

The overall incidence of antibiotic resistance of our isolates was very low compared with clinical isolates [7], while no multi-drug resistant strains were found. All of our isolates (100%) were susceptible to cefepime, ceftazidime, piperacillin (β-lactams), amikacin, gentamicin, tobramycin (aminoglycosides) and ciprofloxacin (fluoroquinolones). The high level of resistance to aztreonam (20.0%) and ticarcillin + clavulanic acid (20.0%) has been mentioned in studies with nosocomial isolates [20]. As for ticarcillin + clavulanic acid, in Greece there are no official data regarding the resistant strains of *P. aeruginosa*. However, according to data obtained from the Vitek2 system in AHEPA Hospital in Thessaloniki during the year 2005, 41% of clinical isolates of *P. aeruginosa* were resistant to these antibiotics. Furthermore, survey data showed emerging

Table 3 Results of susceptibility testing of *Pseudomonas aeruginosa* (*n* = 45 isolates), and minimum inhibitory concentration (MIC) of each antibiotic used

Antibiotic	Sensitive			Intermediate			Resistant		
	No.	%	MIC (μg/mL)	No.	%	MIC (μg/mL)	No.	%	MIC (μg/mL)
Amikacin	45	100.0	≤ 16	0	–	32	0	–	≥ 64
Aztreonam	36	80.0	≤ 8	8	17.8	16	1	2.2	≥ 32
Cefepime	45	100.0	≤ 8	0	–	16	0	–	≥ 32
Ceftazidime	45	100.0	≤ 8	0	–	16	0	–	≥ 32
Ciprofloxacin	45	100.0	≤ 1	0	–	2	0	–	≥ 4
Gentamicin	45	100.0	≤ 4	0	–	8	0	–	≥ 16
Imipenem	44	97.8	≤ 4	0	–	8	1	2.2	≥ 16
Meropenem	44	97.8	≤ 4	0	–	8	1	2.2	≥ 16
Piperacillin	45	100.0	≤ 64	0	–	–	0	–	≥ 128
Ticarcillin/clavulanic acid	36	80.0	≤ 64/< 2	0	–	–	9	20.0	≥ 128/≥ 2
Tobramycin	45	100.0	≤ 4	0	–	8	0		≥ 16

resistance to carbapenems [21], as was the case with 2 of our isolates.

In conclusion, our findings revealed the prevalence of *P. aeruginosa* in recreational waters in northern Greece was relatively low and was compliant with established local microbiological standards. The exception was a high level of contamination in hydrotherapy pools which might be attributed to lack of hygiene practice of the bathers and lack of training of technical personnel.

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