Pseudomonas aeruginosa on vinyl-canvas inflatables and foam teaching aids in swimming pools

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ABSTRACT

Swimming pool-related *Pseudomonas aeruginosa* infections mainly result in folliculitis and otitis externa. *P. aeruginosa* forms biofilms on surfaces in the swimming pool environment. The presence of *P. aeruginosa* on inflatables and foam teaching aids in 24 public swimming pools in the Netherlands was studied. Samples ($n = 230$) were taken from 175 objects and analysed for *P. aeruginosa* by culture. Isolated *P. aeruginosa* were tested for antibiotic resistance by disk diffusion. *P. aeruginosa* was detected in 63 samples (27%), from 47 objects (27%) in 19 (79%) swimming pools. More vinyl-canvas objects (44%) than foam objects (20%) were contaminated, as were wet objects (43%) compared to dry objects (13%). Concentrations were variable, and on average higher on vinyl-canvas than on foam objects. Forty of 193 (21%) *P. aeruginosa* isolates from 11 different objects were (intermediate) resistant to one or more of 12 clinically relevant antibiotics, mostly to imipenem and aztreonam. The immediate risk of a *P. aeruginosa* infection from exposure to swimming pool objects seems limited, but the presence of *P. aeruginosa* on pool objects is unwanted and requires attention of pool managers and responsible authorities. Strict drying and cleaning policies are needed for infrequently used vinyl-canvas objects.

Key words | antibiotic resistance, folliculitis, inflatables, *Pseudomonas aeruginosa*, swimming pools, teaching aids

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ABBREVIATIONS AND NOTATION

AN	Amikacin
ATM	Aztreonam
CAZ	Ceftazidime
CDC	Centers for Disease Control and Prevention
cfu	colony forming unit
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
FEP	Cefepime
GM	Gentamicin
I	intermediate resistant (reduced susceptible)
IPM	Imipenem
MEM	Meropenem
mpn	most probable number
NN	Tobramycin
PIP	Piperacillin
PVC	polyvinylchloride
R	resistant
TIM	Ticarcillin with clavulanic acid
TSA	Trypton Soy Agar
TZP	Piperacillin/tazobactam
UV	ultraviolet

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INTRODUCTION

Pseudomonas aeruginosa is the most important pathogen within the genus *Pseudomonas*. It is an opportunistic human pathogen which is intrinsically resistant to a broad range of antibiotics due to the presence of several resistance mechanisms, but it is also capable of rapidly acquiring

resistance and adaptive resistance (Mena & Gerba 2009; Breidenstein *et al*. 2011). *P. aeruginosa* is a common inhabitant of soil and aquatic environments where it persists and reproduces under low nutrient conditions. *P. aeruginosa* grows within a broad temperature range, from 5 to 42 °C, and thrives in the warm and humid swimming pool environment where it may accumulate in biofilms, especially on surfaces and in plumbing (Mena & Gerba 2009). Introduction of *P. aeruginosa* in the indoor swimming pool environment may occur through human faecal and non-faecal shedding, and carriage by vehicles such as shoes, towels, toys and inflatables (Rice *et al*. 2012).

P. aeruginosa is a major cause of otitis externa (swimmer's ear) in surface water swimmers (Van Asperen *et al*. 1995; Schets *et al*. 2011), whereas *P. aeruginosa* infections of the skin (folliculitis), wounds, the urinary tract and the respiratory tract, are more commonly associated with the use of swimming pools and whirlpools (Ratnam *et al*. 1986; Beckett *et al*. 2000; Mena & Gerba 2009). Exposure routes in swimming pools that can result in infected pool users are contact of the skin with the pool environment, including water, surfaces, and equipment, inhalation of aerosols, and direct ingestion of pool water (Rice *et al*. 2012).

Damaged follicles, for example, through insect bites, blocking or rubbing, may be infected by *P. aeruginosa*, causing folliculitis, with painful, red, itching papules. Many cases of whirlpool- and hot-tub-related *P. aeruginosa*-associated folliculitis have been described, often linked to disinfectant failure in the facilities involved (Beckett *et al*. 2000; Mena & Gerba 2009). Some reports refer to *P. aeruginosa* as the causative agent in folliculitis related to objects in swimming pools, such as bath toys (Buttery *et al*. 1998), and water slides (Centers for Disease Control & Prevention (CDC) 1983). *P. aeruginosa* has also been identified as the cause of a community outbreak of folliculitis related to the use of a pool inflatable in the United Kingdom (Tate *et al*. 2003). A case-control study performed to identify the cause of this outbreak yielded an odds ratio of 12.0 (95% confidence interval; 1.05–136.8) for developing folliculitis having used the only large inflatable present in one of the local swimming pools. *P. aeruginosa* was isolated from swabs taken from the inflatable and from skin rashes of six of the 32 cases. Pulsed field gel electrophoresis demonstrated that the isolates from inflatable and patients were identical.

After use, inflatables, as well as foam teaching aids, are commonly stored wet. Water that remains in or on these objects and a warm environment comprise a favourable habitat for *P. aeruginosa* reproduction (Mena & Gerba 2009; Rice *et al*. 2012). Use of inflatables and teaching aids after wet storage may expose swimmers to high concentrations of *P. aeruginosa* on the surface of these objects, possibly resulting in infections when damaged follicles are present, and subsequent skin conditions. Launching the contaminated objects into the water may lead to short-term elevated levels of *P. aeruginosa* in the water, resulting in a temporary increased risk of ear and eye infections. Dilution and inactivation in well-managed pools will, however, rapidly reduce this risk (Rice *et al*. 2012).

The presence of *P. aeruginosa* on a variety of vinyl-canvas inflatables and foam teaching aids was studied in a selection of swimming pools in the Netherlands. *P. aeruginosa* isolates were analysed for antibiotic resistance. Information was collected on cleaning, storage and replacement policies for the studied objects, and pool managers were asked about reported health conditions related to these objects.

METHODS

Pool and object selection

In July 2011, all public swimming pools ($n = 36$) in the Dutch province Utrecht were asked by letter to participate in the study. In April 2012, public swimming pools in the Leiden region ($n = 30$) in the province South-Holland received the same request.

Swimming pools that agreed to participate were visited once during September–December 2011 (Utrecht) or May–June 2012 (Leiden); all participating pools were indoor pools. During the visits, a questionnaire was administered to the pool manager or operator, including questions on the age, cleaning and storage procedures, replacement policy, frequency of use, and number of users of the inflatables and teaching aids present. Whenever present, samples were taken from large inflatables, like obstacle courses,

vinyl-canvas mats (which float on the water and are used to run on), and foam teaching aids. Foam teaching aids exist in many different types and shapes, for various purposes. In each swimming pool, the most frequently used types of foam teaching aids were selected for sampling. Undamaged or only slightly damaged objects were sampled.

In all swimming pools, pool water quality complied with Dutch swimming pool legislation, indicating that free chlorine levels were 0.5–1.5 mg/l, and pH values were 6.8–7.8 (Anonymous 2010).

Sampling of inflatables and teaching aids

Sampling of the selected objects was done by systematic swabbing with pre-moistened Enviro Swabs (3M, Zoeterwoude, The Netherlands) of a defined sampling area of 10 cm^2 using sterile sampling templates (Copan Diagnostics Inc., Murrieta, CA, USA). Sampling of round structures was done without a sampling template, estimating the sample area to approximate 10 cm^2. Samples were taken from surfaces that could come into contact with human skin. One sample per object was taken from foam teaching aids (two to four objects per swimming pool), whereas three to five samples per object were taken from objects measuring 5–10 m in length and 1–2 m in width, like obstacle courses and vinyl-canvas mats (commonly one object per swimming pool). It was recorded whether objects were dry or wet at the time of sampling (organoleptic observations).

Sample analyses

Enviro Swabs were transported to the laboratory in plastic tubes which were stored in an insulating container with ice-packs. Sample processing started within 24 h from sampling by adding 20 ml 0.1% peptone saline (bioTrading, Mijdrecht, The Netherlands) to the tubes with the Enviro Swabs. The Enviro Swabs were washed in the peptone saline by vigorous mixing by hand, for 1 minute, followed by squeezing out and removal of the swabs, after which the peptone saline was homogenized by vortexing. All samples were examined for the presence of *P. aeruginosa* by membrane filtration (no. HAWG047S1, 0.45 μm pore size; Millipore, Amsterdam, The Netherlands) of 5 ml portions of the homogenate and subsequent incubation of the membrane filters on CN agar (Oxoid, Badhoevedorp, The Netherlands) at 36 ± 2 °C for 44 ± 4 h according to ISO 16266 (Anonymous 2006), and by using Pseudalert (IDEXX Laboratories Inc., Westbrook, ME, USA) according to the manufacturer's instructions. For this, 5 ml of the homogenate was adjusted to 100 ml with sterile demineralized water and mixed with the Pseudalert substrate in Pseudalert mixing vessels, and subsequently incubated in the Pseudalert 51-well quanti-tray at 38 ± 0.5 °C for 24–28 h. After incubation, quanti-trays were read under ultraviolet (UV) light (365 nm), and blue fluorescent wells were counted as positive. A most probable number (mpn) table (provided by the manufacturer) was used to determine the *P. aeruginosa* concentration in the samples. In addition to the above methods, the Utrecht samples were examined by membrane filtration of 5 ml portions of the homogenate and subsequent incubation of the filters on mPA-B agar at 41.5 ± 0.5 °C for 48 ± 4 h (Havelaar *et al.* 1985). The mPA-B agar was prepared from mPA-C agar (Becton Dickinson BBL, Erembodegem, Belgium), which was prepared according to manufacturer's instructions, cooled to 50 °C, and supplemented with 10 ml of sterile antibiotic solution (1.5 g cycloheximide and 1.76 g sulfapyridine in 100 ml) per 990 ml, thus yielding mPA-B agar.

To test whether *P. aeruginosa* bacteria remained in the Enviro Swabs after washing, washed and squeezed out swabs were placed in Pseudalert mixing vessels with 100 ml demineralized water and the Pseudalert substrate, mixed and subsequently incubated at 38 ± 0.5 °C for 24–48 h. After incubation, the vessels were read under UV light (365 nm), and blue fluorescence indicated the presence of *P. aeruginosa*. This was done for the Utrecht samples only.

Confirmation of *P. aeruginosa* isolates

Presumptive *P. aeruginosa* colonies on CN agar and mPA-B agar were cultured on King's B agar (Sigma-Aldrich, Zwijndrecht, The Netherlands) to verify fluorescein production. Incubation was at 36 ± 2 °C for 24 ± 4 h, with an extension of the incubation time to a maximum of 5 days, when no growth was observed at inspection every 24 h. Isolates that produced fluorescein were stored at −80 °C by using the Microbank system (Pro-Lab Diagnostics, Neston, UK).

Stored isolates were subjected to biochemical tests using the API 20E (Amato *et al.* 1980) and API 20NE identification systems (bioMérieux, Marcy L'Etoile, France) according to the manufacturer's instructions, and were tested for oxidative/fermentative metabolism of glucose by stab inoculation of glucose agar tubes (bioTrading, Mijdrecht, The Netherlands), incubated at 36 ± 2 °C and inspected after 24 and 48 h, growth at 42 °C on Trypton Soy Agar (TSA; Oxoid, Badhoevedorp, The Netherlands) incubated overnight, the ability to deaminate acetamide by inoculation of Acetamide Agar Slants (BBL-Becton Dickinson, Erembodegem, Belgium), incubated at 36 ± 2 °C for up to 7 days and inspected every 24 h, and the production of pyocynanin, pyoverdine or pyorubine while grown on TSA at 36 ± 2 °C for 24 ± 4 h.

Antibiotic resistance testing of *P. aeruginosa* isolates

P. aeruginosa isolates were tested for antibiotic resistance by disc diffusion following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2010). Resistance against a set of 12 clinically relevant antibiotics (Magiorakos *et al.* 2012) was tested by using Sensi-Disc susceptibility test discs (BBL-Becton Dickinson, Erembodegem, Belgium). The set consisted of amikacin (no. 231597; 30 μg), aztreonam (no. 231641; 30 μg), ceftazidime (no. 231633; 30 μg), ciprofloxacin (no. 231658; 5 μg), cefepime (no. 231696; 30 μg), gentamicin (no. 231299; 10 μg), imipenem (no. 231645; 10 μg), meropenem (no. 231704; 10 μg), tobramycin (no. 231569; 10 μg), piperacillin (no. 231609; 100 μg), ticarcillin with clavulanic acid (no. 231649; 75/19 μg), and piperacillin/tazobactam (no. 231692; 100/10 μg). Fresh cultures of all isolates were obtained by overnight culture on TSA at 36 ± 2 °C. From these cultures, 0.5 McFarland suspensions were prepared in 0.1% peptone saline; these suspensions were applied to Mueller-Hinton agar plates (Oxoid, Badhoevedorp, The Netherlands) by using cotton swabs to obtain confluent bacterial layers. Sensi-Discs were subsequently applied by using a Sensi-Disc dispenser, and the plates were incubated at 36 ± 2 °C for 24 ± 4 h. *P. aeruginosa* ATCC® 27853™, which is sensitive for all tested antimicrobials, was included as the reference strain. Inhibition zone diameters were measured and compared with CLSI breakpoints (CLSI 2010) to establish resistance.

RESULTS

Swimming pools, objects, and data analysis

Fourteen swimming pools in the Utrecht region (response 39%), and 10 swimming pools in the Leiden region (response 33%) were enrolled. Samples were taken from 87 and 88 specimens of swimming pool aids and inflatables in Utrecht and Leiden, respectively. A total number of 230 samples were taken and analysed. Since initial data analysis showed that percentages of dry and wet samples, percentages of samples from foam or canvas objects, and percentages of positive and negative samples were similar in the two provinces, Utrecht and Leiden data were pooled for final data analysis.

Of the 175 tested objects (Table 1), 125 were made of foam (71%), whereas 50 were made of vinyl-canvas (29%). Fifty-five per cent of the samples were taken from foam objects ($n = 127$), and 45% were taken from vinyl-canvas objects ($n = 103$). Fifty-three per cent of the samples came from dry objects ($n = 121$), and 47% came from wet or moist objects ($n = 109$), either foam or vinyl-canvas.

P. aeruginosa on pool inflatables and teaching aids

Pool inflatables and teaching aids that were contaminated with *P. aeruginosa* were present in 19 of 24 (79%) of the participating swimming pools. *P. aeruginosa* was detected in 63 of the 230 examined samples (27%) with at least one of the used detection methods (Table 1). The 127 samples from foam objects were taken from 125 different objects, yielding 25 (20%) positive foam objects, whereas the 103 samples from vinyl-canvas objects were taken from 50 different objects, yielding 22 (44%) positive vinyl-canvas objects. More samples from wet objects (43%) than from dry objects (13%) were positive, and more samples from vinyl-canvas objects (37%) than from foam objects (20%) were contaminated with *P. aeruginosa*. Wet foam objects that were used just before sampling were less often contaminated than foam objects that were stored wet, for example, at the bottom of a container, in a layer of water. At sampling, the presence of biofilms on large inflatable obstacle courses and inflatable floats was clearly noticeable by organoleptic observations.

On objects that were *P. aeruginosa* positive with at least one of the detection methods, the *P. aeruginosa*

Table 1 | *P. aeruginosa*-positive, dry or wet, foam and vinyl-canvas swimming pool objects in Dutch swimming pools

			No. of samples			No. of positive samples (%)		
	No. of objects	No. of positive objects (%)	Total	Dry	Wet	Total	Dry	Wet
Foam objects								
Swim belt	35	3 (9)	35	22	13	3 (9)	1 (5)	2 (15)
Aqua jogger flotation belt	13	2 (15)	13	10	3	2 (15)	2 (20)	0 (0)
Pool noodle	19	4 (21)	19	11	8	4 (21)	2 (18)	2 (25)
Arm band/disc	14	2 (14)	14	8	6	2 (14)	1 (13)	1 (17)
Float	40	13 (33)	42	15	27	13 (31)	1 (7)	12 (44)
Bricks	4	1 (25)	4	3	1	1 (25)	1 (33)	0 (0)
Total foam	*125*	*25 (20)*	*127*	*69*	*58*	*25 (20)*	*8 (12)*	*17 (29)*
Vinyl-canvas objects								
Inflatable obstacle course	8	5 (62)	34	14	20	17 (50)	3 (21)	14 (70)
Inflatable animal/figure	9	3 (33)	18	11	7	4 (22)	0 (0)	4 (57)
Inflatable float	11	6 (55)	15	8	7	7 (47)	1 (13)	6 (86)
Mat	18	6 (33)	30	18	12	7 (23)	3 (17)	4 (33)
Storage slipcover	3	2 (67)	4	1	3	3 (75)	1 (100)	2 (67)
Various vinyl-canvas	1	0 (0)	2	0	2	0 (0)	0 (0)	0 (0)
Total vinyl-canvas	*50*	*22 (44)*	*103*	*52*	*51*	*38 (37)*	*8 (15)*	*30 (59)*
Total overall	*175*	*47 (27)*	*230*	*121*	*109*	*63 (27)*	*16 (13)*	*47 (43)*

concentrations were variable, and generally higher on vinyl-canvas objects than on foam objects (Table 2). Since some of the samples (particularly those from biofilms on large inflatable obstacle courses) had *P. aeruginosa* concentrations that were too high to enumerate, the true upper limits of the concentration ranges presented in Table 2 are likely to be higher than those that could be calculated. The Pseudalert presence-absence test detected *P. aeruginosa* in 26 of 134 (19%) squeezed out swabs, indicating that the washing procedure did not always remove all *P. aeruginosa* bacteria from the swabs. Moreover, the remaining volume of the washing fluid after removal of the swabs was variable (data not shown), indicating that some swabs retained more fluid than others. Thus, the sampling and sample processing procedure and the subsequent quantitative analysis of the samples only provided an indication of the contamination levels of the tested objects. These were not displayed in colony forming units (cfu) or mpn per sampled

Table 2 | *P. aeruginosa* concentrations in samples taken from vinyl-canvas and foam swimming pool objects in Dutch swimming pools, determined by using different detection methods

	CN-agar[b]		mPA-B-agar[b]		Pseudalert[c]	
	Foam	Vinyl-canvas	Foam	Vinyl-canvas	Foam	Vinyl-canvas
No. of samples positive	23	36	4	25	13	29
Positive samples with count[a]	8	10	3	15	8	10
Concentration range[a]	0.4–38	0.2–90[d]	0.2–20	1–2000[d]	0.2–10	0.2–2026[d]
Mean[a]	10.1	19.7	8.3	494	2.7	339
Median[a]	5.9	2.7	4.8	20	0.85	61

[a]Disregarding all samples with colony numbers too high to count or mpn above the upper detection limit of 40/ml.
[b]Concentrations displayed in cfu per ml.
[c]Concentrations displayed in mpn per ml.
[d]Occasionally, samples were diluted, resulting in a higher upper detection limit.

surface, but per millilitre of the tested washing fluid and were suitable for mutual comparison only.

Antibiotic resistance of *P. aeruginosa* isolates

A total of 312 presumptive *P. aeruginosa* isolates were subjected to biochemical tests; 193 isolates were identified as *P. aeruginosa* and subjected to antimicrobial susceptibility testing. Non-susceptibility (resistance) or reduced susceptibility (intermediate resistance) to one or more of the tested antibiotics was observed in 40 (21%) of the *P. aeruginosa* isolates. Most of these isolates ($n = 28$) were non-susceptible or reduced susceptible to one antibiotic, whereas some of these isolates ($n = 12$) were non-susceptible or reduced susceptible to two to five antibiotics. Multidrug-resistance, which was defined by Magiorakos *et al.* (2012) as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, was not observed. The non-susceptible isolates originated from 11 different objects (6% of all tested objects, and 23% of *P. aeruginosa*-positive objects), of which seven (64%) were vinyl-canvas objects, and four (36%) were foam objects (Table 3). Although several isolates per object were recovered, antibiotic resistance profiles per object were variable for most objects ($n = 8$), suggesting that various clones of *P. aeruginosa* were present. On three objects, the recovered isolates had identical antibiotic resistance profiles per object which may indicate that these isolates were clones. The most frequently observed resistance was to carbapenems (imipenem) and monobactams (aztreonam). Antibiotic resistant *P. aeruginosa* were found in nine (38%) of 24 investigated swimming pools, which is in 47% of the swimming pools where *P. aeruginosa* positive inflatables and foam teaching aids were present.

Use and maintenance of swimming pool inflatables and teaching aids

The age of the inflatables and foam objects was highly variable among the Utrecht swimming pools (no data on object age were obtained for the Leiden swimming pools). All objects were between less than 1 and over 10 years of age, with a most frequently reported age of 1–5 years for vinyl-canvas objects and 1–3 years for foam objects. Vinyl-canvas objects were generally replaced when they were broken (or leaking in case of inflatables), whereas foam objects were replaced when dirty, damaged or broken.

The frequency of use for vinyl-canvas objects ranged from daily to less than once per 3 months. In the Utrecht swimming pools, these objects were mostly used on a weekly basis, whereas in the Leiden swimming pools vinyl-canvas objects were mostly used once every 3 months. Foam objects were commonly used on a daily basis. Once in the water, both vinyl-canvas and foam objects in the Utrecht swimming pools were kept there for 1–2 hours (no data obtained for Leiden swimming pools). The number of users of vinyl-canvas objects in both regions ranged from less than 10 to over 250 persons per occasion of use, with 30–100 persons being the most frequently reported user number.

In five (21%) of the 24 swimming pools, foam objects were cleaned; one pool cleaned once a year by rinsing with tap water, two pools cleaned once every 6 months by rinsing with disinfectant (not specified), two pools cleaned once every 3 months, of which one rinsed with pool water and the other with a non-specified disinfectant. In four of these pools, all tested foam objects were *P. aeruginosa* negative; in one pool, *P. aeruginosa*-positive foam objects were present. Vinyl-canvas inflatables were present in 15 of the 24 swimming pools. In one (7%) of these 15 pools, inflatables were cleaned after use (not specified how); four of the five tested inflatables in this pool were *P. aeruginosa* negative, one was positive. In eight (53%) of the 15 pools the inflatable objects were cleaned and/or dried before storage. In two out of these eight pools, the inflatables were *P. aeruginosa* negative, but in only one of these two pools were the inflatables actually dry during sampling. In one pool, four of the five tested inflatables were *P. aeruginosa* negative; these objects were dry during sampling. The fifth inflatable was positive and wet during sampling. In the other five pools, the inflatables were *P. aeruginosa* positive; in two of these pools the sampled inflatables were dry. Vinyl-canvas mats were present in 16 of the 24 swimming pools. In two (12%) of these 16 pools, the mats were cleaned after use; the frequency of use and of cleaning was once a month. In both pools the mats were *P. aeruginosa* negative. In five (31%) of the 16 pools, mats were cleaned and dried before storage; in four out of five of these pools the mats were *P. aeruginosa* negative, in one pool the mat was *P. aeruginosa* positive.

Table 3 | Antibiotic resistance in *P. aeruginosa* isolates from swimming pool inflatables and teaching aids in Dutch swimming pools

	Antibiotic[a]												
	AN	ATM	CAZ	CIP	FEP	GM	IPM	MEM	NN	PIP	TIM	TZP	No. of isolates with resistance[b]
Foam objects													
Arm band A	R	R			I	R			R				1
Arm band A					R								1
Brick				I									1
Flexibeam A							R	R					1
Flexibeam A							R	I				R	1
Flexibeam A												R	1
Flexibeam A							I	I					1
Float		I	I										1
Vinyl-canvas objects													
Obstacle course A						I							1
Obstacle course A			I										1
Obstacle course B							I						2
Obstacle course B							R						4
Obstacle course B		I					R						1
Obstacle course B		I					I						1
Obstacle course C							R						1
Obstacle course C							R	I					1
Obstacle course C		I											5
Obstacle course C				I									1
Obstacle course D							R						2
Storage slipcover A		I											1
Storage slipcover A		I		I		I	R	I					1
Storage slipcover A		I					R	I				R	1
Storage slipcover A							R	I					1
Storage slipcover A						I	R	I				R	1
Inflatable float											R		5
Mat		I											2
No. of isolates with resistance	1	13	2	3	2	4	19	8	1	0	5	4	

[a]AN: amikacin; ATM: aztreonam; CAZ: ceftazidime; CIP: ciprofloxacin; FEP: cefepime; GM: gentamicin; IPM: imipenem; MEM: meropenem; NN: tobramycin; PIP: piperacillin; TIM: ticarcillin with clavulanic acid; TZP: piperacillin/tazobactam.
[b]R: resistant; I: intermediate resistant (reduced susceptible).

When not used, most objects were stored in a dry place within the building, mostly in an equipment storage room adjacent to the swimming hall. Vinyl-canvas mats were commonly rolled up and stored on a platform next to one of the larger pools. Foam teaching aids were often stored in containers on the platforms in the swimming hall. At sampling, it was regularly observed that there was a layer of water on the bottom of these containers and the bottom-most foam teaching aids were permanently soaked.

Health conditions

All swimming pool managers or operators reported that they were rarely notified of skin conditions by visitors that used

inflatables or foam teaching aids, but they all mentioned self-observed cases of skin rash in children that used inflatable slides or obstacle courses.

DISCUSSION

The frequent presence of *P. aeruginosa* on wet pool inflatables and foam teaching aids in a selection of swimming pools in two regions in the Netherlands was demonstrated. Wet foam objects were less often contaminated than wet vinyl-canvas objects. Foam objects with a high frequency of use (daily or several times a day), like teaching aids, are regularly exposed to pool disinfectants like chlorine. When free chlorine levels are sufficient (World Health Organization (WHO) 2006; Anonymous 2010), *P. aeruginosa* is inactivated and unable to colonize the wet foam (Mena & Gerba 2009). The observed *P. aeruginosa* positive foam teaching aids at the bottom of containers, in a layer of swimming pool water, can be explained by the ability of mucoid *P. aeruginosa* to grow in chlorinated water (Grobe *et al.* 2001; Rice *et al.* 2012), or the decline of the free chorine concentration in the water to below disinfecting levels during longer storage.

Due to their size, and the lack of space in swimming pools, large vinyl-canvas inflatables are often rapidly deflated after use, without drying, and subsequently folded and stored wet. Moreover, these objects generally have a low frequency of use, and the complex shape of the objects, with seams, folds and corners that may retain water, creates a humid environment during storage. These practices advance growth of *P. aeruginosa* (Mena & Gerba 2009), and thus explain the higher frequency of *P. aeruginosa* positive vinyl-canvas objects, and the higher maximum, mean and median concentrations on these objects as compared to foam objects. These outcomes imply that strict drying and cleaning policies are more urgent for vinyl-canvas objects than for foam objects, particularly for those vinyl-canvas objects that are infrequently used and stored for longer periods. Whether the type of material, that is, vinyl-canvas (for some of the studied objects called 'bisonyl', which is polyvinylchloride (PVC)) or foam (which may be polyurethane or polyethylene), influences the growth and biofilm formation of *P. aeruginosa*, needs further study.

Although the frequency of cleaning of foam objects was so low that it unlikely prevented colonization by *P. aeruginosa*, there seems to be a beneficial effect from cleaning foam objects since 80% of the pools that cleaned foam objects had *P. aeruginosa*-negative objects, as opposed to 26% of the pools that did not clean foam objects. In one of the pools that did not clean, but had *P. aeruginosa*-negative foam objects, the foam objects were hung on a rack, leaving them to air dry. This suggests a preventive effect of desiccating and thereby killing *P. aeruginosa*, as opposed to storage in a container, which hinders proper drying of objects.

Vinyl-canvas *P. aeruginosa*-negative mats were cleaned more frequently and dried before storage than *P. aeruginosa*-positive vinyl-canvas mats, indicating that cleaning and drying of these objects before storage prevents colonization, and possibly biofilm formation, by *P. aeruginosa*. However, this was not observed for vinyl-canvas inflatables. For this type of material, there was a discrepancy between what pool operators reported (cleaning and drying before storage) and observations during sampling (wet or moist inflatables in pools that said they cleaned and dried).

Tate *et al.* (2003) found that a contaminated inflatable that caused an outbreak of folliculitis remained heavily contaminated for 2 months despite extensive cleaning and drying, which indicates that cleaning and drying does not completely remove *P. aeruginosa* once contamination has come about and biofilms have been formed. Biofilms protect *P. aeruginosa* against detergents and disinfectants (Vess *et al.* 1993; Goeres *et al.* 2004; Harmsen *et al.* 2010), and to decontaminate equipment, more stringent solutions like cleaning with 70% ethanol (Vess *et al.* 1993) or hydrogen peroxide vapour disinfection (Otter *et al.* 2010; Zoutman *et al.* 2011) may be required. Further study into the effect of various cleaning, drying or disinfection policies on (re-) growth or removal of *P. aeruginosa* on swimming pool inflatables and teaching aids is needed.

The detection of antibiotic-resistant *P. aeruginosa* isolates is in line with other studies that have demonstrated the presence of antibiotic-resistant *P. aeruginosa* in swimming pools (Papadopulou *et al.* 2008), hot tubs, and on surfaces in the swimming pool environment (Lutz & Lee 2011). Swimming pool-related health conditions such as otitis externa and folliculitis are, in the case of otitis externa, treated by local

administration of antibiotic ear drops (McWilliams *et al.* 2012), or, in the case of folliculitis, require no specific treatment (Ratnam *et al.* 1986) because the rash disappears in 1–10 days (Berger & Seifert 1990). Carbapenems, such as imipenem and meropenem, are the first choice antibiotics in treatment of severe *P. aeruginosa* infections due to their broad spectrum of activity and stability against most β-lactamases (Masterton 2009). In the Netherlands, significantly increased imipenem resistance is observed in *P. aeruginosa* strains from patients attending urology departments in hospitals (Croughs *et al.* 2013) and the presence of metallo-β-lactamase (MBL)-producing *P. aeruginosa* in hospitals is considered a nationwide problem (Van der Bij *et al.* 2012). Imipenem resistance was the most frequently observed resistance in *P. aeruginosa* isolates from swimming pool objects. It is unclear whether this reflects the nationwide increase of imipenem-resistant *P. aeruginosa*.

In the Netherlands, there are no officially recorded or reported outbreaks of folliculitis related to swimming pool inflatables and foam teaching aids, suggesting that the risk of contracting such an infection is low. However, this may be an underestimation of the true number of cases because the number of contaminated inflatables is high (Tate *et al.* 2003; this study), large numbers of people use the inflatables, and pool operators consider cases of folliculitis in children a normal consequence of using these objects needing no specific attention or reporting. Moreover, *P. aeruginosa* folliculitis is not unique in its appearance and it may be confused with insect bites, allergy or contact dermatitis (Ratnam *et al.* 1986). Although the immediate risk of contracting a serious *P. aeruginosa* infection from exposure to swimming pool inflatables and foam teaching aids seems limited, the presence of (antibiotic-resistant) *P. aeruginosa* on swimming pool objects is unwanted and requires the attention of pool managers and responsible authorities.

CONCLUSIONS

- Wet storage of vinyl-canvas inflatables and foam teaching aids enhanced growth and biofilm formation of *P. aeruginosa* on these objects.
- Vinyl-canvas objects were more often contaminated with *P. aeruginosa* than foam objects, due to folded and wet storage, lower frequency of use, and less contact with chlorinated water when in use.
- Strict drying and cleaning policies are more urgent for vinyl-canvas objects than for foam objects, particularly for those objects that are infrequently used and stored for longer periods.
- The presence of (antibiotic resistant) *P. aeruginosa* on swimming pool objects is unwanted and requires the attention of pool managers and responsible authorities.

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