J. Hyg., Camb. (1982), **88**, 205
Printed in Great Britain

Hospital hydrotherapy pools treated with ultra violet light: bad bacteriological quality and presence of thermophilic *Naegleria*

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(*Received* 4 *September* 1981; *accepted* 29 *September* 1981)

SUMMARY

The microbiological quality of eight halogenated and two u.v.-treated hydrotherapy pools in hospitals was investigated. The microbiological quality of halogenated hydrotherapy pools was comparable to halogenated public swimming pools, although in some *Pseudomonas aeruginosa* and faecal pollution indicators were more frequent due to bad management.

On the other hand u.v.-treated hydrotherapy pools had very bad microbiological quality. Apart from faecal pollution indicators, *P. aeruginosa* was present in very high numbers.

Halogenated hydrotherapy pools were not highly contaminated with amoebae, and *Naegleria* spp. were never detected. On the other hand u.v.-treated pools contained very high numbers of thermophilic *Naegleria*. The *Naegleria* isolates were identified as *N. lovaniensis*, a species commonly found in association with *N. fowleri*.

Isoenzyme analysis showed a different type of *N. lovaniensis* was present in each of two u.v.-treated pools.

INTRODUCTION

In a recently published review Galbraith (1980) summarized different infections that have been associated with swimming pools. He emphasized that infections occur especially in badly managed baths with inadequately treated water. I wish to report on a 'disinfection' method with ultraviolet light (u.v.), used in hydrotherapy pools, whereby the microbiological quality of the water is the same as though it was not treated at all. While the water of hydrotherapy pools treated with chlorine or bromine normally had good bacteriological quality, the microbiological findings in u.v.-treated hydrotherapy pools always gave cause for concern.

MATERIALS AND METHODS

Eight hydrotherapy pools disinfected with either chlorine or bromine, and two treated with u.v. were sampled. All pools were part of hospital facilities.

Samples were taken just below the water surface in sterile bottles, containing sodium thiosulphate when the water was halogenated. Where possible, samples were also taken from the filters. At site the water temperature was measured and the chlorine (bromine) content was determined by using the diethyl-*p*-phenylenediamine (DPD) test. The pH was recorded in the laboratory. The total count at 37 °C was determined on plate count agar (Oxoid). Membrane-filtered 100 ml samples were incubated 24 h at 37 °C on Tergitol 7 agar supplemented with 2·5% 2,3,5-triphenyltetrazolium chloride (TTC) covered with a thin layer of blue bile agar (Yde & De Maeyer-Cleempoel, 1980) for total coliforms, 24 h at 44 °C on Tergitol 7 agar with 2·5% TTC for faecal coliforms, 48 h at 37 °C on *m*-enterococcus agar (Difco) for faecal streptococci, 48 h at 37 °C on Baird-Parker medium (Oxoid) supplemented with 5% Egg Yolk Tellurite Emulsion (Oxoid) and 0·005% sulphomethazine for *Staphylococcus aureus*, and 48 h at 37 °C on Pseudomonas selective medium (Oxoid) for *Pseudomonas aeruginosa*. Isolates of the latter were sent to Instituut Pasteur van Brabant in Brussels for serotyping and phage typing. The identity of *S. aureus* isolates was confirmed by coagulase and DNase tests. The presence of free-living amoebae was estimated by incubating samples on non-nutrient agar spread with living *Escherichia coli*. Samples of 50 ml either filtered through 0·45 μm membrane filters or concentrated by centrifugation at 2000 ***g*** for 10 min were incubated for comparison. One-millilitre samples and swabs taken from the pool wall were also plated. Samples for amoeba isolation were prepared in duplicate for incubation at 37 °C and 44 °C. A temperature of 44–45 °C is recommended for the isolation of *Naegleria fowleri* (De Jonckheere, 1979*c*).

Naegleria isolates were transferred to serum–casein–glucose–yeast extract medium (SCGYEM) for identification of pathogenic *N. fowleri* (De Jonckheere, 1977*a*). Axenically growing *Naegleria* isolates were further identified using immunofluorescence (Stevens, De Jonckheere & Willaert, 1980) and isoenzyme analysis (De Jonckheere, submitted for publication).

RESULTS

Six hydrotherapy pools disinfected with chlorine and two disinfected with bromine were investigated (Table 1). The total count was low and faecal indicators were absent except when either the chlorine level was too low, the pH too high, or both. *S. aureus* was not found, except in hospital 7 in a small pool not being disinfected. *P. aeruginosa* was encountered especially in the two pools disinfected with bromine and in pool 3, where no free chlorine was detected.

In two hydrotherapy pools sampled the water was 'disinfected' with u.v. in jackets after passing sand filters. The bacteriological quality of the water was discouraging and the pools were therefore sampled on different occasions (Table 2). Apart from very high total counts (up to 10^5/ml), faecal indicators were often identified. *S. aureus* was found only on one occasion in pool B. On the other hand, *P. aeruginosa* was almost always present in high numbers. Different serotypes and phage types were found even on the same date in the same pool (Table 3).

Large differences in the occurrence of free-living amoebae were also observed

Table 1. *Bacteriological results of water from hydrotherapy pools treated with chlorine or bromine*

Hydrotherapy pool	Date (all 1980)	Temp. (°C)	pH	Free chlorine (p.p.m.)	Total chlorine (p.p.m.)	Total count (c.f.u./ml)	Total coliform (c.f.u./100 ml)	Faecal coliform (c.f.u./100 ml)	Faecal streptococci (c.f.u./100 ml)	*S. aureus* (c.f.u./100 ml)	*P. aeruginosa* (c.f.u./100 ml)
1 Inlet	8 July	28	ND	2·0	2·5	1	0	0	0	0	0
Outlet						1	0	0	0	0	3
Filter						232	0	0	0	0	0
2 Inlet†	8 July	34	ND	0·6*	1·0*	75	0	0	0	0	10
Outlet						37	0	0	0	0	>300
Filter						3100	0	0	0	0	4900
3 Inlet	15 July	34	8·05	0	0·2	58800	6	5	4	0	158
Outlet						57500	0	0	3	0	175
Discharge						90500	0	0	5	0	211
4 Pool a	29 July	30	8·6	2·0	2·5	3	43	59	0	0	0
Pool b		33	—	>8·0	ND	1	0	1	0	0	0
Pool c		34	—	>8·0	ND	0	0	0	0	0	0
5 Inlet	29 July	30	8·2	0·1	0·4	7	0	0	0	0	0
Outlet						16	50	68	0	0	0
6 Inlet	5 Aug.	31·5	8·8	>4·0*	ND	1	0	0	0	0	0
Outlet						1	0	0	0	0	0
Filter						44	0	0	2	0	100
Small pool						6	0	0	0	0	22
7 Inlet	18 Nov.	34	7·5	0·4	0·6	30	0	0	0	0	0
Outlet						41	0	0	0	0	0
Small pool		15	—	0	0	6000	42	32	68	700	12
8 Inlet	18 Nov.	36	7·9	>4·0	ND	1	0	0	0	0	0
Outlet						9	0	0	0	0	0
Small pool		34	—	>4·0	ND	1	0	0	0	0	0

c.f.u., colony forming units; ND, not done.

† Stainless steel pool.

* Bromine.

https://doi.org/10.1017/S0022172400070078 Published online by Cambridge University Press

Table 2. *Bacteriological results of water from hydrotherapy pools treated with u.v.*

Date	Place	Temp. (°C)	pH	Total count (c.f.u./ml)	Total coliform (c.f.u./100 ml)	Faecal coliform (c.f.u./100 ml)	Faecal streptococci (c.f.u./100 ml)	*S. aureus* (c.f.u./100 ml)	*P. aeruginosa* (c.f.u./100 ml)
					Hydrotherapy pool A				
4 Feb. 1980	Inlet	35	ND	43200	0	0	54	0	300
	Outlet			41600	0	0	53	0	70
	Filter			133000	0	0	200	0	>10000
5 Feb.	Filter A	ND	ND	×	0	0	2	0	0/ml
	Filter B			×	0	0	0	0	0/ml
18 Feb.	Inlet	38·5	8·5	104000	0	0	3	0	0/ml
	Outlet			91200	0	0	2	0	0/ml
	Filter			80400	0	0	1	0	1/ml
22 June* 1981	Inlet	36	7·3	0	0	0	0	0	0
	Outlet			1	0	0	0	0	0
	Filter			3	0	0	0	0	0
					Hydrotherapy pool B				
25 June 1980	Inlet	31·5	ND	80000	1	2	0	0	3300
	Outlet			89000	4	0	0	0	2200
	Middle			72000	6	0	1	0	2400
4 Nov.	Inlet	32	7·4	7700	12	11	27	0	6800
	Outlet			5500	7	12	27	0	6000
	Middle			9100	9	18	26	0	6000
25 Nov.	Inlet	34	7·2	8400	21	20	13	200	7100
	Outlet			9800	9	24	6	100	4000
	Middle			8300	27	24	6	71	6600
10 Dec.	Inlet	31	7·3	12000	0	2	0	0	1/ml
	Outlet			5000	0	1	0	0	0/ml
	Middle			5400	0	1	0	0	0/ml
24 June 1981	Inlet	31	8·0	17800	16	15	71	0	100
	Outlet			13400	14	11	80	0	180
	Middle			8000	18	12	68	0	90

* Chlorine used (0·8 free chlorine 1·0 total chlorine).
c.f.u., colony forming units; ×, too numerous to be counted; ND, not done.

Table 3. *Serotype and phage type of* P. aeruginosa *isolates*

Source (pool)	Date	Serotype	Phage type
1	8 July 1980	3	21, 24+, 31, 68, F8, 109, 119X, 352, 1214, M4, Col 11+
2	8 July 1980	3	7, 44, 68, F8, 109, 119X, 352, 1214, Col 11
			21, 24, 31, 68, F8, 109, 119X, 352, 1214, M4, Col 11+
3	15 July 1980	1	7, 24, 31, 119X, M4, Col 11
			7, 24, 31, F7, 119X, M4, Col 11
		10	68
6	5 Aug. 1980	6	ND
A	4 Feb. 1980	ND	ND
	18 Feb. 1980	ND	ND
B	25 June 1980	9	31, 44, F8, 109, 119X, 1214, M4, +
			31, 109, 352, 114
		10	119X
	4 Nov. 1980	5	31, 44, 109±
		6	31, 44, 109, 119X
		PANAG	NT
			68+
	25 Nov. 1980	5	31, +
			21, 31, +
	10 Dec. 1980	5	21, 119X, +
	24 June 1981	5	NT
		PANAG	31, 44, F8, 109, 352, 1214, M4
			31

ND, not done; NT, not typable.

between halogenated and u.v.-treated hydrotherapy pools. Although amoebae were isolated from halogenated pools at 37 °C and sometimes even at 44 °C (Table 4), none proved to be *Naegleria* sp. In contrast, amoebae isolated at 44 °C as well as at 37 °C, from the two u.v.-treated pools were mostly *Naegleria* (Table 5). Even 1 ml samples were very often positive (up to 11 *Naegleria*/ml in the filter and 5 *Naegleria*/ml in the pool water). None of the *Naegleria* isolates was identified as a pathogenic *N. fowleri* by axenic cultivation in the selective SCGYEM. Some of the *Naegleria* isolates were further investigated and identified as *N. lovaniensis* by immunofluorescence and absence of virulence for mice. The identity of *N. lovaniensis* strains adapting to the axenic medium was confirmed by isoenzyme analysis of acid phosphatase, leucine amino-peptidase and phosphoglucomutase. Zymograms of leucine amino peptidase (LAP) showed that the *N. lovaniensis* isolates from hydrotherapy pools A and B belonged to different types (Plate 1). Examination of the LAP zymograms of isolates obtained from pool B one year later showed that the same type of *N. lovaniensis* was still present. Pool A was disinfected with chlorine by that time, resulting in the disappearance of *N. lovaniensis*.

Isolation techniques for amoebae were compared during this study. Besides providing a quantitative check on the amoeba occurrence, the centrifugation method was also more often positive than the filtration method. While only 15 (65%) out of 23 of the filtered samples from u.v.-treated pools were positive at

Table 4. *Occurrence in halogenated hydrotherapy pools of amoebae growing at 37 °C (44 °C) on* E. coli

Hydrotherapy pool		On walls	Plaque-forming units in 1 ml	Plaque-forming units in 50 ml concentrated by centrifugation	50 ml concentrated by filtration
1	Inlet	− (−)	0 (0)	0 (0)	+ (−)
	Outlet	− (−)	0 (0)	0 (0)	+ (+)
	Filter	ND (ND)	2 (0)	2 (0)	+ (−)
2	Inlet*	− (−)	0 (0)	2 (0)	+ (+)
	Outlet	− (−)	0 (0)	0 (0)	+ (+)
	Filter	ND (ND)	1 (0)	9 (3)	+ (+)
3	Inlet	− (−)	0 (0)	1 (0)	+ (−)
	Outlet	− (−)	0 (0)	7 (0)	+ (−)
	Discharge	− (−)	0 (0)	0 (0)	+ (−)
4	Pool a	− (−)	0 (0)	0 (0)	− (−)
	Pool b	− (−)	0 (0)	0 (0)	− (−)
	Pool c	− (−)	0 (0)	0 (0)	− (−)
5	Inlet	+ (−)	0 (0)	5 (0)	− (−)
	Outlet	+ (+)	0 (0)	2 (0)	− (−)
6	Inlet*	− (−)	0 (0)	0 (0)	− (−)
	Outlet	− (−)	0 (0)	0 (0)	− (−)
	Filter	− (−)	0 (0)	2 (0)	+ (−)
	Small pool	− (−)	0 (0)	0 (0)	− (−)
7	Inlet	− (−)	0 (0)	2 (0)	+ (−)
	Outlet	− (−)	0 (0)	0 (0)	− (−)
	Small pool	− (−)	0 (0)	4 (0)	− (−)
8	Inlet	− (−)	0 (0)	0 (0)	− (−)
	Outlet	− (−)	0 (0)	0 (0)	− (−)
	Small pool	− (−)	0 (0)	0 (0)	− (−)

* Disinfected by bromine, the others by chlorine.

+, Positive for amoebae; −, negative for amoebae; 0, no plaque-forming units; ND, not done.

37 °C, the number of positives for samples concentrated by centrifugation was 23 (100 %). At 44 °C the numbers are 13 (56 %) and 22 (95 %) respectively. On the other hand, with 24 samples from halogenated pools the centrifugation technique gave 10 positives (42 %) and the filtration technique 11 positives (46 %) at 37 °C. At 44 °C incubation the figures were 17 % and 4 % respectively.

DISCUSSION

The bacteriological quality of halogenated hydrotherapy pool water is comparable to that of halogenated public swimming pool water in Belgium. Also the occurrence of free-living amoebae is comparable for both pool types with a predominance of *Acanthamoeba* (De Jonckheere, 1979*b*).

In cases where bacterial indicators were detected, an ignorance about pool maintenance was noticed in the personnel. This ignorance was manifested by chlorine levels that were too low (pool 3 and 5) or too high (pool 4 and 8) or a pH that was too high (above 8·0). It is worth noting that several of the pool attendants

Table 5. *Occurrence in u.v.-treated hydrotherapy pools of amoebae growing at* 37 °*C* (44 °*C*) *on* E. coli

Date	Place	On walls	Plaque-forming units in 1 ml	Plaque-forming units in 50 ml concentrated by centrifugation	50 ml concentrated by filtration
			Pool A		
4 Feb. 1980	Inlet	+ (+)	0 (0)	NC (NC)	+ (+)
	Outlet	+ (+)	0 (0)	NC (NC)	+ (+)
	Filter	+ (+)	0 (0)	NC (NC)	+ (+)
5 Feb. 1980	Filter A	+ (+)	NC (NC)	NC (NC)	+ (+)
	Filter B	+ (+)	NC (NC)	NC (NC)	+ (+)
18 Feb. 1980	Inlet	+ (+)	4 (2)	× (×)	+ (+)
	Outlet	+ (+)	5 (3)	× (×)	+ (+)
	Filter	+ (+)	11 (3)	× (×)	+ (+)
22 June 1981	Inlet	− (−)	0 (0)	0 (0)	− (−)
(chlorine used)	Outlet	− (−)	0 (0)	1 (0)	− (−)
	Filter	ND (ND)	0 (0)	0 (0)	− (−)
			Pool B		
25 June 1980	Inlet	+ (+)	1 (1)	28 (4)	+ (+)
	Outlet	+ (+)	1 (1)	12 (4)	+ (−)
	Middle	+ (+)	2 (1)	28 (4)	+ (+)
4 Nov. 1980	Inlet	+ (−)	1 (0)	11 (1)	+ (−)
	Outlet	+ (−)	1 (0)	9 (1)	+ (+)
	Middle	+ (−)	1 (0)	16 (0)	− (−)
25 Nov. 1980	Inlet	+ (−)	1 (1)	20 (2)	− (−)
	Outlet	− (−)	0 (0)	9 (4)	− (−)
	Middle	− (−)	0 (0)	12 (2)	− (−)
10 Dec. 1980	Inlet	+ (−)	0 (0)	4 (3)	− (−)
	Outlet	+ (+)	0 (1)	2 (3)	+ (−)
	Middle	+ (+)	0 (0)	2 (3)	− (−)
24 June 1981	Inlet	+ (+)	1 (1)	5 (4)	+ (−)
	Outlet	+ (+)	1 (1)	4 (4)	− (+)
	Middle	+ (+)	1 (1)	3 (5)	− (+)

NC, not counted; ×, too numerous to be counted; +, positive for amoebae; −, negative for amoebae; 0, no plaque-forming units; ND, not done.

were unaware of the fact that bacteriological monitoring of the water has to be performed frequently as is compulsory in public swimming pools. On the other hand, some attendants claimed the water was bacteriologically pure. When tested in my laboratory this appeared not to be the case. The explanation for this was found in the fact that the water had been analysed in the bacteriology laboratory of the hospital. It cannot be stressed enough how different the methods for clinical and water bacteriology are.

Especially bad microbiological results were obtained with the water from hydrotherapy pools treated with u.v. Apart from high total counts in both pools, faecal indicators and *P. aeruginosa* were frequently found in high numbers, especially in pool B. It is worth noting that in pool B the water of the entire pool had been changed the day before sampling on 4 November and 25 November 1980, and yet high numbers of faecal indicators were detected.

The high frequency of *P. aeruginosa* found in hydrotherapy pools (Klenner & Weber, 1979) and swimming pools (Seyfried & Fraser, 1980) when chlorine levels are too low are amply confirmed in this study. *P. aeruginosa* has been isolated more frequently in hospital swimming pools than in other pools (Schindler, 1978). Outbreaks of infections due to *P. aeruginosa* in swimming pools (Reid & Porter, 1981) and health spa whirlpools (Sansker *et al.* 1978) are still being reported. When *P. aeruginosa* was isolated during this study, large numbers were found in the filter. Whether this is due to the concentration effect of the filter or due to growth of the bacteria in the filter as suggested by Botzenhart, Thofern & Külpmann (1974) is not known. The *P. aeruginosa* strains present belonged to different sero- and phage types.

In addition to faecal indicators and *P. aeruginosa*, thermophilic *Naegleria*, identified as *N. lovaniensis* (Stevens *et al.* 1980) were found in high numbers in both u.v.-treated pools. *N. lovaniensis* is frequently found in association with *N. fowleri* and is therefore considered an indicator organism for identifying places where the conditions are favourable for the occurrence of pathogenic *N. fowleri*. Thermally polluted waters where at first only *N. lovaniensis* was isolated (De Jonckheere & van de Voorde, 1977) yielded pathogenic *N. fowleri* upon repeated sampling (De Jonckheere, 1977*b*). Only in aquaria could *N. fowleri* not be identified, while high numbers of *N. lovaniensis* were present (De Jonckheere, 1979*a*). Also in the hydrotherapy pools examined here, the presence of *N. fowleri* could not be confirmed. In Czechoslovakia, pathogenic *N. fowleri* and non-pathogenic thermophilic *Naegleria* sp. were found together in a swimming pool (Kadlec *et al.* 1980) more than 10 years after cases of *Naegleria* meningoencephalitis had occurred in an epidemic manner as a result of swimming in that particular pool. Also in England, thermophilic *Naegleria* were isolated from a warm pool (Warhurst & Mann, 1980) where a girl had been swimming before dying of *Naegleria* meningoencephalitis. In New Zealand *Naegleria* meningoencephalitis has been contracted by swimming in an indoor heat-exchange swimming pool (Cursons *et al.* 1979). In a hydrotherapy pool in Germany, high concentrations of amoebae and cysts were found but thermophilic *Naegleria* were not identified (Michel & Schneider, 1980), probably because free chlorine was present in concentrations of 0·2 p.p.m. In England, an unidentified thermophilic *Naegleria* was demonstrated in a remedial pool (Warhurst & Stamm, 1976).

Although no epidemiological correlation was sought, nosocomial *Naegleria* infections may be possible especially with u.v.-treated hydrotherapy pools. The hospitals involved were therefore urged to alter the disinfection method in order to obtain a safe microbiological quality of the water.

As a result the water of pool A was chlorinated by addition every morning of Javel water to give a level of approximately 1·0 p.p.m. free chlorine as determined by the DPD test. The bacteriological quality of the water improved immediately (Table 2). Also *Naegleria* were no longer present although one unidentified amoeba was detected (Table 5). This result is in accordance with laboratory-scale experiments where it was found that free chlorine concentrations of 0·5 p.p.m. kill *Naegleria* cysts (De Jonckheere & van de Voorde, 1976), while even lower doses are known to destroy the trophozoite stage immediately.

In hydrotherapy pool B the u.v. 'disinfection' method remained in use. The bacteriological quality of the water continued to be bad (Table 2), and thermophilic *N. lovaniensis* were still present. The strains of *N. lovaniensis* were of the same type as those isolated from the same place one year earlier. Because of the favourable conditions for *N. fowleri* growth (high water temperature, no disinfectant and bacteria for food), hydrotherapy pool B remains a threat to human health.

The results of the comparison between two isolation methods show that the centrifugation technique is not more favourable when applied to halogenated waters, while it is much better when applied to u.v.-treated waters. This can be explained by the difference in genera isolated and the life stage wherein they are present. In halogenated baths amoebae are mostly *Acanthamoeba*, probably because they are in the cystic stage and as such can resist the disinfecting agent. Being in the cyst stage they can also better withstand the filtration procedure. On the other hand, amoebae isolated from the u.v.-treated hydrotherapy pools were probably in the trophozoite stage as no efficient disinfectant was present. Trophozoites are probably damaged by the stresses of filtration, although the filter never became dry at the end of the filtration procedure. During studies on the occurrence of *Acanthamoeba* spp. (De Jonckheere, 1981) and *N. fowleri* (De Jonckheere, 1978) in thermal discharges, it was also found that the centrifugation method yields more positives than the filtration method. It may be assumed that amoebae in thermally polluted discharges and surface waters are mostly in the trophozoite stage as they are not under adverse conditions.

Although stringent standards exist for public swimming pools controlled by the Public Health Services nothing comparable seems to exist for hydrotherapy pools in hospitals. However, in hospitals more stringent standards should be applied and controlled even more, because patients coming into contact with the water may be more susceptible to infection. Therefore a disinfection with halogens should be properly applied. It was found during the study that this was not always the case.

Although u.v. might be considered a valuable method for disinfection of secondary effluents (Severin, 1980), where no complete sterilization is needed, our results clearly indicate that it should not be used in hydrotherapy pools nor swimming pools, where residual protection is needed in the pool water.

The technical assistance of H. Coppens, J. Weyckmans and F. Vogeleer is acknowledged. I thank Dr C. Godard for *P. aeruginosa* typing.

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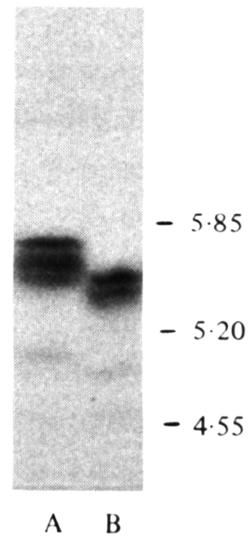
EXPLANATION OF PLATE 1

Leucine amino peptidase isoenzyme patterns using agarose isoelectric focusing of *N. lovaniensis* from hydrotherapy pool A and B. The reference isoelectric points on the right are obtained with bovine carbonic anhydrase B (pI 5·85), β-lactoglobulin A (pI 5·20) and soybean trypsin inhibitor (pI 4·55).

https://doi.org/10.1017/S0022172400070078 Published online by Cambridge University Press



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