BY T. M. S. REID AND I. A. PORTER Regional Laboratory, City Hospital, Aberdeen

(Received 27 November 1980)

SUMMARY

Pseudomonas aeruginosa was isolated from the ears of 18 of the 25 members of a team of competitive swimmers who complained of painful discharging ears. This group of swimmers trained twice daily in the pool, in the early morning and late afternoon. In contrast swabbing of the ears of a similar group of 54 competitive swimmers who used the pool only in the afternoon revealed only one swimmer with P. aeruginosa. Investigation of the swimming pool revealed that chlorination was often inadequate when the first group of swimmers were training in the early morning. Strains of P. aeruginosa were isolated from various sites around the pool and from the bag of a vacuum used to clean the pool.

Pyocin typing, serotyping and phage typing were performed on all isolates. The dominant strain recovered from the swimmers' ears was found to be almost identical to that from the vacuum bag and belonged to serotype 0-11 which has been particularly associated with outbreaks of *P. aeruginosa* infection in whirlpools in the United States.

These results support the hypothesis that there is a direct correlation between the development of otitis externa and swimming in water contaminated with P. *aeruginosa*.

INTRODUCTION

There is renewed interest in the association of infections in swimmers and the microbiological quality of swimming pool waters (Galbraith, 1980). In particular the recent outbreaks of skin infection which have occurred in the United States as a result of the increasing popularity of heated whirlpool baths have highlighted the potential hazards of *Pseudomonas aeruginosa* in bath waters (McCausland & Cox, 1975; Jacobson, Hoadley & Farmer, 1976; Washburn, Jacobson & Narstib, 1976; Sausker *et al.* 1978).

P. aeruginosa can be isolated from swimming pool waters, particularly if there is a high bather load, inadequate chlorination or increased water temperature (Brodsky & Nixon, 1974; Hoadley, Ajello & Masterson, 1975; Schindler, Metz & Hellwig, 1978; Kush & Hoadley, 1980). Under certain conditions such strains can become resistant to chlorine treatment (Black *et al.* 1970; Seyfried & Fraser, 1980).

P. aeruginosa is frequently isolated from the ears of swimmers with otitis externa (Hoadley & Knight, 1975; Weingarten, 1977; Seyfried & Fraser, 1978) and has recently been implicated in outbreaks of otitis externa in saturation divers in

the North Sea (Alcock, 1977). However there is still debate about the relative importance of local trauma, the degree of hydration of the ear canal and the presence of P. aeruginosa in the pathogenesis of otitis externa (Wright & Dineen, 1972; Wright & Alexander, 1974; Hoadley & Knight, 1975).

This report describes an outbreak of otitis externa in a group of competitive swimmers and illustrates the use of serotyping, pyocin typing and phage typing techniques to attempt to define the epidemiology of the strains of P. aeruginosa isolated from the swimmers' ears and the environment of the swimming pool.

MATERIALS AND METHODS

The swimming pool

The swimming pool had a capacity of 135000 gallons with a water change of 45000 gallons per hour. Chlorination was performed by means of cylinder chlorine and filtration by air agitated sand. Water entering the pool was first heated then filtered and finally chlorinated. The pool was normally closed from midday on Saturday until 06.00 hours on Monday during which time there was no chlorination. Free chlorine levels and pH were routinely measured at the poolside and frequent water samples taken for estimation of free chlorine by the diethyl-pphenylene diamine method. Samples were taken for bacteriological testing every two months. During the investigation of the outbreak repeated water samples were taken from various sites throughout the day and were analysed.

Bacteriological examination of water samples was performed by the membrane filtration method (Report 1969). Any growth of non-coliform organisms was subcultured on to Pseudomonas agar (Oxoid) and milk agar (Brown & Scott Foster, 1970) for identification of P. aeruginosa.

The swimmers

The group of swimmers (Squad A) who had reported the 'ear problem' consisted of 25 members (ages 9–17). This squad trained in the pool twice daily for 2 hours in the early morning and evening five days a week. A similar squad of 54 swimmers (Squad B) also used the pool but only in the afternoon five days a week. Ear swabs were obtained from all members of both groups. These were plated on blood agar and MacConkey agar and enriched in Robertson's meat broth.

The environment

Swabs were taken from the surrounds of the pool, kayaks, foot baths, waste channelling, pumps, hoses, the vacuum and a mop used by the pool attendant. The pool was occasionally used for training offshore personnel in survival techniques. The inflatable rafts, pumps, anchors and life jackets used in the pool during these sessions were also examined for the presence of pseudomonads.

Typing of isolates

The strains of *P. aeruginosa* isolated from the swimmers and the environment were serotyped (Habs, 1957) and phage typed (Asheshov, 1974) at the Central Public Health Laboratory, Colindale. In addition pyocin typing (Govan, 1978) was performed at the Department of Bacteriology, University of Edinburgh.

RESULTS

P. aeruginosa was isolated from one or both ears of 18 members of squad A, all but two having experienced ear discomfort. Of the remainder, three had complained of symptoms and had been treated prior to swabbing. *P. aeruginosa* was isolated from only one swimmer in Squad B. He had complained of a painful discharging ear. Seven others who gave a history of ear problems had been treated with 'ear drops' in the month prior to swabbing. No occasional users of the pool had complained of ear symptoms. The cultures from the vacuum bag, mop and one of the inflatable offshore rafts yielded growths of *P. aeruginosa*. None of the other environmental samples grew *P. aeruginosa*.

Specific enquiry at the time of the outbreak revealed that the pH of the water varied from 7.3 in mid afternoon to 7.6 early on Monday morning. Similarly peak levels for free chlorine were found in mid afternoon (1.5-2.5 mg/l) while samples taken at 06.00 hours on a Monday morning had free chlorine levels of 0.2-0.4 mg/l. The temperature of the pool water varied from 79 to 83 °F. The bather load rarely exceeded 100 bathers per day.

None of the water samples from the pool showed any growth of E. coli at 37 °C or faecal coli at 44 °C. However specimens taken early on Monday morning when the free chlorine was low (0.2-0.4 mg/l) produced a heavy growth of non-coliform organisms on the membrane filter none of which proved to be *P. aeruginosa*.

Typing of isolates

Serotyping revealed eight different serological types but serotype 11 predominated (Table 1). It was isolated from 23 of the 28 isolates from the swimmers' ears and from the vacuum bag. However, when pyocin typing was performed it was found that the isolates from the vacuum bag and 1 swimmer were 10/h in contrast to the majority of serotype 11 strains which were 1/h. In addition the latter group failed to show any S-type inhibition whereas the three 10/h strains inhibited indicator strains 3, 6 and 7.

Finally phage typing confirmed that the majority of ear isolates were similar and that the 10/h (3s, 6s, 7s) serotype 11 strains were distinct as they were lysed by three phages not active against the 1/h group namely 21, F7 and Col 18.

Source of isolate	Number of isolates	Serological type			
		́О	н	Pyocin type	Bacteriophage type
Swimmers' ears	22	11	1,2,5	1/h	44, 68, 119X, 1214 68, 119X, 1214 68, 1214 68
Swimmer's ear	1)	11	1,2,5	10/h	21, 68, F7, 119X, Col 18
Vacuum bag	2∫			$(3s, 6s, 7s)^*$	
Swimmer's ear	1	NT	2	NT	68
Swimmer's ear	1	NT		1/h	NT
Swimmer's ear	1	8	1	1/c	21, 31, 68
Swimmer's ear	1	1	1,2	47/F	21, 68, F7, F8, 109, 119X 332, 1214, M4, Col 11
Swimmer's ear	1	5d	2,3	1/e	16, 21, 68, F8, 109, 1214
Мор	2	8	6	10/c	NT
Survival raft	1	3	3	35/v	7, Col 11

Table 1. Summary of typing results

* s - type inhibition. NT denotes not typable.

DISCUSSION

Analysis of the typing data indicates that this outbreak was caused by a single predominant strain of P. aeruginosa (Table 1). In addition the environmental sampling showed that a strain identical to that recovered from the ear of one swimmer was found in the vacuum bag. This strain appeared very similar to the epidemic strain but pyocin and phage typing demonstrated distinct differences. These findings simply underline the importance of using more than one typing method when investigating an outbreak.

Despite the relative frequency with which pseudomonad species other than P. aeruginosa can be isolated from the swimming pool waters they are rarely implicated in human infection (Von Graevenitz, 1973). This would seem to support the view that the presence of P. aeruginosa in the ear canal is of critical importance in the pathogenesis of otitis externa in swimmers.

Furthermore certain serotypes of P. aeruginosa may be more pathogenic for man than others. It is of interest that serotype 11 which is often present in pool waters has been the dominant strain in this outbreak, the whirlpool associated infections (Jacobson et al. 1976, Kush & Hoadley, 1980) and in the outbreaks of otitis externa in divers (Alcock, 1977). It seems likely that the infecting strain of P. aeruginosa was either derived from the skin surface of a swimmer or was carried by feet into the pool and its surrounds from the environment as would appear to be the case in the whirlpool outbreaks (Hoadley, 1978). Having gained access to the pool the contaminating organism was able to survive and perhaps multiply due to the inadequate chlorination. The importance of not only maintaining adequate levels of free chlorine in pool waters but also ensuring that the surrounds and facilities are kept in a hygienic condition cannot be over emphasised. We wish to thank all those who assisted in the investigation of this outbreak and in particular Dr H. N. M. Thomson, the Environmental Health Officers Aberdeen District Council and the Staff, Regional Laboratory, City Hospital, Aberdeen. We are indebted to Dr J. R. W. Govan, Department of Bacteriology, University of Edinburgh and Dr T. L. Pitt, Central Public Health Laboratory, Colindale for typing the isolates of *P. aeruginosa*.

REFERENCES

- ALCOCK, S. R. (1977). Acute otitis externa in divers working in the North Sea; a microbiological survey of seven saturation dives. *Journal of Hygiene* 78, 395-409.
- ASHESHOV, E. H. (1974). An assessment of the methods used for typing strains of *P. aeruginosa*. Proceedings of the VIth International Congress of Bacteriology, Athens, pp. 9-22.
- BLACK, A. P., KEIRN, M. A., SMITH, J. J., DYKES, G. M. Jr. & HARLAN, W. E. (1970). The disinfection of swimming pool water. II. A field study of the disinfection of public swimming pools. American Journal of Public Health 60, 740-50.
- BRODSKY, M. H. & NIXON, M. C. (1974). Membrane filter method for the isolation and enumeration of *Pseudomonas aeruginosa* from swimming pools. *Applied Microbiology* 27, 938-43.
- BROWN, M. R. W. & SCOTT FOSTER, J. H. (1970). A simple diagnostic milk medium for *P. aeruginosa*. Journal of Clinical Pathology 23, 172-7.
- GALBRAITH, N. S. (1980). Infections associated with swimming pools. *Environmental Health* 88, 31-3.
- GOVAN, J. R. W. (1978). Pyocin typing of *Pseudomonas aeruginosa*. Methods in Microbiology **10**, 61–91.
- HABS, I. (1957). Research on O-antigens of P. aeruginosa. Zeitschrift für Hygiene und Infectionskrankheiten 144, 218–22.
- HOADLEY, A. W. (1978). Potential health hazards associated with Pseudomonas aeruginosa in water. In Bacterial Indicators/Health Hazards Associated with Water. American Society for Testing and Materials Special Technical Publication 635, pp. 80-114.
- HOADLEY, A. W., AJELLO, G., & MASTERSON, N. (1975). Preliminary studies of fluorescent pseudomonads capable of growth at 41 °C in swimming pool waters. Applied Microbiology 29, 527-31.
- HOADLEY, A. W. & KNIGHT, D. E. (1975). External otitis among swimmers and non-swimmers. Archives of Environmental Health 30, 445-8.
- JACOBSON, J. A., HOADLEY, A. W. & FARMER, J. J., III. (1976). Pseudomonas aeruginosa serotype 11 is the cause of pool associated skin rash. American Journal of Public Health 66, 1092-3.
- KUSH, B. J. & HOADLEY, A. W. (1980). A preliminary survey of the association of *Pseudo-monas aeruginosa* with commercial whirlpool bath waters. American Journal of Public Health 70, 279-81.
- McCAUSLAND, W. J. & Cox, P. J. (1975). Pseudomonas infection traced to motel whirlpool. Journal of Environmental Health 37, 455-9.
- REPORT (1969). The Bacteriological Examination of Water Supplies. Reports on Public Health and Medical Subjects no. 71. London: H.M.S.O.
- SAUSKER, W. F., AELING, J. L. FITZPATRICK, J. E. & JUDSON, F. N. (1978). Pseudomonas folliculitis acquired from a health spa whirlpool. Journal of the American Medical Association 239, 2362-5.
- SCHINDLER, P. R. G., METZ, H. & HELLWIG, R. (1978). Pseudomonas aeruginosa im schwimmbeckenwasser. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene Abteilung 1/Originale B 167, 462-9.
- SEYFRIED, P. L. & FRASER, D. J. (1978). Pseudomonas aeruginosa in swimming pools related to the incidence of otitis externa infection. Health Laboratory Science 15, 50-7.
- SEYFRIED, P. L. & FRASER, D. J. (1980). Persistence of Pseudomonas aeruginosa in chlorinated swimming pools. Canadian Journal of Microbiology 26, 350-5.
- VON GRAEVENITZ, A. (1973). Clinical microbiology of unusual Pseudomonas species. Progress in Clinical Pathology 5, 185-218.

WASHBURN, J., JACOBSON, J. A. & MARSTON, E., (1976). Pseudomonas aeruginosa rash illness associated with a whirlpool. Journal of the American Medical Association 235, 2205-7.

WEINGARTEN, M. A. (1977). Otitis externa due to Pseudomonas in swimming pool bathers. Journal of the Royal College of General Practitioners 27, 359-60.

- WRIGHT, D. N. & ALEXANDER, J. M. (1974). Effect of water on the bacterial flora of swimmers' ears. Archives of Otolaryngology 95, 15-8.
- WRIGHT, D. N. & DINEEN, M. (1972). A model for the study of infectious otitis externa. Archives of Otolaryngology 95, 243-7.