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Staphylococci in swimming pool water

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SUMMARY

During a period of five years 1192 water samples from swimming pools were examined for staphylococci and 338 for coliform organisms only. Eighty-nine different pools were sampled.

Numbers of staphylococci, estimated by the membrane filtration technique did not bear any significant relation to either bathing load or concentration of free chlorine.

Wide variation in the staphylococcal count was observed when different parts of a pool were sampled on the same occasion.

The only practicable standard for pool samples in relation to staphylococci would appear to be that these organisms should be absent from 100 ml. water when the pool has been out of use during at least ten hours before sampling if filtration and chlorination are adequate.

INTRODUCTION

There have been several suggestions that coliform organisms were not the best indicators of bacterial quality of chlorinated swimming pool waters (Victorin, 1974). Determination of staphylococci in particular was advocated by Ferramola & Elena Durieux (1951) but they neither made counts nor reported free chlorine concentration. Robinton, Mood & Elliot (1957) suggested examining a sample of 100 ml. using a membrane filter for cocci; they found that staphylococci predominated in samples with low residual chlorine and suggested that estimation of free chlorine was a sufficient test for safety because of the rapid rate of kill with a high concentration. A Report (1953) by the Public Health Laboratory Service Water Subcommittee concluded that staphylococci were too resistant to chlorine to be satisfactory indicator organisms. Favero, Drake & Randall (1964) advocated the use of staphylococci as indicators of pool pollution and proposed a standard of fewer than 100 staphylococci per 100 ml. water. Keirn & Putnam (1968) considered 30 staphylococci/100 ml. in less than 15 per cent of samples to be a realistic standard. Robinton & Mood (1966) showed that bathers shed staphylococci most consistently and in the largest number compared with other organisms. Villa & Zaffino (1970) concluded that no single test was adequate but that both coliform

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organisms and staphylococci should be looked for concurrently in assessing the results of chlorination of pool water samples. Barnard (1972) examining samples from a hydrotherapy pool found a high count of Gram positive cocci whenever bathers were in the water whatever the free chlorine concentration.

This paper describes an investigation to determine whether enumeration of staphylococci in chlorinated swimming pool water provides a reliable indication of swimming pool contamination which might be used as a guide to management of water treatment.

METHODS

Chlorine estimations were made at the pool side by using the diethyl-*p*-phenylene diamine (DPD) test (Palin, 1957). Samples for culture were taken about two inches below the surface in sterilized bottles containing sodium thiosulphate and transported immediately to the laboratory. Presumptive coliform organisms were estimated by the multiple tube method using MacConkey broth with Teepol 610 substituted for bile salts. Staphylococcal counts were made by the membrane filtration technique described in Report (1969) using Oxoid staphylococcal medium 110 with addition of bromo-thymol blue, membranes being incubated for 48 hr. at 30 °C.

RESULTS

During 1967, between May and September, 227 water samples from 20 swimming pools of various types were examined both for most probable number (MPN) of presumptive coliform organisms and for numbers of staphylococci. Seventy-eight samples (34 %) were found to contain neither coliform organisms nor staphylococci; 60 samples (26 %) contained both and 84 samples (37 %) contained staphylococci but no coliform organisms. Five samples (3 %) yielded coliform organisms but no staphylococci. Thus 144/227 samples (63 %) contained staphylococci. Numbers of staphylococci were less than 100/100 ml. in 62 samples (43 %) and 100 or more/100 ml. in 82 (57 %) samples. Of staphylococci isolated 65 % were found to be coagulase positive.

In 1968 a study was made of the distribution of coliform organisms and staphylococci in 133 samples grouped according to total (free and combined) chlorine concentration. In 44 samples (33 %) neither coliform organisms nor staphylococci were found, a figure close to that of 1967. Table 1 shows the results. It will be noted, summing appropriate entries of the table, that staphylococci were detected in 84/133 samples (63 %), a figure identical with that obtained in 1967, but only 21/37 (57 %) samples with 1·0 parts per million (ppm) or more total chlorine contained staphylococci whereas 41/56 (73 %) samples with less than 0·5 ppm did so. In only five samples (3·8 %) were coliform organisms found in the absence of staphylococci, all these samples being taken from pools which were out of use and whose water received no treatment.

In 1969 an investigation of coliform organisms, including *Esch. coli*, in relation to the presence of bathers at the time of sampling was made. Table 2 shows results on 338 samples grouped according to the free chlorine concentration from which it

Table 1. *Numbers of samples showing association between coliforms and staphylococci in three ranges of chlorine concentration. Results of* 1968

	Total chlorine (ppm)			
	< 0·5	0·5–0·9	1·0 and above	
Coliforms absent Staphylococcus absent	13	17	14	44
Coli absent Staphs present	21	15	16	52
Coli present Staphs present	20	7	5	32
Coli present Staphs absent	2	1	2	5
Totals	56	40	37	133

Table 2. *Numbers of samples with coliforms and* E. coli *according to the presence of bathers and two ranges of chlorine concentration. Results of* 1969

	Free chlorine < 1·0 ppm		Free chlorine ⩾ 1·0 ppm		
	No bathers	Bathers present	No bathers	Bathers present	Totals
Total samples	78	114	54	92	338
Samples with coliforms	29	45	8	8	90
Samples with *Esch. coli*	6	22	0	0	28

can be seen that among 192 samples with free chlorine of less than 1·0 ppm 74 (38·5 %) yielded coliform organisms and 28 (14·5 %) *Esch. coli*, whereas 146 samples with free chlorine of 1·0 ppm and over yielded coliform organisms in only 16 (10·9 %) but none proved to be *Esch. coli*. It is evident that, as expected, coliform organisms are reduced in number by increasing free chlorine concentration. From Table 2 it can also be seen that of 206 samples taken when bathers were present 53 (25·7 %) yielded coliform organisms and 22 (10·7 %) *Esch. coli*. Samples taken when no bathers were present numbered 132 of which 37 (28 %) yielded coliform organisms and only 6 (4·5 %) *Esch. coli*. It can be concluded that the presence of bathers at the time of sampling increases the frequency of isolation of *Esch. coli* if the free chlorine concentration is less than 1·0 ppm.

In 1970 samples from 89 pools, including those previously sampled, were examined by the staphylococcal count only and classified according to the free chlorine concentration and the number of bathers using the pool at the time of sampling to determine whether numbers of staphylococci in samples were associated with a low chlorine concentration or with the presence of bathers (Tables 3 and 4). These pools were of all varieties of size and chlorination methods but all were filtered. The best straight line of multiple regression fitted to the data of Table 3 was:

$$\text{count of staphylococci} = 22{\cdot}1 - 1{\cdot}67 \times \text{chlorine concentration} + 4{\cdot}56 \times \text{presence}$$

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Table 3. *Some results of* 1970 *showing numbers of samples with fewer than* 100 *staphylococci per* 100 *ml. according to presence of bathers and amount of free chlorine*

Staphylococcal count (mean of range)	Chlorine		Bathers	
	Low (< 1 ppm)	High (⩾ 1 ppm)	Absent −	Present +
0	28	32	35	25
5	45	55	58	42
15	19	25	26	18
25	8	15	10	13
35	14	5	11	8
45	8	9	8	9
55	6	2	4	4
65	5	7	5	7
75	5	7	6	6
85	5	7	6	6
95	4	3	3	4
Total	147	167	172	142

Table 4. *Some results of* 1970 *showing mean counts of* 87 *samples with* 100 *and more staphylococci per* 100 *ml. and chlorine* ⩾ 1 *ppm.*

Staphylococcal mean count	Chlorine			
	1	1·5	2·5	3·5
	Number of samples			
302	—	—	—	2
378	—	—	9	—
456	50	—	—	—
530	—	26	—	—

of bathers. Neither 1·67 nor 4·56 differs significantly from zero (t = (respectively) 0·06 and 0·17 and would have been exceeded by chance in 80–90 % of trials).

Table 4 shows the number of samples of which the chlorine concentration was high and the mean staphylococcal count 100 or more per 100 ml. Thirty-seven samples with 1·5 or more ppm chlorine concentration gave a mean count of 302 or more staphylococci. The best straight line was:

count of staphylococci = $466{\cdot}5 - 42{\cdot}6 \times$ chlorine concentration. 42·6 does not differ significantly from zero (t = 0·11 and would be exceeded by chance in 90 per cent of trials).

From these two sets of findings it can be concluded that the association of staphylococcal numbers with absence of bathers and concentration of chlorine was not significant.

In 1971 samples examined numbered 431 (Table 5) of which 156 contained less than 1·0 ppm free chlorine and 275 over this amount. In the group with less than 1·0 ppm there were 67 samples. (42·9 %) containing fewer than 10 staphylococci per 100 ml. whereas in the group with 1·0 ppm and over 148 (53·8 %) contained fewer than 10 staphylococci/100 ml. Of 75 samples yielding 100 staphylococci or

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Table 5. *Showing distributions of counts of staphylococci at low and high free chlorine concentrations of samples of* 1971. *Number of samples at each range.*

Count (mean of range)	Free chlorine (< 1ppm)	Free chlorine (⩾ 1 ppm)
0	33	75
5	34	73
15	14	27
25	10	21
35	3	13
45	0	10
55	5	4
65	3	5
75	5	5
85	3	1
95	4	8
137	16	14
277	10	6
43	6	6
705	6	1
1292	4	6

over/100 ml. 42 had less than 1·0 ppm and 33 had more than this concentration of free chlorine. The regression line fitted to all the results of Table 5 was:

count of staphylococci = $148 - 52{\cdot}4 \times$ concentration of chlorine. 52, unlike the regression of Tables 3 and 4, differs significantly from zero ($t = 2{\cdot}35$ and $0{\cdot}02 > p > 0{\cdot}01$).

Variability amongst samples

The results so far obtained were calculated as the mean of counts on two samples of pool water taken from different places, usually the deep and shallow ends, on the same occasion.

To assess the variability of staphylococcal numbers in different parts of a pool at the same time, two pools (A and B) were examined by taking three samples on each occasion, bathers being absent. Results were highly variable indicating a far from homogeneous staphylococcal distribution probably due to aggregation of organisms on skin squames and in mucus. It will be seen (Table 6) that on 12 occasions the count at the inlet was higher than counts at the middle and outlet. The total variances of the numbers of staphylococci in pools A and B were analysed into those ascribable to position in the pool, (α) those to occasion of sampling (β) and those to error (γ). For neither pool were the variance ratios α or β divided by γ significant at the 5% point; indeed for pool A (Table 6) variance due to γ exceeded that due to α and β. When samples were taken from pool A before use by the first bathers of the day, after water circulation through the filters and chlorination had continued all night, it was found that staphylococci were present, if at all, only in small numbers (5/100 ml.). When the free chlorine was raised to 1·0 ppm no staphylococci were found after all night water treatment during 10 hours or more. Similar results were obtained with other pools.

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Table 6. *Counts of staphylococci on 32 sampling occasions and three places at Pool A*

Inlet	Middle	Outlet	Inlet	Middle	Outlet
0	564	29	14	1	0
103	5	61	24	64	0
0	4	1	13	66	86
25	79	55	4	4	5
0	0	0	38	5	6
13	42	0	0	6	0
8	28	9	12	10	4
0	0	3	0	5	64
1	2	5	350	1	1
10	0	0	13	66	86
60	12	18	36	45	57
0	48	400	7	1	9
152	8	7	7	19	114
48	191	46	140	64	87
106	18	21	332	25	54
0	0	0	6	4	0

DISCUSSION

A review of the literature reveals that the use of swimming pools is occasionally associated with subsequent illness. Hellerström (1951) reported skin nodules containing acid-fast bacilli occurring after small abrasions while bathing in swimming pools. There have been later accounts of granulomata following injury by the rough sides of pools (Tolmach & Frank, 1953; Thomas, 1967). In the Netherlands, Sluyter (1973) found tuberculin conversion in children associated with use of a swimming pool from which Runyon group IV organisms, but not *Mycobacterium balnei*, were isolated. English & Gibson (1959) found swimming pools an important source of tinea pedis infection. Plantar warts occurred more commonly among children using heated, covered pools than among users of other types of pool (Allen & Dickinson 1968). Gentles & Evans (1973) showed that both verruca and tinea pedis infection spread among the users of a covered swimming pool. In the U.S.A. Bell and co-workers (1955) investigating outbreaks of conjunctivitis in summer camps found the incidence 50% greater among users of camp pools than among non-users. It has been long established that inclusion conjunctivitis is associated with the use of swimming pools, the TRIC agent responsible, a member of the *Chlamydia* (*Bedsonia*) genus, evidently being initially derived from vaginal or urethral discharges of bathers and subsequently from conjunctival washings of those infected. Non-infective conjunctivitis may be caused by combined chlorine. Ormsby & Aitchison (1955) in Canada reported pharyngoconjunctival fever spread by swimming pool use. van der Veen & van der Ploeg (1958) associated adenovirus infection with swimming in an unchlorinated pool as did Kallings & Madsen (1961) and Foy, Cooney & Hatlen (1968). McLean (1963) isolated parainfluenza 1 virus from swimming pool water. Clarke, Stevenson & Kabler (1956) concluded that transmission of adenovirus in a 'well chlorinated' pool was unlikely because water without demonstrable coliform organisms was

probably free from adenovirus. Anderson & Jamieson (1972) described a case of meningoencephalitis due to *Naegleria fowleri* in swimming pool water from which the organism was not eradicated by chlorination to 10 ppm.

Epidemiological evidence is slowly accumulating that bathers using badly managed pools are at risk from cross-infection. It must be remembered that even if pool water is of satisfactory standard the floors of the pool surround and changing rooms must also be kept at a high standard of cleanliness.

Results reported here show that staphylococci, of which about 65% are *Staph. aureus*, are always present in water of a pool in use if coliform organisms are also present and that their numbers are unlikely to be a useful indication of the hygienic condition of a pool. The number found was associated in the expected way with the absence of bathers and the presence of chlorine, both of which might be regarded as justifiable measures of the hygiene of a pool, but for two sets of results the regression was not significant (Tables 3 to 5). It is clear that counts from one or two samples, which are likely to be submitted in practice, could never be used for a judgement. This view is reinforced by the results from samples of pools *A* (Table 6) and *B* which show that no significant part of their variance could be ascribed to situation in the pools or occasion of sampling. However, it was observed that staphylococci were absent from 100 ml. samples when a pool had been out of use during the previous 10 hr. if filtration and chlorination were adequate, that is to say the water circulation time was not more than 4 hours with free chlorine maintained at 1·0 ppm by breakpoint chlorination. Used in these circumstances, a count of staphylococci might be useful in detecting poor filtration, inadequate chlorination or prolonged circulation time since, when these are satisfactory, staphylococci are absent after ten hours treatment. Otherwise our results confirm the conclusion of the Public Health Laboratory Service Water Subcommittee (Report, 1953) that staphylococci are too resistant to chlorine to be useful indicator organisms in examination of the water of swimming pools.

No evidence of illness attributable to the use of the pools examined was found during five seasons of the investigation. Phage typing of 48 strains of *Staph. aureus* yielded types in all three groups but none were resistant to both penicillin and tetracylcine.

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