Comparative Study of Microbial Flora of Iodinated and Chlorinated Pools

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WITHIN the last two decades the use of iodine as a swimming pool disinfectant has been proposed by a few investigators (*1–5*). The usual method for comparing chlorinated and iodinated swimming pools has been to use pools of similar construction, bathing load, and other factors and then to test their sanitary quality by the use of the standard plate count, the coliform test and, in a few instances, by examinations for fecal streptococci. The results have shown that iodine is equal to, or better than, chlorine as a swimming pool disinfectant.

Recently it has been demonstrated that certain bacteria may accumulate in large numbers in iodinated swimming pools. Mood (*6*) stated that *Alcaligenes faecalis* was found to persist in some iodine-treated pools. Marshall and coworkers (*4*) pointed out that a gradual buildup of certain types of bacteria occurred in an iodinated pool when a cross-connection existed between the overflow gutters and the deck drains. It was determined that 99 percent of this high microbial population consisted of organisms closely resembling *A. faecalis* (*7*). These organisms were found to be more halogen resistant than both the coliform bacteria and the fecal streptococci, so that high total counts in the absence of these indicator organisms were thought to be of little significance with respect to the sanitary condition of swimming pool waters. It was shown that these bacteria were not pathogenic to white mice or rabbits.

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In addition to germicidal considerations these investigations have shown that iodine has several advantages over chlorine. It does not combine with nitrogen to form iodamines so that iodine concentrations are less dependent on bathing loads and the amount of organic matter in the swimming pool water. Iodine has been shown to be an effective microbicide over a relatively large pH range in contrast to chlorine, and the amount of eye and skin irritations is significantly reduced. Although the pH is fairly stable in an iodinated swimming pool, it requires strict control. In many cases too low a pH is accompanied by a disagreeable yellow color.

Byrd and associates (*8*) have recently studied the possibility of iodine in swimming pools being absorbed by bathers. Protein-bound iodine and urinary total iodine determinations were taken on 30 selected swimmers. No significant blood or urine changes were observed as a result of swimming in iodinated pools. The majority of these swimmers preferred iodine over chlorine, and medical examinations of both eyes after a 1-month period revealed complete absence of conjunctivitis.

There are two basic methods for iodinating swimming pools. The most common one is the so-called banking method in which a relatively large amount of potassium iodide (KI) is added to the swimming pool every 5 to 10 days, and a small amount of hypochlorous acid, derived either from gaseous chlorine or hypochlorite solutions, is added continuously. In the second method, the one employed in this study, a small amount of KI is added daily along with a relatively larger amount of a chloramine, dichlorodimethyl hydantoin. The chloramine acts to provide a slow release of hypochlorous acid which, in turn, releases free iodine from the iodide ions. In both methods the chlorine functions primarily as an activator rather than as a microbicide.

The objective of this study was to compare the predominant microbial flora of iodinated and chlorinated swimming pools. Since data consisting of only coliform counts and total counts contribute little to information about the sanitary condition of swimming pools, a relatively large spectrum of micro-organisms consisting of the total viable count, coliform bacteria, enterococci, staphylococci, *Streptococcus salivarius* and *Pseudomonas aeruginosa* were investigated. For quantitative determinations the membrane filter method was used with suitable selective and differential media (*9*).

Methods

Five recirculation-type swimming pools were studied. Two (pools E and F) were 150,000-gallon indoor pools (sand filters), one (pool D) was a 88,000-gallon outdoor public pool (diatomaceous earth filters), and two were 30,000-gallon outdoor private pools (pool H had a sand filter and pool V a diatomaceous earth filter). All pools were sampled during both chlorinated and iodinated periods. Pools E and F were chlorinated for 4 months and then iodinated for 4 months. Pools D, H, and V were chlorinated for 1 month and iodinated for 2 months.

Samples of 500-ml. volume were obtained from each pool, using sterile bottles containing sodium thiosulfate. Colorimetric halogen determinations were done at the time of sampling and pH determinations were performed electrometrically. Samples were immediately brought to the laboratory and processed within 20 to 60 minutes after collection.

The following microbiological examinations were made. Total viable counts were determined by the pour plate method and total viable counts on larger volumes by the membrane filter technique on standard plate count agar. These plates were incubated at 35° C. and counted at 24 hours and in some instances again at 96 hours.

Coliform bacteria and the enterococci were determined by the membrane filter method according to the 11th edition of "Standard Methods for Examination of Water and Wastewater" (*10*).

Staphylococci were determined by the membrane filter technique on both Chapman-Stone agar (Baltimore Biological Laboratory) and phenol red mannitol salt agar (Difco) (*9*).

Numbers of *Streptococcus salivarius* were determined by the membrane filter technique on Drake-Chaplin medium (*11*) modified by the addition of 2,3,5-triphenyltetrazolium chloride. One ml. of a sterile 1 percent solution was added to each 100 ml. of sterile, melted, and cooled medium.

Pseudomonas aeruginosa were determined by two methods. The first was by the use of tech medium (*12*) plus 0.05 percent hexadecyltrimethyl ammonium bromide in conjunction with the membrane filter method. Colonies of *P. aeruginosa* on this medium were typically translucent and produced green or blue pigments. This method is considered to be more a qualitative than a quantitative test because a single cell of *P. aeruginosa* may not grow or produce a pigmented clone on this selective medium. Also, if more than 20 colonies of *P. aeruginosa* were present, counting was difficult because of the increased amount of diffused pigments which colored the entire membrane filter blue or green.

For quantitative determinations of *P. aeruginosa* the method of Drake (*9*) was used. This technique employs the most probable number method. The primary medium was asparagine enrichment broth and positive tubes (the production of blue or green pigment or very good growth at 35° to 37° C.) were confirmed in acetamide confirmatory broth.

All media were incubated for 48 hours at 35° C. except for the standard plate count agar which was incubated for 24 and 96 hours, m–Endo broth for 18 hours, and phenol red mannitol salt agar for 36 hours. Media with membrane filters were incubated in a humidified incubator.

Pools D, E, and F were chlorinated with gaseous chlorine; calcium hypochlorite tablets

Table 1. Average test findings for four pools during chlorinated and iodinated periods

Water factors	Chlorination			Iodination		
	Average	Range	Number positive samples	Average	Range	Number positive samples
	Institution pool F (39 samples of chlorinated water, 28 samples of iodinated water)					
Halogen concentration (ppm)	0. 54	0. 1–1. 0	--------	0. 87	0. 5–1. 2	--------
Total viable count per ml	11	0–300	37	123	2–2, 000	28
Staphylococci per 100 ml	70	1–658	39	24	0–60	25
Coliforms per 100 ml	1. 6	0–58	4	0	0	0
Streptococcus salivarius per 100 ml	3. 1	0–52	8	0	0	0
Enterococci per 100 ml	. 9	0–32	3	. 5	0–10	3
Pseudomonas aeruginosa per 100 ml	0	0	0	0	0	0
Number of bathers per day	80	75–100	--------	75	50–101	--------
pH	7. 7	7. 4–8. 0	--------	7. 8	7. 6–7. 9	--------
	Institution pool E (23 samples of chlorinated water, 47 samples of iodinated water)					
Halogen concentration (ppm)	. 38	0. 2–1. 0	--------	. 67	0. 3–1. 4	--------
Total viable count per ml	52	0–500	20	69	2–241	47
Staphylococci per 100 ml	204	0–1, 159	19	23	0–254	41
Coliforms per 100 ml	1. 1	0–10	5	0	0	0
Streptococcus salivarius per 100 ml	3. 5	0–40	5	. 1	0–4	2
Enterococci per 100 ml	1. 8	0–22	4	. 1	0–4	2
Pseudomonas aeruginosa per 100 ml	0	0	0	. 1	0–3. 5	1
Number of bathers per day	150	75–300	--------	75	51–102	--------
pH	7. 5	7. 3–7. 8	--------	7. 7	7. 5–8. 0	--------
	Outdoor public pool D (26 samples of chlorinated water, 31 samples of iodinated water)					
Halogen concentration (ppm)	. 24	0. 1–1. 0	--------	. 68	0. 2–2. 0	--------
Total viable count per ml	18	0–330	25	267	1–1, 744	31
Staphylococci per 100 ml	84	1–1, 180	26	32	1–305	31
Coliforms per 100 ml	. 4	0–8	2	0	0	0
Streptococcus salivarius per 100 ml	1. 6	0–16	7	. 1	0–4	1
Enterococci per 100 ml	. 1	0–2	2	. 2	0–2	4
Pseudomonas aeruginosa per 100 ml	0	0	0	. 4	0–3. 3	4
Bathing load per day	120	44–228	--------	167	12–346	--------
pH	7. 5	7. 0–8. 1	--------	7. 8	7. 6–8. 1	--------
	Private pool V (10 samples of chlorinated water, 30 samples of iodinated water)					
Halogen concentration (ppm)	. 17	0–1. 0	--------	. 76	0–1. 4	--------
Total viable count per ml	73	0–590	9	3. 2	1–13	30
Staphylococci per 100 ml	132	2–562	10	45	0–27	22
Coliforms per 100 ml	. 2	0–2	1	0	0	0
Streptococcus salivarius per 100 ml	. 8	0–8	1	0	0	0
Enterococci per 100 ml	2	0–20	2	. 07	0–2	1
Pseudomonas aeruginosa per 100 ml	0	0	0	. 5	0–12	2
Number of bathers per day	3	0–11	--------	2	0–8	--------
pH	8. 0	7. 6–8. 5	--------	7. 7	7. 4–7. 9	--------

were used in the two private pools H and V. For the iodinated periods, potassium iodide and dichlorodimethyl hydantoin were applied daily in the ratio of 1 to 2 grams of KI to 6 to 10 grams of chloramine per 1,000 gallons of water.

Results

Table 1 shows that the numbers of staphylococci, enterococci, *Streptococcus salivarius*, and coliforms were less in the iodinated periods of all the pools tested. *Pseudomonas aeruginosa* was isolated on a few sporadic occasions while pools E, D, and V were on iodine. The total counts were significantly higher during the iodinated periods of all the pools tested with the exception of private pool V. The data from private pool H, which are not shown, were similar to pool V, except that the total counts were higher during the iodinated period. Although iodine concentrations were two to three times as high as the chlorine concentrations in all five pools, complaints of eye irritation were rare. Algal growth occurred sporadically in the three outdoor pools during the chlorinated periods when the chlorine levels were very low, but none occurred during the iodine treatment.

Table 2. Findings for public outdoor pool D during iodinated period

Number of days on iodine	Iodine concentration (ppm)	Total count per 2 ml.	Number bathers
3	1.0	3	15
4	.9	3	20
8	.4	3	320
9	.4	3	354
10	.6	3	262
11	.7	2	272
13	1.3	1	285
15	.5	5	50
16	1.4	2	69
17	----------	3	92
18	.8	22	97
22	1.0	15	346
24	1.0	27	333
25	2.0	28	306
29	.7	233	325
30	.4	596	342
31	.2	536	311
38	.3	1,160	12
39	.3	1,920	75
43	.7	343	150
44	.4	339	143
45	.3	2,600	96
46	.2	1,060	71
51	.9	1,340	24
52	.8	1,627	82
53	.2	3,484	76

Table 3. Findings for institution pool E during once-a-day use, iodinated period

Water factors	Morning no bathers [1]	Afternoon no bathers	Afternoon bathers
Total count per ml	149	41	97
Staphylococci per 100 ml	0	5	29
Iodine concentration (ppm)	.2	.6	.6
Number of samples tested	4	5	6

[1] Samples were taken prior to the addition of iodine.

There was a gradual increase in the total viable count in pool D during the iodinated period (table 2). This increase was not accompanied by a decrease in the free iodine concentrations or an increase in the bathing load. There was also no corresponding increase in the number of indicator organisms. This phenomenon occurred in all pools except pool V. Unfortunately, the full significance of these higher total counts was not realized until the end of the bathing season. However, pool E was still being used by one group of bathers once a day, and relatively high total counts were observed. Several samplings were conducted during the last week of the pool's use to determine if the total counts were high only at the time of bathing or at all periods.

Even when no bathers were present at both the morning and afternoon samplings, the total counts were relatively high (table 3). In the authors' experience, total counts of chlorinated pools, even at low concentrations of free chlorine, are very low especially when the pool has been free of bathers for a long period of time. Total counts of less than 10 bacteria per ml. are not uncommon.

A total of 150 isolates were picked at random from plates inoculated with water from iodine-treated pools. All were gram-negative rods and their biochemical activities were identical to *A. faecalis*. However, staining by Leifson's method (*13*) revealed that the cells had a single polar flagellum which would place them in the order Pseudomonadales. These observations were confirmed by electron micrographs. Classification of these bacteria is incompletely established, but the data indicate that they are similiar to *Pseudomonas alcaligenes* recently described by Ikari and Hugh (*14*).

Table 4 illustrates a phenomenon that many investigators and present standards (*10, 15*) overlook. Favero and Drake (*16, 17*), studying the survival rates of certain bacteria in chlorine solutions, noted that some chlorine-injured cells of *Staphylococcus aureus* would not exhibit visible growth in 48 hours when plated in trypticase soy agar. As much as a sixfold increase in the number of visible colonies occurred when these plates were recounted after 96 hours of incubation. With this in mind, colonies were counted at the normal 24-hour period, reincubated, and counted again at 96 hours. The percent increase was calculated and averaged. The data (table 4) indicate that the increase was significant, although there does not seem to be any correlation with the counting method or type of swimming pool.

Discussion

It is evident from the study findings that iodine was an efficient microbicide in the swimming pools tested. Iodine appeared to be more effective than chlorine against both the standard fecal indicators, the coliform bacteria and the enterococci, and against staphylococci derived from the mouth, nose, and skin areas. This may be partly explained by the fact that high iodine concentrations were easily maintained and were relatively more stable than chlorine concentrations in the pools.

Table 4. Effect of extended incubation on the standard plate counts of halogen-treated swimming pools

Pool	Number samples tested	Average increase in number of colonies from 24 to 96 hours at 37° C. (percent)	Range (percent)
Institution pool E:			
Pour plate	16	712	49–3, 460
Membrane filter	3	516	316–773
Institution pool F:			
Pour plate	13	207	5–690
Membrane filter	4	145	39–277
Public outdoor pool D:			
Pour plate	16	645	0–2, 075
Membrane filter	10	1, 211	42–4, 460
Private pool V:			
Pour plate	13	551	0–4, 337
Membrane filter	5	128	36–333

The significance of the higher total counts of viable organisms in the iodinated pools is difficult to assess. Obviously, there was a flora of certain types of bacteria able to withstand iodine levels that were bactericidal to the indicator organisms used. The fact that high total counts did not occur until after iodine treatment had been used for a certain period of time suggests that the bacteria were either naturally iodine resistant or that iodine-resistant mutants were being selected for and were accumulating in these pools.

Unlike the studies of Marshall and co-workers (*4*) the high total counts in the indoor pools could not be correlated to cross-connections between the deck and gutter drains. In pool E the decks drain to waste while the gutters drain back into the main recirculation system. In pool F the decks and gutters both drain to waste. However, it is possible that cross-connected deck and gutter drains could serve as a reservoir of contamination in these swimming pools.

Since the identity of these bacteria is still in question, it is difficult to determine the significance of their presence. As was stated previously, they are physiologically identical to *A. faecalis* which is not considered pathogenic. A relatively high density of naturally iodine-resistant nonpathogenic bacteria in swimming pools is probably not significant with respect to human health. However, such high total counts are undesirable for a number of reasons. The theoretical possibility of these micro-organisms being potential pathogens, however remote, cannot be overlooked (*18*). Also it has long been the tradition for sanitary microbiologists to strive to keep the total counts in all systems relatively low as a means of evaluating contamination. Consequently, these high total counts would interfere with the interpretation of tests now used to evaluate the sanitary quality of swimming pool waters.

The presence of *P. aeruginosa* requires special consideration. Since this organism is a common but often overlooked cause of otitis externa and other infections, its presence in swimming pools is highly undesirable. The limited data presented show its occasional presence in iodinated pools and its absence in the chlorinated pools. A larger series of observations, not yet reported, shows its frequent presence in chlorinated pools whenever the chlorine level drops below a critical point. Since chlorine levels fluctuate over a far wider range than do iodine levels, *P. aeruginosa* will frequently appear unless the pool receives meticulous attention, a condition that we have yet to observe in regularly operated pools. During this comparative series we apparently succeeded in limiting the fluctuations in chlorine levels so that this organism did not occur. Further work is indicated with respect to the comparative chlorine and iodine resistance of *P. aeruginosa*.

In addition to the standards already proposed (*9*) we would suggest that iodinated pools which develop a flora of iodine-tolerant organisms, as indicated by high total counts with low or negative findings for indicator organisms, receive a temporary treatment with free chlorine.

The data presented in table 4 indicate that extended incubation has a significant effect on the total viable count in swimming pool waters as determined by either the pour plate method or the membrane filter technique. Many halogen-injured bacteria cannot exhibit visible growth in the normal 24-hour period. The metabolic injuries and the subsequent extended lag phase require up to 96 hours of incubation before visible growth occurs. However, these injured bacteria in another type of medium or a richer natural growth environment, such as the body, may show excellent growth. It is suggested that if various health agencies continue to advocate the use of the standard plate count to determine the sanitary quality of halogen-treated waters, this phenomenon should be taken into consideration.

Summary

In a comparative study of the predominant microbial flora of five swimming pools alternately iodinated and chlorinated, there were fewer staphylococci, enterococci, coliform bacteria, and *Streptococcus salivarius* during the iodinated period, but total viable counts were significantly high. Iodine-resistant pseudomonads, physiologically identical to the nonpathogenic *Alcaligenes faecalis*, were responsible for the high total viable counts. These organisms were tentatively identified as *Pseudomonas alcaligenes*. The membrane filter technique in conjunction with suitable media was used in making quantitative determinations.

Effects of extended incubation on the standard plate count of chlorinated and iodinated swimming pool waters were investigated by counting plates after 24 hours and again after 96 hours incubation at 35° C. Increases as high as 4,460 percent occurred in the 72-hour interval.

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Bibliography on Fluoride in Public Health

"The Role of Fluoride in Public Health," an annotated bibliography, by Irene R. Campbell, assistant professor of industrial health, has been published by the Kettering Laboratory, College of Medicine, University of Cincinnati.

The references tend to establish the basic principles of the role of fluoride in dental health. The book contains a glossary and an index for the 158 abstracts of 1958–62 publications. They were selected on the basis of scientific reliability and usefulness to those responsible for public water supplies. The compilation is based on a continuous review of the literature covered by the medical and scientific abstracting and indexing services and those of the legal and social sciences, as well as of reports of health organizations and universities. This and a 1958 comprehensive bibliography on "The Occurrence and Biological Effects of Fluorine Compounds, Volume I, The Inorganic Compounds," were supported by a grant from the National Institute of Dental Health, Public Health Service.

Both are available from the Division of Bibliographic Research, Kettering Laboratory, College of Medicine, Eden Ave., Cincinnati, Ohio. Also, a continuing abstract card file of the comprehensive bibliography is available to libraries of health departments and of interested research organizations at a nominal annual subscription fee.