Use of Staphylococci as Indicators of Swimming Pool Pollution

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THE USE of coliform organisms as indicators of contamination has long dominated the thinking of sanitary bacteriologists, and there would appear to be more than a chance relationship to the great reduction in enteric infections during this period. However, the equally logical use of indicator organisms derived from the nose, mouth, and related structures has lagged behind. It is obvious that contamination of air, eating utensils, swimming pool waters, and similar materials is not necessarily best measured by determining the presence of organisms derived from the intestinal tract. The total count, an alternate method sanctioned more by use than logic, is often substituted for or combined with the determination of coliform organisms. Unfortunately, such use of the total count gives little useful information. A high total count does not distinguish among biological growth in a system containing some type of utilizable nutrient, the introduction of large numbers of relatively harmless extraneous organisms from dust, soil, and similar sources, and the introduction of organisms from the mouth, throat, nose, and skin surfaces of persons using the facilities. Clearly, high total counts of organisms derived from nose, mouth, and skin sources, especially in swimming pools, imply the direct danger of transmission of potential pathogens of many types.

A further reason for questioning the methods now used to determine the sanitary quality of swimming pools lies in the fact that various organisms derived from the mouth, nose, throat, and skin areas have a relatively higher resistance to the chlorine concentrations common in swimming pools than do the coliform organisms. Experience has shown that chlorine levels which eliminate the coliform bacteria will also eliminate *Salmonella* and *Shigella*. However, there is no evidence that the staphylococci, streptococci, and various viral agents are equally susceptible. Indeed there is strong evidence to the contrary. Ritter and Treece (*1*) showed that *Staphylococcus aureus*, *Streptococcus salivarius*, and the enterococci were 5 to 20 times more resistant to chlorine than the coliform bacteria. Favero (*2*) and Favero and Drake (*3*) showed that *S. aureus* was relatively more resistant to chlorine than *Escherichia coli* and *Pseudomonas aeruginosa*. Part of these results are illustrated in the chart.

Dick noted in data on an artificial swimming pool seeded with salivary specimens, which were further inoculated with staphylococci, that the staphylococci were quite chlorine resistant. He also noted that staphylococci could be found in some fairly well-run swimming pools with high chlorine residuals (personal communication, E. C. Dick, 1962).

During the past 40 years several investigators have maintained that the coliform bacteria or other intestinal micro-organisms are an inadequate measure of the sanitary quality of swimming pools. Mallmann (*4–6*) and others (*7,8*) have advocated streptococci as indicators of

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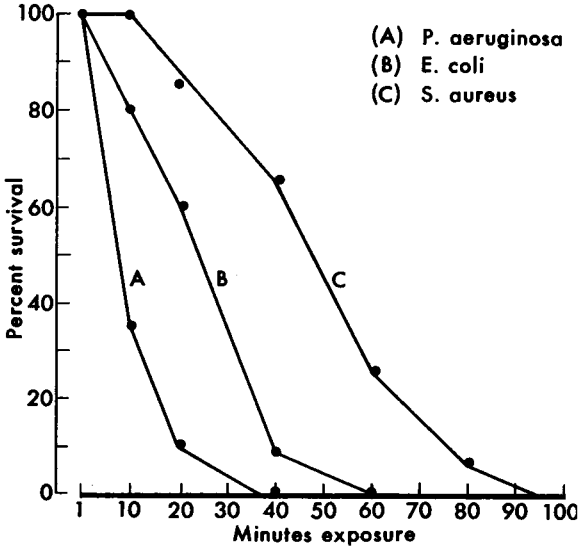
swimming pool pollution. A "coccus" index was proposed by Seligmann in 1951 (*9*). This index was determined by the most probable number method using azide dextrose broth. A test tube was presumed positive if there was sufficient growth to give visible turbidity. Confirmatory tests were done by staining the sediment and demonstrating gram-positive cocci in chains of four or more. In examining 132 isolates from 15 positive tubes of azide dextrose broth, it was shown that the positive tubes consisted of 43 percent *Micrococcus* (*Staphylococcus*) *epidermidis*, 27 percent *Streptococcus mitis*, 15 percent *Streptococcus salivarius*, 10 percent *Streptococcus faecalis*, and 5 percent unidentified micrococci. Seligmann suggested that the "coccus" index of swimming pools should be less than 15 per 100 ml.

In a study of the effect of free and combined available chlorine upon bacteria in swimming pools, Mood (*10*) showed that streptococci were more resistant to both free and combined available chlorine than were the coliform bacteria.

Robinton and co-workers (*11*) reported that the micro-organisms most apt to survive high free-chlorine residuals were those of the *Bacillus* and *Micrococcus* (*Staphylococcus*) genera. They also found that the high free-chlorine method, that is employing free chlorine residuals well above 1 ppm in pool water of pH 8 to 8.2, efficiently destroyed organisms as rapidly as they enter the water. They proposed that bacteriological examinations be replaced by chemical tests which determine the concentration of free chlorine.

Percentage survival of organisms suspended in 0.03 ppm free chlorine at pH 7.0 and 23° C.



Dick and co-workers (*12*) suggested that uniform streaking of blood agar plates be substituted for coliform tests as an index of swimming pool pollution from human sources. They based their proposal on the relative chlorine resistance of many pharyngeal organisms.

Other workers (*13,14*) demonstrated that several viruses were more or equally resistant to free chlorine than the coliform bacteria.

In an attempt to determine the validity of present methods and to develop a more logical procedure, the predominant bacterial flora of swimming pools of different types and use loads were studied. Pertinent data concerning these pools are given in table 1.

Methods

This 2-year study covered 12 recirculation type swimming pools of varied size, use load, and filtration systems. Samples of 500-ml. volume were obtained from each pool in sterile bottles containing sodium thiosulfate. Colorimetric chlorine determinations were performed at the time of sampling, and pH determinations were done electrometrically. Data were gathered on the use load of the pool at the time of each sampling. The pools were sampled during the peakload periods, and samples were taken in those areas of the pool where most of the bathers had congregated. The collected samples were immediately brought to the laboratory and processed, usually within 20 minutes and never more than 1 hour after collection. Several bacteriological examinations were made.

Total counts were determined by the pour plate method and total counts on larger volumes by the membrane filter technique on standard plate count agar.

Coliform bacteria and enterococci were determined by the membrane filter method according to the 11th edition of "Standard Methods for the Examination of Water and Wastewater" (*15*).

S. salivarius numbers were determined by the membrane filter technique using Drake-Chaplin medium (*16*) modified by the addition of 2,3,5-triphenyltetrazolium chloride (1 ml. of a sterile

Table 1. Operational data on 12 swimming pools

Pool and type	Capacity in gallons	Type of chlorination	Type of filtration system
A. Private	35, 000	Calcium hypochlorite	diatomaceous earth
B. Public outdoor	150, 000	gas	diatomaceous earth
C. Public outdoor	190, 000	gas	pressure, sand
D. Public outdoor	88, 000	gas	diatomaceous earth
E. University indoor	150, 000	gas	pressure, sand
F. University indoor	150, 000	gas	pressure, sand
G. Semipublic outdoor	75, 000	Calcium hypochlorite [1]	pressure, sand
H. Private outdoor	30, 000	Calcium hypochlorite	pressure, sand
K. Private outdoor	20, 000	Sodium dichloroisocyanurate	pressure, sand
L. Private outdoor	30, 000	Sodium dichloroisocyanurate	pressure, sand
M. Public outdoor	200, 000	gas	pressure, sand
R. Private outdoor	35, 000	Calcium hypochlorite	pressure, sand

[1] Also used sodium dichloroisocyanurate for half of the bathing season.

1 percent solution was added to each 100 ml. of sterile, melted, and cooled medium). On this selective medium *S. salivarius* appear as gumdrop-like colonies which are tinted pink.

Staphylococci were determined by the membrane filter technique. At least two media, Chapman-Stone agar (Baltimore Biological Laboratory) and phenol red mannitol salt agar (Difco) were employed for each sample analysis. In some instances Vogel-Johnson agar (Baltimore Biological Laboratory) was substituted for one of these media.

P. aeruginosa was quantitatively determined by the method of Drake (*17*). Ten-ml. samples were inoculated into each of 3 to 5 test tubes of double-strength asparagine enrichment medium. Pigment production or good growth at 35° to 37° C. was considered a positive presumptive test. Positive tubes were confirmed in acetamide confirmatory medium. The numbers of *P. aeruginosa* were determined by the most probable number method.

The membrane filter technique was also employed to determine the presence of *P. aeruginosa*. "Tech" medium (*18*) plus 0.05 percent hexadecytrimethyl ammonium bromide was found to be a good selective medium for these organisms. Colonies of *P. aeruginosa* were translucent with blue or blue-green soluble pigments easily recognized on the white membrane filter. At present this method is considered qualitative rather than quantitative, since single cells may not grow or produce pigmented clones on this selective medium. When more than 20 colonies were present, counting was difficult because of the increased amount of diffusable pigments which colored the entire membrane.

The standard plate count agar was incubated for 24 hours, m-endo broth for 18 hours, phenol red mannitol salt agar for 36 hours, and all other media for 48 hours at 35° C. Media upon which membrane filters had been placed were incubated in a humidified incubator.

During the study it was found desirable to test the efficiency and specificity of the three selective media used for the enumeration of staphylococci from swimming pools. Isolates were obtained from four series of water samples. Series A consisted of 300 colonies isolated from pool samples taken at random intervals during a period of 3 months. One hundred colonies were picked from membrane filters incubated on each of the three media. No attempt was made to choose colonies typical of staphylococci. Series B, C, and D included a total of 51 isolates, and each of these series was composed of isolates from one pool sample.

All isolates were stained and tested for glucose and mannitol fermentation. Coagulase tests were carried out, using reconstituted desiccated coagulase plasma (Difco). Classification of gram-positive cocci as the genus *Staphylococcus*, rather than as the genus *Micrococcus*, was based on the ability to ferment glucose with copious production of acid. Those isolates which produced acid from both glucose and mannitol and which elaborated coagulase were classified as *S. aureus*. Staphylococci which neither fermented mannitol nor produced coagulase were classified as *Staphylococcus epi-*

Table 2. Typical findings from 3 different types of swimming pools

Chlorine (ppm)	Total count per ml.	Staphylococci per 100 ml.	Coliforms per 100 ml.	Enterococci per 100 ml.	*Streptococcus salivarius* per 100 ml.	*Pseudomonas aeruginosa* per 100 ml.
	Public outdoor pool C (daily average of 300–400 bathers and pH of 7.5)					
T[1]	1, 450	2, 400	24	34	78	0
T[1]	1	26	0	0	2	0
0.3	2	33	0	0	0	0
0.4	2	84	0	0	2	0
0.4	1	47	0	0	2	0
T[1]	500	2, 600	12	28	184	2
0.3	13	90	0	0	0	0
0.15	190	60	0	0	4	0
0.15	760	766	108	2	4	0
0.1	10	391	0	0	50	0
0.2	7	317	0	0	72	0
0.4	2	48	0	0	12	0
0.5	2	65	0	0	12	0
0.4	18	340	3	0	50	0
0.3	7	434	0	0	0	0
T[1]	235	766	4	32	402	2
0.4	9	528	0	0	0	0
	University indoor pool E (daily average of 150–200 bathers and pH of 7.5)					
1.0	4	10	0	0	0	0
1.0	2	125	0	0	0	0
0.4	1	72	0	2	0	0
0.2	12	252	0	0	0	0
0.2	6	188	0	0	0	0
0.2	9	5	0	0	0	0
0.2	11	533	0	9	0	0
0.2	66	122	2	0	0	0
0.2	1	92	0	0	0	0
0.2	1	14	0	0	0	0
0.5	0	0	0	0	0	0
0.2	6	90	0	0	0	0
0.4	400	1, 000	0	14	0	0
0.4	6	139	0	0	0	0
0.3	3	0	0	0	2	0
0.3	1	24	0	0	0	0
0.2	500	231	0	0	0	0
	Private outdoor pool K (daily average of 0–1 bathers and pH of 7.9)					
1.0	1	4	0	0	0	0
1.0	11	5	10	0	0	2
1.0	4	0	0	0	0	0
T[1]	91	2	28	0	6	168
0.5	10	0	0	0	0	9
0.1	367	0	0	0	0	10
0	500	8	0	0	0	100
T[1]	15, 000	5	0	0	0	56
T[1]	108	0	2	0	0	36
0.75	24	12	0	0	0	32
0.5	7	16	0	0	0	6
0.2	3, 000	1	0	0	0	6
T[1]	6, 000	12	19	48	0	4
0.75	4, 000	80	2	0	0	232
0.15	100	26	0	0	0	40
0.75	2	0	0	0	0	5
1.0	190	0	0	0	0	8

[1] Trace amounts.

dermidis. Classification of organisms which were either mannitol-negative and coagulase-positive or mannitol-positive and coagulase-negative was not attempted beyond the generic level.

Results

Table 2 shows typical results for several swimming pools. Because of the large number of samples taken, all data for each pool are not shown. Typical data for pool C, an outdoor public pool with a heavy load, show that the number of staphylococci was often very high. They were especially numerous when coliform bacteria or enterococci were present. In addition staphylococcal counts were often high when the chlorine residuals were great enough to eliminate the intestinal bacteria and to reduce greatly the total counts. Essentially the same information was obtained from pool F.

Pool K is a small private pool with a very light load that employed sodium dichloroisocyanurate as a pool disinfectant. It is not surprising that with the very light bathing load there were few staphylococci, enterococci, or *Streptococcus salivarius*, but coliform bacteria were as frequent as in pools with heavy use. The most surprising finding was the frequent presence of the potential pathogen, *P. aeruginosa*, which in laboratory studies was much more susceptible to free chlorine than the coliform bacteria (*2,3*). Also, the total counts tended to be high for no obvious reason. These data suggest that this disinfection system, which is claimed to release free chlorine, does not have as great an effect on *P. aeruginosa* and some other types of bacteria as does free chlorine derived from hypochlorites or gaseous chlorine. The reason for this is not apparent at the present time.

Private pool G used calcium hypochlorite granules for half of the 1961 bathing season. Because adequate free chlorine residuals could not be maintained, the operator changed to sodium dichloroisocyanurate. Although the

Table 3. Average findings for three selected swimming pools

Factor	University indoor pool F (83 samples)		Outdoor public pool C (33 samples)		Outdoor private pool K (21 samples)	
	average	range	average	range	average	range
Chlorine concentration (ppm)	0. 53	0. 1–1. 0	0. 37	0. 1–0. 75	0. 59	0–1. 0
Total count per ml	8	0–300	111	0–1450	1, 150	1–7500
Staphylococci per 100 ml	47	1–658	291	0–2600	8	0–80
Coliforms per 100 ml	. 8	0–58	4. 8	0–108	3	0–28
Streptococcus salivarius per 100 ml	1. 9	0–52	27	0–402	. 28	0–6
Enterococci per 100 ml	. 7	0–32	3	0–34	2. 5	0–48
Pseudomonas aeruginosa per 100 ml	0	0	. 36	0–10	39. 4	0–232
Bathing load per day	90	30–250	300	112–960	. 5	0–4
pH	7. 7	7. 4–8. 0	7. 5	7. 1–8. 3	7. 9	7. 6–8. 9

Table 4. Comparison of efficiency in the primary isolation of staphylococci from membrane filters on three media

Type of isolate	Chapman-Stone agar		Phenol red mannitol salt agar		Vogel-Johnson agar	
	Number	Percent	Number	Percent	Number	Percent
Total isolates	120		120		111	
Staphylococcus isolates	119	99. 1	114	95. 0	106	95. 5
Staphylococcus mannitol-positive	78	65. 6	57	50. 0	104	98. 1
Staphylococcus coagulase-positive	69	58. 0	43	37. 7	104	98. 1
Staphylococcus mannitol- and coagulase-positive	65	54. 6	41	36. 0	104	98. 1

apparent free chlorine residuals were raised and could be maintained for long periods of time, the sanitary quality of the pool water did not improve. The total count increased, as did the concentrations of both the staphylococci and *P. aeruginosa*. As stated previously, *P. aeruginosa* were seldom found in pools which employed gaseous chlorine or hypochlorites and were maintained at moderate chlorine residuals.

Table 3 summarizes the data on three different types of swimming pools. These data further emphasize the marked difference in staphylococcal counts in pools with different load uses. The higher the bathing load, and consequently the more pollution from the mouth, nose, and skin areas of the bathers, the higher the staphylococcal count. While detailed study suggests that enterococci were more resistant to chlorine than were coliform organisms, they do not add significant information not already available from the coliform count in regard to the presence of intestinal bacteria.

Results of the study of the efficiency and specificity of the three selective media used to determine the number of staphylococci are shown in table 4. The data indicate that Chapman-Stone agar was slightly superior to either phenol red mannitol salt agar or Vogel-Johnson agar for the isolation of staphylococci from swimming pools when the membrane filter method was used. Of 120 colonies picked from membrane filters incubated on Chapman-Stone agar, 99.1 percent (119) met the criteria for classification as *Staphylococcus*. The one colony which did not fall into this group was identified as a *Bacillus* sp. Of 120 isolates from membrane filters incubated on phenol red mannitol salt agar, 95.0 percent (114) were classified as *Staphylococcus*, the other 6 as *Bacillus* sp. In both instances the *Bacillus* sp. colonies could be differentiated easily from the typical staphylococcal colonies. Vogel-Johnson agar was found

Table 5. Comparison of media efficiency and specificity in the isolation of staphylococci from equal aliquots of water from the same pool

Series and isolate	Chapman-Stone agar		Phenol red mannitol salt agar		Vogel-Johnson agar	
	Number	Percent	Number	Percent	Number	Percent
Series B						
Total growth on filter	22	----------	20	----------	0	----------
Number viable isolates [1]	6	----------	10	----------	0	----------
Staphylococci isolated	6	100. 0	8	80. 0	0	----------
Staphylococci mannitol-positive	5	83. 3	4	50. 0	0	----------
Staphylococci coagulase-positive	4	66. 6	1	12. 5	0	----------
Staphylococci mannitol- and coagulase-positive	4	66. 6	1	12. 5	0	----------
Series C						
Total growth on filter	4	----------	2	----------	1	----------
Number viable isolates [1]	4	----------	2	----------	1	----------
Staphylococci isolated	3	75. 0	0	----------	1	100
Staphylococci mannitol-positive	0	----------	0	----------	0	----------
Staphylococci coagulase-positive	2	66. 6	0	----------	0	----------
Staphylococci mannitol- and coagulase-positive	0	----------	0	----------	0	----------
Series D						
Total growth on filter	18	----------	23	----------	12	----------
Number viable isolates [1]	10	----------	8	----------	10	----------
Staphylococci isolated	10	100	7	88	9	90
Staphylococci mannitol-positive	6	60	4	57	9	100
Staphylococci coagulase-positive	4	40	2	28. 6	9	100
Staphylococci mannitol- and coagulase-positive	4	40	2	28. 6	9	100

[1] 10 colonies picked where possible.

to be approximately equal to phenol red mannitol salt agar in its selectivity for the genus *Staphylococcus*. Of 111 isolates picked from membrane filters on Vogel-Johnson agar, 95.5 percent (105) were *Staphylococcus*. No *Bacillus* sp. were isolated from this medium, but five pinkish yeast colonies were found and tentatively classified as *Rhodotorula* sp.

For the selection but not necessarily detection of mannitol- and coagulase-positive *S. aureus*, Vogel-Johnson agar was superior to the other two media. The data indicate that 98.1 percent of the staphylococci isolated from membrane filters on Vogel-Johnson agar were *S. aureus*, while 54.6 percent of those isolated from Chapman-Stone agar and 36 percent of those isolated from phenol red mannitol salt agar met these criteria.

Results of series B, C, and D, each representing organisms isolated from equal volumes of water from the same pool, are shown in table 5. Because of the extremely small number of isolates in series C, these data could not be regarded as representative. In series B and D the greater efficiency of Chapman-Stone agar for the isolation of staphylococci was again apparent. The high specificity of Vogel-Johnson agar for *S. aureus* was especially evident in series D. In series B the failure of this medium to isolate any organisms from a water sample in which the presence of staphylococci was amply demonstrated by growth on membrane filters incubated on the other two selective media suggests that Vogel-Johnson agar may be specific for certain strains within the species.

This hypothesis was further substantiated by the fact that throughout this investigation total colony counts on membrane filters incubated on Vogel-Johnson agar consistently averaged far less than either of the other two media. In one series of samples Chapman-Stone agar averaged 28.5 colonies per sample, phenol red mannitol salt agar averaged 26.1, and Vogel-Johnson agar averaged only 1.7. Vogel-Johnson agar may require many staphylococcal cells in order to initiate growth.

Of the 339 gram-positive clumped or clustered cocci isolated, 100 percent fermented glucose and were classified as *Staphylococcus*. Of these 70.5 percent (239) were found to be mannitol-positive as well. Of the 339 isolates, 63.7 percent (216) were coagulase-positive. The correlation between mannitol fermentation and coagulase production is summarized in table 6. Although the isolates which were mannitol-positive and coagulase-negative or mannitol-negative and coagulase-positive were not definitely classified as to species, it is interesting that the percentages in both instances agree almost exactly with the results reported in 1962 by Kilmer (*19*).

Discussion

Although the data available from this study raise as many new questions as they answer old ones, they do indicate that present methods for determining the sanitary quality of swimming pool waters are inadequate. The 3d revision of the Ordinance and Regulations Covering Swimming Pools prepared by the Public Health Service for submission to the Joint Committee on Swimming Pools of the American Public Health Association in 1961 states that, "Not more than 15 percent of the samples covering any considerable period of time shall either (*a*) contain more than 200 bacteria per milliliter, as determined by the standard (35° C.) agar plate count, or (*b*) show positive test (confirmed test) for coliform organisms in any of five 10-milliliter portions of a sample or more than 1.0 coliform organisms per 50 ml. when the membrane filter test is used."

Our results indicate that this standard does not offer any particular degree of safety in clas-

Table 6. Percentage correlation between mannitol fermentation and coagulase activity

Type of *Staphylococcus* isolate	Chapman-Stone agar	Phenol red mannitol salt agar	Vogel-Johnson agar	Total
Total number of isolates	119	114	106	339
Mannitol- and coagulase-positive	54. 6	36. 0	98. 1	61. 9
Mannitol-positive and coagulase-negative	10. 9	14. 0	0	8. 6
Mannitol-negative and coagulase-positive	3. 4	1. 8	0	1. 8
Mannitol- and coagulase-negative	31. 1	48. 2	1. 9	27. 7

sifying swimming pools of good or poor sanitary quality. It is evident from the data that if coliform bacteria were present, the pool was of poor sanitary quality, not because of the presence of the coliform organisms but because of the fact that when these organisms were present, the numbers of staphylococci and streptococci were very high. However, lack of coliform bacteria did not necessarily indicate that the sanitary quality of the pool was adequate. On the contrary, in many instances when the numbers of staphylococci were high, no coliform bacteria were found.

The use of the total count is also in question. In several of the small private pools the chlorine concentrations, at times, fell to a very low level. During these periods, even with no bathers present, the total counts were very high but were not accompanied by high counts of staphylococci or streptococci. A few coliforms were isolated during these periods, but they could have been introduced by dust, hay, grass, and other types of vegetation surrounding these private pools. Since little or no chlorine was present, microbial growth in the pool waters could have occurred very easily. It is our belief that high total counts in such low-use pools with erratic care are of little significance. On the other hand the data in table 2 show that it was not uncommon for a sample from a large, well-operated public pool to pass readily the present standards based on the total count, although it contained large numbers of staphylococci. Also, as will be indicated in a subsequent publication, iodinated swimming pools may have very high total counts. Some were found to contain, on occasion, 3,000 to 10,000 organisms per ml. However, 99 percent of these high total counts consisted of the apparent harmless saprophytes *Alcaligenes faecalis* and *Pseudomonas alcaligenes*.

A fallacy exists in the application of the total plate count by the "Standard Methods" procedure to the enumeration of bacteria present in the water of treated pools. The specified incubation period of 24 to 48 hours, depending on the temperature of incubation, gives erroneously low results. Favero (*2*) and Favero and Drake (*3*) in a study of the effect of chlorine on various micro-organisms noted that extending periods of incubation beyond those recommended in "Standard Methods" greatly increased the plate counts, presumably because of the slow recovery of chlorine-damaged cells. The use of richer media also gave higher counts during the shorter incubation period, since the chlorine-damaged cells could initiate growth on such media more rapidly than on the standard medium. Since the treatment of swimming pool waters with halogens for germicidal effects would be quite comparable to such experimental procedures, the effect of increased incubation was tested on samples from such pools. In tests of 17 to 26 samples from each of 4 pools, total counts increased from 128 to 1,211 percent during the interval between a 24-hour and a 96-hour incubation period.

P. aeruginosa usually did not occur in the large, well-chlorinated pools unless the chlorine residuals were low. However, in private pools which used sodium dichloroisocyanurate, the incidence of *P. aeruginosa* was high. This organism has been identified as one of the principal causative agents of otitis externa among swimmers. Cothran and Hatlen (*20*) isolated several cultures of *P. aeruginosa* from a swimming pool and also isolated cultures of the same organisms from several bathers suffering from otitis externa who had been using the pool facilities. The cultures were sent to this laboratory where Hoff (*21*, *22*), who had developed the first large-scale *P. aeruginosa* phage typing system, showed that both the ear isolates and the pool isolates were of the same phage type.

The authors suggest that at present the best indicator organisms for evaluating the sanitary quality of public and semipublic pools are staphylococci. They are readily detected and have a higher resistance to chlorine than do coliform bacteria so that the absence of staphylococci in numbers implies the absence of coliforms and, consequently, enteric pathogens. In addition to being valid indicators of pollution from the mouth, nose, throat, and skin surfaces of bathers, they are themselves potential pathogens. The selective media used with the membrane filter technique afford an easier, simpler, more accurate, and quantitative means of measuring the pollution in swimming pools than does the most probable number method. What numerical standard should be accepted is problematical, but it is suggested that the

Table 7. Comparison of pool water samples under proposed standard of less than 100 staphylococci per 100 ml. using membrane filter procedures and the present standard of a total count below 200 per ml.

Pool and number of samples	Passed both	Failed both	Passed present, failed proposed	Failed present, passed proposed
Heavy or moderate use:				
B. 33	22	2	9	0
C. 33	21	4	8	0
D. 29	23	1	5	0
E. 57	23	14	19	1
F. 90	72	7	11	0
M. 9	8	0	1	0
Light use:				
A. 10	8	0	1	1
G. 16	5	3	4	4
H. 9	7	0	0	2
K. 21	10	0	1	10
L. 9	3	1	0	5
R. 4	1	0	0	3

numbers of staphylococci allowable should be less than 100 per 100 ml. of sample water. Sampling by the membrane filter technique is suggested, using at least 50 ml. of water and incubating the filter on either Chapman-Stone agar or phenol red mannitol salt agar for 48 hours at 35° C. in a humidified incubator.

Tables 7 and 8 show the results if either the present or the proposed staphylococci criteria for sanitary quality were applied to samples from the 12 pools. The present methods frequently failed to detect pollution in the pools with heavy-to-moderate bathing loads. In almost all instances where pools failed the present standards, they also failed the proposed staphylococcal standard.

It is suggested that in addition to the routine testing for staphylococci, periodic qualitative or quantitative tests for *P. aeruginosa* should be employed at times of obviously heavy contamination or at periods when ear infections occur. The isolation of *P. aeruginosa* in 100 ml. or less of swimming pool water would warrant closure of the pool until an adequate chlorine residual could be maintained. In this study it was found that at free chlorine concentrations of more than 0.5 ppm, *P. aeruginosa* was rarely found except in those pools which used sodium dichloroisocyanurate as a pool disinfectant.

It is of particular interest that in a study of the relation between the incidence of disease and the sanitary quality of bathing waters, Stevenson (*23*) reported a 100 percent increase in disease incidence in swimmers as compared to nonswimmers. His findings particularly support our contention that the eye, nose, throat, and the ears are of special significance, since more than half of the illnesses he reported were ailments of these structures.

Summary

The predominant bacterial flora of 12 public, university, and private swimming pools were studied for a 2-year period to determine the validity of present standards for measuring the water's sanitary quality. Selective media used with the membrane filter method provided a means for the quantitative determination of staphylococci, coliform bacteria, enterococci, *Streptococcus salivarius*, and *Pseudomonas aeruginosa*. Both Chapman-Stone agar and phenol red mannitol salt agar were highly efficient and specific for the isolation of staphylococci.

Intestinal bacteria in pool waters were always accompanied by large numbers of staphylococci and streptococci. However, large numbers of

Table 8. Comparison of pool water samples under proposed standard of less than 100 staphylococci per 100 ml. using membrane filter procedures and the present standard of a coliform count of less than 1 per 50 ml.

Pool and number of samples	Passed both	Failed both	Passed present, failed proposed	Failed present, passed proposed
Heavy or moderate use:				
B. 33	20	1	10	2
C. 33	18	6	6	3
D. 29	22	2	4	1
E. 57	23	17	15	2
F. 90	68	6	13	3
M. 9	7	0	1	1
Light use:				
A. 10	6	0	1	3
G. 16	9	1	6	0
H. 9	7	0	0	2
K. 21	14	0	0	7
L. 9	5	1	0	3
R. 4	4	0	0	0

staphylococci were frequently found when no coliform bacteria or enterococci were present and when the total count per ml. was low. These results indicated that the present standards used to determine the sanitary quality of swimming pools based on the presence of the coliform bacteria and the total count are inadequate.

It is suggested that the staphylococci be adopted as indicators of pollution in swimming pools. They are valid indicators of pollution derived from the mouth, nose, throat, and skin surfaces of bathers and are obviously of concern since they are potential pathogens. Because they are more chlorine resistant than coliform bacteria, the absence of large numbers of staphylococci implies the absence of intestinal bacteria. The authors propose an allowable maximum of less than 100 staphylococci per 100 ml. of water.

Three private swimming pools using sodium dichloroisocyanurate as a pool disinfectant were found to contain large numbers of the potential pathogen, *P. aeruginosa.*

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