

CHARACTERIZATION OF *A. FAECALIS* ISOLATED FROM AN IODINE-DISINFECTED SWIMMING POOL

John D. Marshall, M.S.
Claire B. Wolford, M.S.
John E. Faber, Ph.D.

SOME bacteria whose sanitary significance is obscure have been found to resist concentrations of halogens bactericidal to fecal streptococci and coliform bacteria, the accepted indicators of swimming pool sanitation (1). Many instances of high plate counts in the absence of coliforms are known. The identification of species within these populations is lacking in the literature, although there are several reports concerning the bactericidal properties of iodine disinfection on known intestinal pathogens in water (2-4). We have been unable to discover any information concerning the occurrence in water of a microorganism highly resistant to halogens.

This paper supplies data on the basic physiological characteristics of an organism identified as *Alcaligenes faecalis*, together with information on its resistance to various levels of iodine and chlorine disinfection. The operation of a large swimming pool and the rates and methods of feeding are discussed in another paper (1).

Occurrence and Characterization

During routine analysis (1) of swimming pool water disinfected with iodine, pinpoint colonies, those less than 0.25 mm. in diameter, were consistently observed after 24 hours of incubation at 35° C. on trypticase glucose extract (TGE) agar plates. An increase to 1.0 mm.

in diameter occurred after 48 hours. Standard plate counts made on samples of swimming pool waters containing between 0.2 and 0.8 ppm iodine ranged from 8 to 17×10^2 . Approximately 99 percent of the organisms encountered were of the colony type. During the entire 8 months of this study, not a single positive test for fecal streptococci or coliform bacteria was obtained. In view of the fact that the flora recovered on TGE agar appeared to be almost homogeneous, our interest was directed toward further characterizing these organisms. It should be pointed out that similar observations have been made on waters from pool samples taken at Baltimore, Md., Buffalo, N.Y., Denver, Colo., and elsewhere.

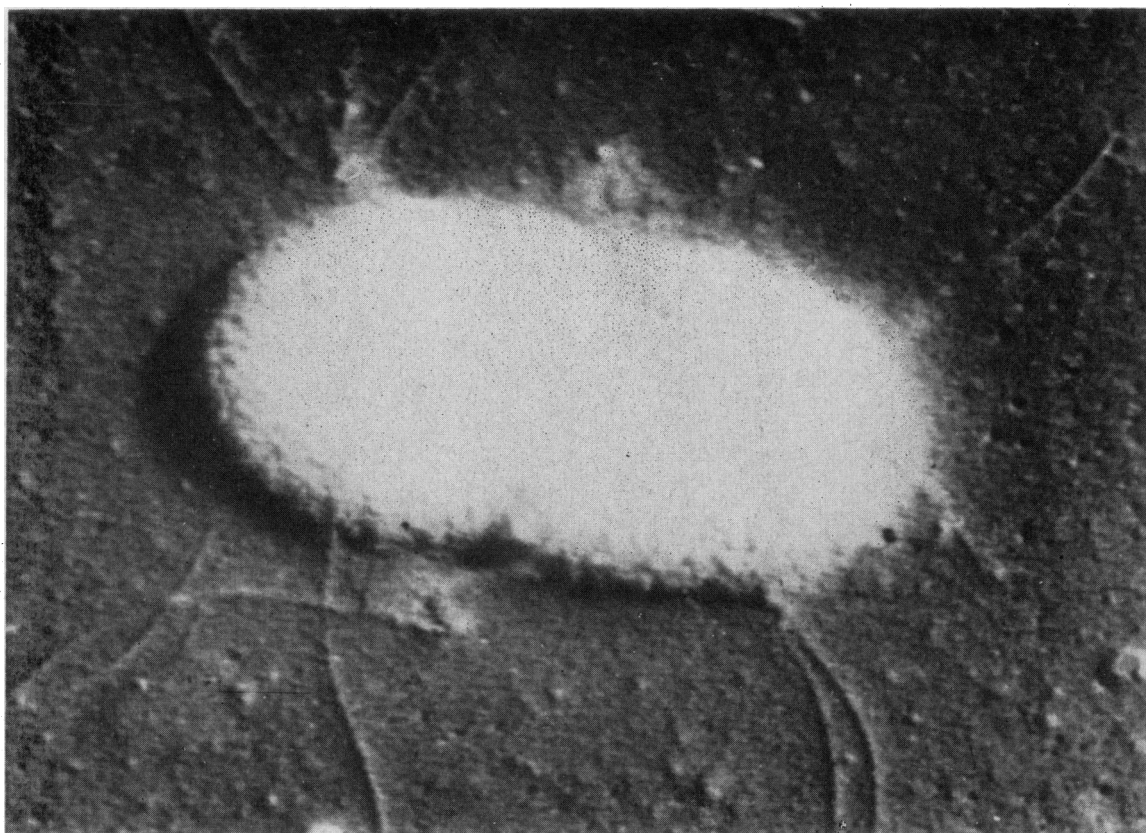
Macroscopic Morphology

As stated above, after 48 hours at 35° C. the colonies were about 1.0 mm. in diameter. They were glistening, convex, mucilaginous, and with no apparent pigmentation. Plates containing pure cultures of these organisms possessed an unclean, characteristic odor upon removing the covers initially. After 10 days at 20° C. the colonies increased in size to approximately 2 to 3 mm. in diameter. The centers were dome shaped, and a faint buff coloration was apparent. In liquid media, the organism grew with the formation of a slight pellicle and a heavy mucilaginous sediment. The sediment formed a characteristic strand when the tube was swirled.

Microscopic Morphology

Twenty-four cultures, grown on either TGE agar or in phenol red broth (Baltimore Biolog-

The authors are with the department of microbiology, University of Maryland, College Park, Md. This investigation was supported by a grant from the Chilean Iodine Educational Bureau, Inc.



***Alcaligenes faecalis* (×35,600)**

ical Laboratory), were found to be gram-negative, bipolarly staining rods, occurring singly or in pairs. The organisms were approximately 2μ in length and 0.9μ in width, as determined by electron microscopy. They possessed a distinct capsule when stained by Maneval's technique. The organism was actively motile when observed in wet preparations and on hydrogen sulfide indole motility medium (Baltimore Biological Laboratory). Spores were never observed. An electron micrograph showing the morphology and flagellar arrangement of these organisms is presented in the photograph.

Physiological Reactions

Physiological reactions were obtained by tests described in the Manual of Microbiological Methods. The organism may be characterized as follows: There was no acid production from xylose, arabinose, rhamnose, glucose, mannose, galactose, fructose, sorbose, glycerol, mannitol, sorbitol, inositol, sucrose, maltose, lactose, raffinose, salicin, inulin, or dextrin.

Citrate was used as the sole carbon source, and the organism grew on desoxycholate agar but not on tellurite agar. Litmus milk was made alkaline without digestion. Gelatin was not liquefied after 21 days' incubation. Tests for indol, nitrate reduction, and hydrogen sulfide were negative. Urea was hydrolyzed, and the methyl red and Voges-Proskauer tests were negative. The organism was not hemolytic on horse blood agar, and it failed to grow in the presence of 3 percent sodium chloride. Colorimetric tests for cytochrome oxidase were positive after 30 seconds. Growth was optimum between 25° and 35° C., and slight at 20° C. There was no growth at 10° or 45° C. Routine sensitivity tests against various antibiotics and sulfa compounds gave the following results: not inhibited by bacitracin, oleandomycin, penicillin, erythromycin, altafur, or furadantin; inhibited by dihydrostreptomycin, triple sulfa, sulfathiazole, albamycin, neomycin, polymixin B, terramycin, mandelamine, dimethylchlortetracycline, and novobiocin.

Pathogenicity Tests

Five 30-gm. white mice were inoculated intravenously with 0.2 ml. of an 18-hour trypticase soy broth culture of the organism. All of the mice survived, and none showed symptoms of any kind. The test dose was then raised to 0.5 ml., and a second set of five mice was inoculated intraperitoneally. Again all mice survived and were asymptomatic. One-half milliliter of broth culture was placed under the eyelids and in the ears of each of three white rabbits weighing 6 to 7 pounds each, and no irritation was observed in any of the sites. Intradermal inoculation of rabbits with 0.1 ml. of broth culture resulted in a slight reddening of the skin at the inoculation site, but this disappeared in 5 days without ulceration.

The growth from a 24-hour culture on a TGE agar slant was suspended in 0.85 percent saline, and standardized to the No. 3 tube of the McFarland nephelometer. Two-tenths milliliter of this solution was injected into the yolk sacs of embryonated eggs, as follows: six eggs with 5-day embryos, seven eggs with 9-day embryos, and four eggs with 12-day embryos. Among the 5-day embryos, one died within 24 hours, two died within 48 hours, and the remaining three survived. Among the 9-day embryos, two died within 48 hours, one died after 72 hours, and the remaining 4 survived. Among the 12-day embryos, one died within 24 hours, and the remaining three survived.

Halogen Tolerance

Stock chlorine demand-free water was prepared in accordance with Standard Methods for the Examination of Water and Waste Water (Fisher Scientific Co. standard iodine solution I_2/I^-). Stock solutions of iodine and chlorine were prepared as follows: N/50 iodine solution was diluted with chlorine demand-free water to give a concentration of approximately 0.3, 0.6, 1.2, 1.8, 3.6, and 4.8 ppm iodine. Iodine solutions in the form of hypiodous acid were prepared by adding 4 ml. of a potassium iodide solution (0.416 mg./ml.) to an aged solution of 1,3-dichloro-5,5-dimethylhydantoin (8.54 mg./l.) and diluting to provide 1.0, 2.0, and 4.0 ppm iodine.

Five percent sodium hypochlorite (NaOCl)

was diluted to give 0.3, 0.5, 0.8, and 1.6 ppm solutions of free chlorine. Combined available chlorine solutions were prepared from a stock solution of $(NH_4)Cl$ and NaOCl to provide final concentrations of 1.0, 5.0, 10.0, 20.0, and 50.0 ppm.

Halogen levels were determined prior to inoculation of the test suspension of bacteria and again at the end of the experiments by amperimetric and iodometric titrations.

To 400 ml. of test solution, 0.25 ml. of a suspension of the test organism was added so that the final count was approximately 4 to 6×10^6 organisms per milliliter. One-ml. samples were withdrawn at intervals of 1, 5, and 10 minutes and placed into 2 ml. of N/4 sodium thiosulfate solution. At the conclusion of the

Table 1. Bactericidal efficiency of halogens against *Alcaligenes faecalis*

Halogen concentration (ppm)		Growth after exposure (minutes)		
Start	30 minutes	1	5	10
Iodine:				
0.32	0.02	+	+	+
.69	.35	+	+	+
1.39	.67	+	+	+
1.86	1.20	+	+	+
3.42	2.54	+	0	0
4.52	3.49	0	0	0
Hypiodous acid:				
1.04/0.77 ¹	1.04/0.48	+	+	+
2.08/0.89	2.08/0.74	+	+	0
4.16/1.72	4.16/1.47	0	0	0
Chlorine (free):				
0.36	0.18	+	+	+
.49	.27	+	+	+
.88	.45	+	+	+
1.60	.75	+	+	0
Chlorine (combined available):				
1.22	1.24	+	+	+
5.14	4.96	+	+	+
10.37	10.10	+	+	+
19.86	19.59	+	+	0
49.99	48.04	+	0	0

¹ Numerator refers to actual iodine concentration; denominator, to residual available chlorine from dichlorodimethylhydantoin.

NOTE: Temperature, 28° C.; pH, 7.85; initial cell concentration, 5×10^6 /ml.

Plus sign indicates growth in glucose-phenol red broth after 72 hours at 35° C.; 0, no growth.

The large decrease in halogen concentration during the 30-minute period was due to the addition of washed bacteria to the flasks (1.6 – 2.4×10^9 organisms). Duplicate flasks treated in like manner but without addition of bacteria showed no drop in halogen concentration during the 30-minute period.

experiment, 0.1 ml. of these treated suspensions was inoculated into glucose-phenol red broth base medium, incubated for 72 hours at 35° C., and observed for visible growth after 24, 48, and 72 hours. In addition, 1 and 0.1 ml. of these treated suspensions were plated in milk protein hydrolysate agar (Baltimore Biological Laboratory) and incubated 48 hours at 35° C. with counts being made after both 24 and 48 hours. Results of these experiments are presented in tables 1 and 2.

Table 1 shows that at least 2.54 ppm of iodine was required to kill 100 percent of the organisms within 10 minutes as determined by failure to grow in glucose-phenol red broth. Iodine in the form of hypiodous acid gave similar results. A residual-free chlorine concentration of at least 0.75 ppm was required to kill 100 percent of the organisms within 10 minutes as determined in the same manner. Combined available chlorine was completely ineffective unless the concentration was at least 19.59 ppm.

Results of the plating experiments are shown in table 2. In order to kill 99.99 percent of the test organism in 10 minutes, the following initial concentrations in ppm were required: iodine, 1.8; hypiodous acid, 1.04; free chlorine, 0.88; and combined available chlorine, 10.37.

Discussion of Results

The characteristics of the organism described above most closely approximate those detailed for *Alcaligenes faecalis* in Bergey's Manual of Determinative Bacteriology. The only excep-

tion noted was the ability of the isolated organism to hydrolyse urea. This isolant apparently possesses no pathogenic properties, as shown by the tolerance of rabbits and mice to relatively large inoculums. In addition, in a previous study (1) no adverse effects were observed on swimmers when the pool water contained as many as 6×10^3 organisms per milliliter.

One of the most important results of this study was the discovery that the organism possesses an extremely high resistance to halogen disinfectants. The tolerance to iodine and chlorine was not only of a greater magnitude than has been hitherto reported in the literature for bacteria, but also was approximately six times greater than that required to kill the "indicator bacteria," namely, *Escherichia coli* and *Streptococcus faecalis*. Additionally, the resistance of our isolant to halogen disinfectants was approximately four times greater than that of various species of the *Salmonella-Shigella* group.

It has been stated above that at no time in this study were fecal streptococci or coliform bacteria detected. About 1 percent of the colonies appearing on standard plates were not *A. faecalis*. This group, mainly pseudomonads, and chromogenic gram-negative rods, was found to be killed by the normal operating concentrations of 0.2 to 0.6 ppm of iodine and chlorine.

The source of *A. faecalis* in swimming pools is obscure, although Bergey's reports that it has occurred in decomposing organic matter.

Table 2. Initial concentration of halogen required to kill at least 99.99 percent of *Alcaligenes faecalis*

Halogen	Time and concentration (ppm) to kill					
	1 minute		5 minutes		10 minutes	
	Concentration	Percent kill	Concentration	Percent kill	Concentration	Percent kill
Iodine as I ₂ ¹	1. 8	99. 991	1. 8	99. 999	1. 8	99. 9999
Iodine as hypiodous acid.....	2. 08	99. 990	2. 08	99. 998	1. 04	99. 992
Chlorine (free).....	1. 60	99. 996	. 88	99. 992	. 88	99. 9999
Chlorine (combined available).....	49. 99	99. 991	19. 86	99. 993	10. 37	99. 992

¹ The next lower dilution, 1.4 ppm, killed 98.25 percent of the organisms in 10 minutes.

NOTE: Temperature, 28° C.; pH, 7.85. Initial concentration, 5×10^6 /ml.

Studies are in progress in our laboratory to determine possible modes of entry of this organism in such waters. Stevenson and Wetzler investigated the environmental contamination of pool water by total plate counts (personal communication). Pool decks, overshoes, duckboards, stair wells, shower stalls, and locker rooms were found to be reservoirs of *A. faecalis*. Such investigation pointed up the absolute need for total environmental sanitation of all pool premises, often neglected in pool management.

The significance of *A. faecalis* in total counts of swimming pool water is difficult to assess. If, as in our experience, 99 percent of the colonies appearing on standard plates are of this type, it would be presumptuous to condemn such water for sanitary reasons, even if the numbers of colonies exceeded generally accepted values for total bacterial counts. We would stress the qualitative rather than the quantitative aspects of such water, particularly in the light of the results presented here.

Summary

An organism comprising approximately 99 percent of the total bacteria count of swimming pool water has been identified as *Alcaligenes faecalis*. This organism was found highly resistant to halogen disinfectants. Apparently it has no great significance in the sanitary analysis of swimming pool water.

REFERENCES

- (1) Marshall, J. D., Faber, J. E., and Campbell, W. R.: Advantages and limitations of iodine disinfection of a large controlled indoor swimming pool. *Am. J. Pub. Health*. In press.
- (2) Chambers, C. W., Kabler, P. W., Malaney, G., and Bryant, A.: Iodine as a bactericide. *Soap and San. Chem.* 28: 149-151, 153, 163, 165 (1952).
- (3) Chang, S. L., and Morris, J. K.: Elemental iodine as a disinfectant for drinking water. *Indust. & Eng. Chem.* 45: 1009-1012 (1953).
- (4) Chang, S. L.: The use of active iodine as a water disinfectant. *J. Am. Pharm. A.* 47: 417-423 (1958).

Uniform Personal Health Record for Migrant Workers

A uniform personal health record form for migrant agricultural workers has been produced by the Public Health Service. The form was recommended by the Association of State and Territorial Health Officers at their 1960 conference in San Francisco.

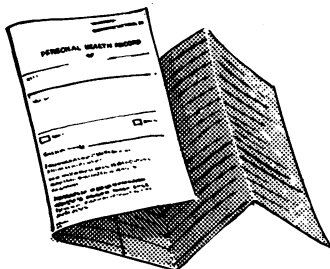
On the cover, space is provided for the worker's name, home address, sex, and birth date or age. A note in English and in Spanish advises him to show the record whenever he goes to a doctor or a nurse. Physicians and nurses are requested to enter pertinent information and return the record to the bearer.

The entry sections of the form provide space

for records of immunizations, laboratory tests and results, obstetrical history, and clinical conditions, which are important in future care of the worker. In the last two sections, the name and address of the person making the entry are requested.

The forms are 10½ by 4 inches and have space on both sides for information pertinent to the worker's health. They fold, as illustrated, to 2⅞ x 4 inches, a size which the worker can carry easily in his billfold or in his pocket.

Local health departments and voluntary agencies may obtain these forms from their State health departments.



Program Notes

The Connecticut State Department of Health is providing new educational and consultant services to assist administrators of nursing homes and other long-term care institutions in improving restorative and dietary services to their patients.

The restorative services program is surveying existing recreational and occupational activities and needs in licensed nursing homes and chronic disease facilities as a first step.

« »

A leaflet issued in Spanish, by the Minnesota Department of Health, to advise mothers on preparing their children for school, has been republished in revised form by the Michigan Health Department for the Spanish-speaking population of that State, including migrant agricultural workers and industrial workers of Mexican or Puerto Rican origin.

Minnesota also has published a book of recipes in English and Spanish to help Spanish-speaking families order ingredients for Spanish dishes in American markets.

« »

The National Science Foundation on March 20, 1961, announced the award of 1,537 graduate fellowships in the sciences, mathematics, and engineering for the academic year 1961-62. Of the awards, 347 were made in the life sciences; 1,156 in the physical sciences, including a number in interdisciplinary fields; and 34 in certain areas of the social sciences.

« »

The Doctor's Association of the Department of Health has been formed in New York City. Bargaining collectively, the association will work for salary increases, pension rights, relief of dentists and physicians from clerical work, more nurse assistance, and reestablishment of reorientation courses for public health doctors.

Mothers who smoked a pack or more of cigarettes a day had a rate of premature births more than double that among nonsmoking mothers, it was observed in a 2½-year study of 2,736 Negro women by the Baltimore City Health Department aided by a PHS grant.

Three possible reasons were cited: Smoking may curb the appetite and reduce weight of mother and baby; it may constrict blood vessels which nourish the baby, causing a decrease in blood supply to the placenta; and smoking itself may be linked to other characteristics which contribute to premature births.

« »

A report on the Conference on Leprosy, at Carville, La., November 7-10, 1960, is being printed by the *Star*, published by patients in the Public Health Service Hospital. There is no charge for reprints requested from the *Star*, Carville, La.

« »

The Minnesota Health Department's last budget statement used charts to demonstrate the investment potential outlays for alcoholism, genetics, and fluoridation.

The alcoholism chart contrasted a request for \$35,000 for a preventive program with a disbursement of \$435,000 for institutional treatment of alcoholics, \$600,000 spent on alcoholics in two city workhouses, and the uncalculated number of accidents associated with drunken driving. Taxes levied on alcohol within the State total \$45 million a year.

The chart for the genetics budget presents one example of an inherited disease, phenylketonuria. Faribault State Hospital has 46 patients with this disease, representing a lifetime earning loss of approximately \$6,900,000 and cost to the State for care of about \$3,450,000.

« »

A census of public health nurses, compiled for the first time by the Public Health Service, appears in *The American Journal of Nursing*, February 1961.

It is contrary to the custom of this journal to announce awards. An exception is made for Pupone, 2-year-old pointer, awarded a silver medal by the National Society for Medical Research for contributions to the study of leptospirosis at the PHS Communicable Disease Center. A carrier of leptospires, Pupone is personally in excellent health. As for the disease, he says, "It shouldn't happen to a man."

« »

Following the pattern set on the State level, New York City's executive director of medical care services in the health department will serve also as medical welfare administrator in the welfare department.

« »

The New Jersey State Public Health Council has set forth what are to be considered the recognized public health activities of local health departments in New Jersey and the minimum standards of performance they must follow. This action was in accordance with 1947 legislation which also provides for the enforcement of the requirements by the State commissioner of health. The detailed requirements were initially prepared by a committee of local health officers.

« »

The first progressive patient care hospital to be sponsored by the Hill-Burton program is now in operation in Grand Junction, Colo., with 15 beds for intensive care, 57 beds for intermediate care, and 12 beds for self-care.

« »

In 1955, when the LeRoy, N.Y., sewage program was defeated at the polls, the project would have cost \$1,380,000. Today, the cost is \$2,150,000.

« »

Ohio's Health, published by the State department of health devotes an entire issue to dental health. Articles on dental care for needy children, emphasis on dental health in 4-H clubs, mouth protectors in athletics, and school programs and one titled "Fluoridation—the 65 Percent Answer to Tooth Decay" are included.