Unusual Organism Which Gives a Positive Elevated Temperature Test for Fecal Coliforms

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Organisms apparently not of fecal origin isolated from a sulfur hot spring gave a positive elevated temperature test for fecal coliforms.

At the present time, a distinction is commonly made between coliforms of "fecal" and "nonfecal" origin on the basis of the elevated temperature test (4). This test is based upon the ability of fecal coliforms to produce gas from lactose within 24 hr at 44.5 C. This test may be run by determination of most probable numbers (MPN) in EC broth or by the membrane filter technique on M-FC broth-soaked pads. Nonfecal or soil coliforms do not give a positive reaction under these conditions.

Geldreich (2) discussed the significance of fecal coliforms and suggested that these organisms be used as indicators of fecal pollution of recreational waters. Hendricks (3) found that 24% of the Enterobacter (Aerobacter) group (nonfecal in origin) which he isolated from a "relatively unpolluted river" were positive in the elevated temperature test for fecal coliforms. Data presented here describe an organism from a sulfur hot spring which gives a positive elevated temperature test for fecal coliforms.

 Cultures were isolated and Gram-stained from eosin methylene blue (EMB) agar plates (Difco) inoculated from positive Brilliant Green lactose bile (BGB) broth tubes (Difco), from EC broth tubes (Difco), or from colonies on membrane filters (Millipore Corp., Bedford, Mass.) incubated on M-FC broth (BBL)-soaked pads at 44.5 C. All cultures were retested in EC broth, and these inoculations were made by means of a needle. Indole production, hydrogen sulfide production, and motility were performed in SIM medium (Difco). Methyl red and Voges-Proskauer tests were conducted on 48-hr cultures incubated at 35 C in MR-VP medium (Difco). Citrate utilization was determined on Simmons citrate agar slants (Difco). Gelatin liquefaction was studied on nutrient agar supplemented with 0.4% gelatin (5). Cytochrome oxidase tests were performed with test strips (Pathotec, Warner Chilcott Laboratories). Flagellation was determined by use of a flagella stain. Samples were obtained from a sulfur hot spring, from the Laramie river, and from an aerated sewage lagoon.

The data in Table 1 show results of studies conducted in April 1970. The finding that 17 of 19 isolates of Escherichia coli produced gas at 44.5 C in EC broth supports the idea that "typical" E. coli isolates give a positive elevated temperature test. In contrast, the fact that 25% (5 of 20) of the Enterobacter aerogenes isolates from the lagoon and river also gave a positive elevated temperature test, a result similar to the value found by Hendricks (4), suggests that a single or few samples are insufficient to judge water quality, since nonfecal coliforms might be falsely confused with coliforms of fecal origin. This could be particularly important in situations in which great distances might limit the number of samples taken.

In marked contrast to the results obtained from other sources, 9 of 10 apparent isolates of E. aerogenes from the hot spring drainage produced gas in EC broth at 44.5 C. This result indicates that the incidence of apparent E. aerogenes capable of giving a positive elevated temperature test may depend upon the environment. The isolates that produced gas in EC broth at 44.5 C were obtained throughout the hot spring drainage as soon as the temperature of the water had dropped to 46 C. This along with the visual observation of distance from the spring suggested that these organisms were not of fecal origin. To determine if this was a fortuitous result, another series of samples was taken in November 1970. Data in Table 2 show that 28 of 58 isolates in November were posi-
in the elevated temperature test, confirming the April result that an unusually high percentage of falsely positive fecal coliforms were found in the hot spring drainage. Four "typical" E. coli cultures were recovered in November, and all were positive in the elevated temperature test.

Approximately half of the November isolates were lightly golden in color when grown on nutrient agar at 35 C, and, upon storage at room temperature, these cultures became bright yellow. This finding was not observed in April. The yellow cultures exhibited typical Enterobacter colonies on EMV agar; gave typical indole, methyl red, Voges-Proskauer, citrate (IMViC) reactions; produced gas in BGB broth; were motile; and were gram-negative rods. However, they failed to produce hydrogen sulfide or to liquefy gelatin and were cytochrome oxidase-negative. Flagellation was typical of the Aeromonas group (1). Old cells had predominantly a single polar or subpolar flagellum, whereas young (6- to 8-hr) cells showed additional lateral flagella. The exact taxonomic position of these organisms is thus unclear.

Data in Table 2 also show that these organisms displayed a positive elevated temperature test when the membrane filter procedure was employed. Moreover, they may be nage" (yellow colony) by the membrane filter method and positive by the EC broth method. It should be noted, however, that the color of these organisms by the membrane filter method was not as bright blue as typical E. coli colonies.

Regardless of the taxonomic position of the yellow organisms, it is clear that, except for color, they are not easily distinguished from E. aerogenes, particularly in a routine bacteriological water analysis. It, therefore, appears that the use of multiple criteria in determining water quality is desirable.

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