SANITARY SIGNIFICANCE OF COLIFORM AND FECAL COLIFORM ORGANISMS IN SURFACE WATER

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THE SCIENCE of sanitary water bacteriology began in 1880 when von Fritsch described *Klebsiella pneumonia* and *K. rhinoscleromatis* as organisms characteristic of human fecal contamination. A short time later Escherich identified *Bacillus coli* as an indicator of fecal pollution. Both observers considered human feces as dangerous pollution while the feces of other warm-blooded animals were not so regarded. From this origin the current "coliform group" developed to include numerous micro-organisms of diverse biochemical and serologic characteristics, natural sources and habitats, as well as controversial sanitary interpretations.

Investigators continued to enlarge the number of organisms classified within the coliform group by applying all available physical and biochemical procedures. In 1908, Bergey and Deehan (*1*) expanded the classification to include 16 groups composed of 256 types. The difficulties associated with application of so many subdivisions caused Jackson (*2*) to propose classification into only 16 groups based on sucrose, dulcitol, raffinose, and mannitol fermentation. A tremendous amount of information was available on the physical and biochemical characteristics of the coliform group, but the correlation between the source of the various types, such as fecal, vegetable, and soil, and these tabulated characteristics left much to be desired in sanitary interpretation.

Biochemical Tests

Harden and Walpole (*3*) demonstrated that the hydrogen to carbon dioxide production ratio was 1:1 for *Escherichia coli* and 1:2 for *Aerobacter aerogenes*. Rogers and associates (*4, 5*) then showed that coliform strains isolated from bovine feces fermented glucose, producing equal volumes of hydrogen and carbon dioxide (1:1), while coliform strains from grains produced two or more times as much carbon dioxide as hydrogen (1:2 or 1:3). They concluded that differences in gas ratios formed a basis for differentiating coliform strains isolated from warm-blooded animal feces from those associated with grains. In further attempts to develop tests for strain classification, Clark and Lubs (*6*) described the methyl red test; Voges and Proskauer (*7*), a test for acetylmethylcarbinol; and Koser (*8*), the citrate utilization procedure. Numerous tests for indole were suggested that showed considerable differences in sensitivity and specificity. None of these procedures were entirely satisfactory in relating the specific group or individual strain with its source.

In an attempt to develop an improved classification of the coliform group, Parr (*9*) studied the information available on the separation of strains from fecal and nonfecal sources. He chose the indole, methyl red, Voges-Proskauer, and citrate tests as the combination of reactions that would yield the best classification. This combination of four procedures was designated by the mnemonic "IMViC test."

Parr suggested sanitary interpretations for the 16 possible types as follows:

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Escherichia group: Included IMViC types + + − −, + − − −, and − + − −, and were to be considered of fecal origin.

Acrobacter group: Included IMViC types − − + +, − − + −, and − − − +, and probably represented the majority of soil types.

Intermediate group: Included the remaining 10 possible IMViC types.

The four reactions designated by Parr yielded the best correlations at the time he developed the IMViC classification of the coliform group. The fecal or nonfecal classification of the group showed good correlations when a statistically significant number of strains from a single source were examined, but unexplained discrepancies occurred when a decision was made on the results from a few coliform cultures. This unexplained relationship between source (fecal, soil, vegetation, and so forth) and coliform types made the sanitary interpretation of a few strains an uncertain procedure. Experimental data have demonstrated that the indole and Voges-Proskauer reactions may undergo changes in an artificial environment. The possibility is suggested that similar changes cannot be excluded in waste waters.

Elevated Temperature Test

The elevated temperature test for the differentiation of the fecal from the nonfecal coliform group was originally proposed by Eijkman (*10*). It was based on his findings that the coliform bacteria derived from the gut of warm-blooded animals produced gas from glucose at 46° C., while the coliform strains from nonfecal sources failed to grow. The Eijkman reaction, or one of its many modifications, has been studied by many investigators, but conclusions have differed concerning its sensitivity, specificity, and the interpretation of results.

Perry and Hajna (*11*) proposed an elevated temperature test that used a buffered bile salts medium and air incubation at 45.5° C. This procedure showed improved sensitivity with slight loss in specificity but was not generally accepted. Vaughn, Levine, and Smith (*12*) recommended a buffered boric acid-lactose broth for the enrichment and identification of *E. coli*. They believed that the reduction of the incubation temperature to 43° C. for 48 hours increased sensitivity and that the addition of boric acid inhibited the growth of the *Aerobacter* genus and the intermediate-aerogenes-cloacae (IAC) for the maintenance of specificity.

From the information available and from our own investigations (*13*, *14*) we have reached the following conclusions:

1. The most acceptable temperature of incubation for separation of the fecal coliform group is 44.5° C. in a water bath.
2. A small percentage of the fecal coliform strains will be excluded and an equal percentage of nonfecal coliforms will be included.
3. EC medium, described by Perry and Hajna, will give the most rapid results, as it requires only 24 hours' incubation.
4. The test can be used only as a confirmatory procedure from coliform cultures growing on a nonselective medium.
5. In the evaluation of results, all coliforms from the feces of warm-blooded animals must be considered as fecal coliform strains, and all cultures isolated from unpolluted soils must be considered as nonfecal coliform strains.

Applying the above concepts as guides, Geldreich and associates (*15–17*) studied the coliform organisms isolated from the feces of several warm-blooded animals including humans, cows, sheep, pigs, chickens, ducks, and turkeys; from 223 soil samples with no known fecal pollution, collected in 26 States; from 28 fecally polluted soil samples from feed lots or locations recently flooded with domestic sewage; and from 152 species of plants and 40 species of insects. Coliform strains isolated from these samples were purified, and their reactions to standard method's completed test, to IMViC, and to 44.5° C. temperature tests were determined. Results of these studies showed:

1. Coliform strains from feces gave good correlation of the elevated temperature test (fecal coliform) with the + + − − IMViC type and with the Parr fecal types (+ + − −, + − − −, and − + − −).
2. Coliforms from soils showed good correlation of the elevated temperature test with the IMViC type + + − − and with the Parr fecal types, but attempts to classify the coliform group by individual reactions were unsuccessful.
3. The most prevalent IMViC types found in unpolluted soils were 48.1 percent − + − + type and 18.8 percent − − + +.

4. There were low numbers of coliforms on plants but very few of these were classified as fecal coliforms.

5. The number of coliforms recovered from insects showed wide variation. Relatively few fecal coliforms were found.

6. No IMViC type was predominant on either vegetation or insects.

It appears that separation of fecal coliforms from nonfecal coliforms can be obtained with essentially equivalent results by using the IMViC types and interpreting the results according to the "Parr classification," or by using the elevated temperature test, which was superior in simplicity of technical procedure and in time required to complete it.

Sanitary Significance

Fecal coliform organisms may be considered indicators of recent fecal pollution by warm-blooded animals. Because no satisfactory method is currently available for differentiating fecal coliform organisms from human and other animal origin, it is necessary to consider all fecal coliform organisms as indicative of dangerous contamination. The presence of the intermediate-aerogenes-cloacae group organisms in untreated water may be the result of relatively less recent fecal pollution or of soil runoff water. The presence of any type of coliform organism in treated drinking water suggests either inadequate treatment or access of undesirable materials to the water after treatment.

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