NOTES

Detection of False-Positives among Total and Fecal Coliform Counts by Factorial Analysis of Correspondence

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Application of an analysis of correspondence to the biochemical characteristics of total and fecal coliforms isolated in the Ivory Coast permitted us to separate two small clusters of isolates different from the main clusters, which included isolates from human and animal feces. The isolates grouped in the small clusters were from water samples. An analysis of the biochemical characteristics which permitted the segregation of the “water-specific” isolates from the main clusters indicates that water-specific total coliforms were citrate positive, indole negative, and amygdaline positive. Water-specific fecal coliforms were either citrate positive, indole negative, amygdaline positive, and inositol negative or indole negative, amygdaline positive, and inositol positive. Any isolates not fitting the above patterns could be considered of fecal origin. If this observation is confirmed under temperate climates and for a greater number of isolates, these simple tests could be used to confirm the fecal origin of coliforms.

Although underestimation of bacterial indicators utilized as indices of sanitary water quality is of obvious concern, the overestimation of these indicators could also be deleterious, especially if the result is the elimination of the only available drinking water source (7, 13). Total coliform (TC) and fecal coliform (FC) counts are presently the most widely used techniques for the assessment of drinking water quality (1, 2, 5, 9, 19). Although Escherichia coli is recognized as the best indicator of fecal water pollution (1, 8), it has been shown that feces contain other members of the family Enterobacteriaceae (3, 6, 8, 12, 15). It has also been shown that coliform tests (TC and FC) are not necessarily specific for E. coli (4, 11, 12, 14, 6, 20). This paper presents the results obtained from a factorial analysis of correspondence (10, 17, 18) of the biochemical characteristics of TC and FC isolated in the Ivory Coast (12). The results indicate that the “water-specific” coliforms could be differentiated from the “real” coliforms by a combination of three tests for TC (citrate-indole-amygdaline [CIA]) and four tests for FC (CIA plus inositol [CIA-IN]).

The coliform strains (TC and FC) were isolated by membrane filtration and purified, as described previously (12), from water and fecal samples. The biochemical characteristics were determined with the API-20E system (Analytab Products, Inc., St-Laurent, Quebec). A total of 430 isolates were analyzed, 120 TC and 310 FC. Of the TC, 34 were isolated from water samples and 86 were isolated from human feces. The origin of FC isolates was as follows: 85 from water samples, 107 from human feces, 49 from goat feces, 32 from oxen feces, and 37 from sheep feces.

A correspondence factor analysis is analogous to a principal-component analysis, except that it is done on qualitative data, such as contingency or logical tables (containing only 0's and 1's). This difference has deep implications: the notion of Euclidean distance is replaced by chi-square distance, and sample variance is replaced by sample inertia. However, the aims are the same: represent the sample points in a space of lower dimension (ideally a two-dimensional space) with the smallest loss of information as measured by the difference between the sample variability of the original data and the sample variability of its representation.

A brief description of the method is contained in reference 17. For more complete information and extensions, see reference 18 (chapters 7 and 8). In this application the data are a logical table with dimensions of 120 by 34 for TC and 310 by 34 for FC. A line in one of these tables may be represented as a vector (X1, Y1; X2, Y2; ⋯; X17, Y17), where, for J = 1 to 17, Xj = 0, if there is no reaction to the biochemical test; and Xj = 1, if there is a positive reaction to the biochemical test and Yj = 1 − Xj. The analysis was performed on a computer with a package called ANAFACOR (10).

The results of the factorial analysis of correspondence of the biochemical characteristics of the TC are presented in Fig. 1. We obtained similar results with the FC analysis. Figure 1 represents the data on the first two-factorial axis, accounting for 34% of the sample variability of the original data. It can be observed that two clusters appeared: a main cluster which represents the majority of the isolates analyzed and a distinct, small cluster. The interesting point is that this small cluster is composed only of isolates from well water samples (Fig. 1): 19 TC and 18 FC. These represent 56% of the TC and 21% of the FC isolated from water samples. The isolates forming these two clusters were identified as reported in Table 1.

We then extracted the biochemical variables characterizing the isolates which formed these separate clusters. The TC isolates differing from the main cluster could be recognized as being citrate positive, indole negative, and amygdaline positive. The specific characteristics of the FC isolates differing from the main cluster were indole negative, amygdaline positive, and inositol positive. However, if the inositol
reaction was negative, the isolates had to be citrate positive to be considered water specific.

The identification of the coliform isolates to the species level does not permit one to distinguish fecal strains from those indigenous to water because the origin of certain species regrouped under the term "coliform" is still uncertain (3, 4, 6, 8, 11, 12, 14, 15, 20). Our results indicate that three to four simple tests could differentiate between these two groups. The CIA tests seem capable of distinguishing between water-specific (CIA + - +) and feces-specific (other CIA patterns) TC isolates. To detect the false-positives in the FC test, we have to add the inositol (IN) fermentation test. The water-specific isolates are thus distinguished by one of the following patterns of reactions, IA IN (- + +) or CIA-IN (+ - +). The strains which do not fit the above-mentioned patterns could be considered of fecal origin.

The isolates reported here as water-specific could be of two types. They could represent a group of indigenous environmental flora, in which case they could be recognized as false-positives in the coliform tests. On the other hand, these isolates could be survivors of old fecal contamination. The group of isolates designed here as water specific could be normal inhabitants of the fecal flora of humans and other animals which survived longer than the other coliforms in the aquatic environment. In that case, they could be recognized as pollution indicators, but certainly not to the same extent as the isolates grouped in the main clusters (see Fig. 1), which include fecal isolates.

The results presented in this paper apply to coliform strains isolated in a tropical climate, and we do not know if the same criteria would hold for strains isolated in a temperate climate. Furthermore, the present analyses were performed on a limited number of isolates (310 FC and 120 TC) and would have to be confirmed with a greater number of strains. However, if the relationship between the water-specific strains and both the CIA (for TC) and CIA-IN (for FC) tests holds, these two series of tests could be used in conjunction with membrane filter techniques as a confirmatory test for the evaluation of the bacteriological quality of water.

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LITERATURE CITED


![Diagram](image-url)