

Escherichia Coli as an Indicator of Fecal Pollution in Oysters and Oyster Waters*

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IN this paper data are presented which should indicate the relative value of *Escherichia coli* and the colon group as indicators of significant fecal pollution. While the discussion is concerned only with fresh shell oysters, the question of a more specific indicator of significant fecal pollution than the colon group is of even more importance in the examination of market oysters. Obviously the same problem exists whether the object is to determine fecal pollution in shell oysters or market oysters. Market oysters are, after all, the same oysters except for handling and storage. Those members of the colon group which confuse the examination as to pollution in shell oysters confuse it even more perhaps in market oysters because these are often subject to non-fecal contamination from dirty equipment and are subsequently held. The result is that colon group types (not *Escherichia coli*) are found in excessive numbers, though they probably have no more significance as indicators of significant fecal pollution than a total colony count of bacteria.

The question of whether *Escherichia*

coli or the colon group should be used as a basis for estimating pollution in oysters and other mollusks is a broad one of the utmost importance, for the colon group has been found confusing and unreliable, and some means of determining probable fecal pollution is essential as a check on the certification system, as a test for market oysters and for oysters taken from waters in which they grow or in which they are temporarily placed.

During the 10 years 1925 to 1935, thousands of samples of oysters and oyster waters (oysters 4,152, oyster waters 12,689) have been collected from the 20 different oyster areas into which the Chesapeake Bay, Md., has been divided. These data have already been studied extensively and have been the basis for several special reports by various members of the staff of the Maryland State Department of Health.^{1, 2, 3} The one inescapable fact in these studies has been the lack of any significance which may be attached to individual oyster scores as indicators of pollution. So useless have oyster scores been found for this purpose that an agreement between those concerned was arrived at about 18 months ago to discontinue the oyster scores on the basis of the colon group in the sanitary oyster survey in Maryland and to test

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the value of *Escherichia coli* as an indicator of fecal pollution. During the past 2 years 1,463 samples of oysters and oyster waters have been examined for both their *Escherichia coli* and colon group scores.

The term *Escherichia coli* is construed as indicating any member of the colon group which is unable to grow in Koser's citrate medium. Such organisms are invariably methyl red positive, Voges-Proskauer negative, produce indol, cannot ferment cellobiose, and produce gas in suitable media at 46° C.

Examinations for *Escherichia coli* were made using both the modified Eijkman medium of Perry and Hajna⁴ and standard lactose broth, as presumptive tests. Determinations for the colon group were made in accordance with the standard procedures of the American Public Health Association. A report of this work up to January 1, 1935, has been given in the *Journal* for June, 1935. The Eijkman test in its present form has been found so superior to lactose broth as a presumptive test for the isolation of *Escherichia coli* from oysters and oyster waters, that it is being recommended for trial in other laboratories in consideration of its inclusion as part of a new standard procedure for the examination of shellfish.

Through a grant from the American Public Health Association, it has been possible to compile and study the more than 1,000 *Escherichia coli* results, and more than 4,500 colon group results from certain oyster areas in Maryland. An attempt has been made to present these data in a way that should show directly what correlation, if any, there is between quantitative *Escherichia coli* and colon group concentrations in oysters and oyster waters and pollution as judged from sanitary survey data. The data have been studied in two ways. In one approach, the average number of *Escherichia coli* and colon

group organisms for a number of sampling stations were "spotted" on sanitary survey maps for the Choptank, Chester, Severn, and Patuxent Rivers in Maryland. In this way, the relationship both of *Escherichia coli* and the colon group to known pollution could be studied.*

No extensive comments on the significance of these data are necessary. The important point is the close agreement evident between the concentration of *Escherichia coli* in oysters and observed pollution. In order to make this relationship clearer in some respects, the data for the 4 rivers were also prepared in linear fashion; that is, the rivers were straightened out and the data summarized for mile sections and studied in relation to the principal sources of pollution. It is unfortunate that a larger number of *Escherichia coli* results as well as those for more sampling stations were not available. It is believed, however, that in spite of the comparatively small number of *Escherichia coli* results (1,463—see footnote Table I) available at the time, they do attest the superior value of this organism as an indicator of significant fecal pollution.

An attempt to clarify further the relationship between pollution in oysters and oyster waters as judged from sanitary survey information and as expressed by *Escherichia coli* and the colon group has been by giving average concentrations of *Escherichia coli* and the colon group organisms for pollution classified as "not significant," "slight," "moderate," and "heavy." (See Table I.) While it is impossible to classify accurately pollution at sampling stations on the basis of sanitary survey data alone as "not significant," "slight," "moderate," or "heavy," it is possible to do this in a roughly accurate manner.

* It has been found impossible to reduce the size of these maps to that suitable for inclusion in this article.

Such a method has afforded the only approach to an evaluation of the bacteriological data. If the classification could have been more accurately made on other than bacteriological grounds, the data would be more convincing. Pollution varies for any sampling station with tides, weather, and temperature. The biology of the oyster further complicates the relationship and makes and accurate evaluation difficult.

in the oysters are still very high, but *Escherichia coli* is practically absent.

Temperature affects greatly the number of colon group organisms present in both oysters and water. During the summer months when the temperature of the water rises above 50° F., there is a large increase in the colon group in both water and oysters. In oysters this increase may be enormous. When the temperature of the water is below 50°

TABLE 1

RELATIONSHIP OF *ESCHERICHIA COLI* AND THE COLON GROUP TO FECAL POLLUTION
IN OYSTERS AND OYSTER WATERS

VARIOUS TRIBUTARIES OF CHESAPEAKE BAY

Pollution as Indicated Bacteriologically
(Most Probable Numbers per 100 c.c.—McCrary)

Fecal Pollution as Judged by Sanitary Survey	Pollution as Indicated Bacteriologically (Most Probable Numbers per 100 c.c.—McCrary)			
	Oysters		Oyster Waters	
	<i>Esch. coli</i>	Colon Group	<i>Esch. coli</i>	Colon Group
Not significant	4.5 (422)	1,158 (694)	1.5 (238)	61 (1,026)
Slight	49.0 (230)	1,979 (476)	5.5 (287)	100 (1,752)
Moderate	196.0 (97)	1,634 (184)	36.0 (92)	134 (686)
Heavy	167.0 (30)	1,509 (97)	67.0 (67)	376 (413)

Figures in parentheses represent number of examinations.

Escherichia coli determinations: Oysters 779, Water 684, Total, 1,463.

Colon Group determinations: Oysters 1,451, Water 3,877, Total, 5,328

In general, the classification of a sampling station as not significantly, slightly, moderately, or heavily polluted, was based on the proximity to towns, sewers, privies, and on the population of towns, type of sewage disposal, and the volume of water. In the Chester River for example, the major source of pollution is from Chestertown, a town of more than 2,800 population with untreated sewage. A secondary source of pollution is Centerville (population 1,291) on the Corsica River, a tributary of the Chester River. Very little, if any, pollution from Centerville reaches the Chester River. The only oysters showing any significant pollution on the basis of *Escherichia coli* are those from the upper part of the river nearest Chestertown. In the lower part of the river, many miles from any pollution, concentrations of colon group organisms

F., the concentration of colon group approaches that of the *Escherichia coli*, but during the summer, the non-fecal types of the colon group apparently find a most suitable environment for multiplication, probably due to organic matter present. The environment in the oyster is doubtless even more suitable, and results in high colon group scores on oysters taken during the period of active feeding. It is possible that *Escherichia coli* may also increase somewhat both in water and oysters when the temperatures of the water are unusually high, but water temperatures are always relatively low for the growth of this organism, and this, together with its inability to utilize carbon from organic matter (as indicated by its inability to use carbon from citrate and other organic salts) places it in an unfavorable environment for multiplica-

tion. The data presented show that *Escherichia coli* tends to disappear gradually as self purification occurs, while the non-fecal colon group types increase. This is in agreement with the observations recently reported on the Whangpoo River in China.⁵

It will be noted in Table I that the average *Escherichia coli* (most probable number per 100 c.c.) results for 422 samples of oysters from waters judged to be without any significant pollution, is 4.5, the average for 230 samples from slightly polluted waters was 49, while the average of those from moderately polluted water (97 samples) was 196 and heavily polluted (30 samples) 167. In contrast to this, there is little if any difference in the average results based on the colon group. In oysters from heavily polluted waters the average was 1,509 against 1,634 for moderately polluted waters, 1,979 for only slightly polluted waters, and 1,158 for waters free of any noteworthy pollution. There is, therefore, a close agreement between pollution as observed and as expressed quantitatively by *Escherichia coli* for oysters. On the basis of the colon group, no such correlation exists.

For oyster waters there is rather a nice transition from 1.5 *Escherichia coli* per 100 c.c. in unpolluted water, to 5.5 for only slightly polluted, to 36 for moderately polluted, and to 67 for heavily polluted water. These numbers seem in much closer agreement than the colon group results which are 61, 100, 134, and 376 respectively. It might be inferred from these figures that the *Escherichia coli* results indicate a lowering of the margin of safety. That this is not true is attested by the fact that more individual *Escherichia coli* have been recovered from fresh raw sewage where the Eijkman presumptive test has been used than total colon group types where lactose broth was used as a presumptive test.⁶

Escherichia coli, as has been repeatedly demonstrated, constitutes over 95 per cent of the colon group types found in fresh human feces. The increase in the colon group both in water and oysters may be considered, therefore, to indicate that self purification is going on. The colon group tends to distort and magnify the picture of real pollution in water while in oysters it may indicate gross pollution where there is none.

A more specific idea of the magnitude of the differences between the numbers of *Escherichia coli* and of colon group organisms in oysters may be obtained from Table II. The results from individual samples indicate the enormous differences between the number of *Escherichia coli* and of colon group organisms which frequently occur, and the greater efficiency of the presumptive Eijkman test over lactose broth for the isolation of *Escherichia coli*. The data are representative of those obtained during the warmer months when the temperature of the Chesapeake Bay water is above 50° F. (April to November) and especially when the temperature is above 70° F. (June to September). The degree of pollution as judged from sanitary survey information is also given. The data indicate a relative correlation between the most probable numbers of *Escherichia coli* and a lack of such correlation on the basis of the colon group.

Colony counts of bacteria growing at 37° C. on standard nutrient agar were also studied. These counts have been found to parallel the concentration of colon group bacteria and to indicate pollution in a general and similar manner.

SUMMARY AND CONCLUSIONS

Data are presented for the concentration of *Escherichia coli* and the colon group in oysters and oyster waters for

TABLE II

COMPARATIVE EFFICIENCY OF EIJKMAN TEST AND LACTOSE BROTH FOR ISOLATION OF ESCHERICHIA COLI FROM OYSTERS AND THE RELATIONSHIP OF ESCHERICHIA COLI AND COLON GROUP ORGANISMS TO POLLUTION

No.	Eijkman Test <i>Esch. coli</i>	Lactose Broth		Estimated Pollution- Sanitary Survey
		<i>Esch. coli</i>	Colon Group	
135	1,800+	1,800+	1,800+	moderate
136	1,800+	200	1,800+	moderate
137	1,600	500	1,800+	moderate
138	1,600	110	1,800+	moderate
140	20	0	1,800+	slight
141	80	50	170	slight
429	20	0	9,000	negative
431	20	20	20	negative
435	20	20	350	slight to negative
504	170	50	130	moderate
506	900	900	1,400	moderate
509	900	0	1,800+	moderate
510	80	20	500	moderate
524	50	0	80	slight
525	20	20	80	slight
529	0	0	500	negative
531	0	0	9,000	slight
532	0	0	350	negative
534	0	0	9,000	negative
539	0	0	9,000	negative
545	130	20	140	slight
550	20	20	50	negative
581	0	20	50	negative
610	50	70	90	negative
611	40	0	40	negative
Average	312	117	907	

the Choptank, Chester, Severn, and Patuxent Rivers, in Maryland, together with sanitary survey information. No significant correlation between the concentration of colon group bacteria in oysters and various degrees of pollution has been found in any of the areas studied. In the case of oyster waters, there is a fair correlation between the concentration of colon group bacteria and pollution. During the summer months there is a large increase of colon group bacteria in both water and oysters. Enormous increases in the colon group content of oysters are found when the water is warm, in both grossly pol-

luted and relatively unpolluted areas. If, therefore, the quality of oysters was determined, especially when the temperature of the growing waters was relatively high, and on the basis of the colon group, those from waters of unquestionable purity would in many instances be judged as grossly polluted. The colon group cannot, therefore, be relied upon to indicate significant fecal pollution in oysters.

A close correlation has, however, been found between the concentration of *Escherichia coli* in both oysters and water and the amount of pollution. The results indicate that *Escherichia*

coli is superior to the colon group as an indicator of significant fecal pollution in both oysters and oyster waters.

REFERENCES

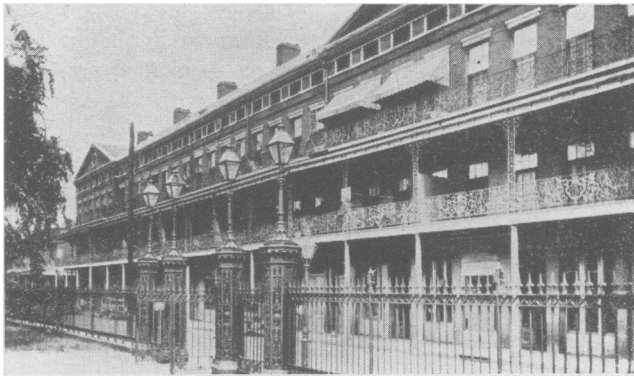
1. Speer, Carl, Jr. Special Report (unpublished). Oyster Investigation—Sanitary Engineering Aspects of Shellfish Pollution. Bureau of Sanitary Engineering, Maryland State Department of Health. June 1, 1926.
2. Wolman, Abel. Special Report (unpublished). A Statistical Summary of the Oyster Findings in the Chesapeake Bay and Its Tributaries. Bureau of Sanitary Engineering, Maryland State Department of Health. Feb. 15, 1927.
3. Perry, C. A. Studies Relative to the Significance of the Present Oyster Score. *Am. J. Hyg.*, 8:694-722 (Sept.), 1928.
4. Perry, C. A., and Hajna, A. A. A Modified

Eijkman Medium. *J. Bact.*, 26:419-429 (Oct.), 1933.

5. Hamilton, W. A Study of the Pollution of the River Whangpoo as Affecting Its Use as a Source of Water Supply. The Shanghai Waterworks Co., Ltd., The Mercantile Printing Co., Ltd., Shanghai, 1935.

6. Hajna, A. A., and Perry, C. A. A Comparison of the Eijkman Test with Other Tests of Determining *Escherichia coli* in Sewage. Accepted for publication in *J. Bact.*

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