Detection and Significance of Escherichia coli in Commercial Fish and Fillets*

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THE use of marine fish as food has increased tremendously in recent years. Better distribution facilities, increased advertising, and the general recognition that fish is a nutritious food and an important source of essential minerals, have all contributed toward popularizing fishery products as a regular part of the diet.

Changes in the methods of handling and marketing certain marine fish have occurred coincidently with their increased use. Formerly very little fresh fish was sold except "in the round," *i.e.*, as caught, or else eviscerated. A large proportion of the catch of cod, haddock, and hake was eviscerated, split, salted, dried, and sold as dried salt fish or "fish flakes."

Today a large proportion of the fresh or frozen fish is "packaged," that is, it is sold prepared either as fillets or in other ready-to-use form. According to the statistics of the U. S. Bureau of Fisheries,[1] 49,000,000 lb. of fish, chiefly haddock, valued at about $5,500,000, were sold as fresh or frozen fillets in 1933. The fish are usually caught by otter trawls, hand lines, or purse seines, and are eviscerated on the vessel, packed in ice in bins or cribs, and brought to port. After being sold to commercial dealers the fish are washed, scaled, and filleted. The fillets are washed, usually in a salt brine, wrapped in parchment, and those sold fresh are packed in 10 and 25 lb. quantities in tin boxes, which in turn are packed in ice for retail shipment.

So much handling, between catching and purchase by the consumer, naturally increases the chances of contamination and the rapidity of subsequent spoilage, particularly in fillet form.

Laboratory tests for the sanitary quality of various food products have come into wide use. However, the bacteriological examination of marine fish has not become common and there are no standard methods for such examination.

There are two possible standards on which the quality of market fish might be based; one the total bacterial count on the fish flesh, and the other the detection of some specific type of bacteria, such as *Escherichia coli*, sanitary significance of which could be interpreted.

REVIEW OF LITERATURE

The majority of investigators agree that the flesh of live fish and of freshly

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caught fish is sterile. Hunter [2] and Fellers [3] found that the digestive tract and flesh of migrating salmon was usually sterile. Proctor and Nickerson,[4] in examining the flesh of 96 fish, frozen with dry ice immediately upon being caught, and examined upon reaching the laboratory, reported all except one sample to be sterile.

The slime of fish is not sterile and, after the death of the fish, serves as an excellent medium for the rapid multiplication of bacteria. Gee [5] made isolations from slime of live haddock and found numerous organisms. Stewart [6] examined the slime content of 22 haddock. Of the 247 cultures isolated, 140 corresponded in general characteristics with the genus Achromobacter. Organisms of the mammalian intestinal type were not found, and only 4 cultures belonging to the genus Aerobacter were isolated.

All investigators agree that bacterial invasion of the flesh occurs soon after the death of the fish. The number of organisms present and the rate of spoilage depend on several factors, temperature of storage and carefulness of handling probably being most important. Griffiths and Stansby [7] in studying the correlation between chemical and bacterial tests for freshness of fish flesh found that with market haddock, caught from 4 to 10 days, the bacterial count was between 10,000 and 100,000 per gm., while counts of 500,000 to 1,000,000 were typical of very stale and unmarketable fish. The maximum period during which haddock stored in ice could be kept in an edible condition was between 16 and 21 days. Fillets, because of their greater initial contamination, spoil more rapidly.

Because of the public health significance of *Escherichia coli* in water and foods as an indication of potentially dangerous contamination, the possibility of its occurrence in the intestinal tract of lower animals and of fish has been investigated. Gibbons [8] in a review of literature and as the result of his own investigations concluded:

> *E. coli, E. communior* and *A. aerogenes* are not normal inhabitants of the intestinal tract of marine fishes. Representatives of the genera Escherichia and Aerobacter may be found in marine fishes, but they seldom occur in fish taken some distance off-shore. The fecal forms are particularly rare, except in fish taken near shore or in contaminated waters.

Griffiths [9] did not find fecal *Escherichia coli* in the slime or flesh of fresh haddock, but found typical fecal *E. coli* on 5 commercial fillets examined. Hunter [10] considered the presence of *E. coli* in cooked crab meat an indication of contamination due to faulty handling.

PURPOSE OF THE PRESENT INVESTIGATION

It was the purpose of the present study to investigate the occurrence of bacteria of the coli-aerogenes group on market fillets and whole fish, and to determine the proportion of these organisms which could be identified as fecal (mammalian) *Escherichia coli.*

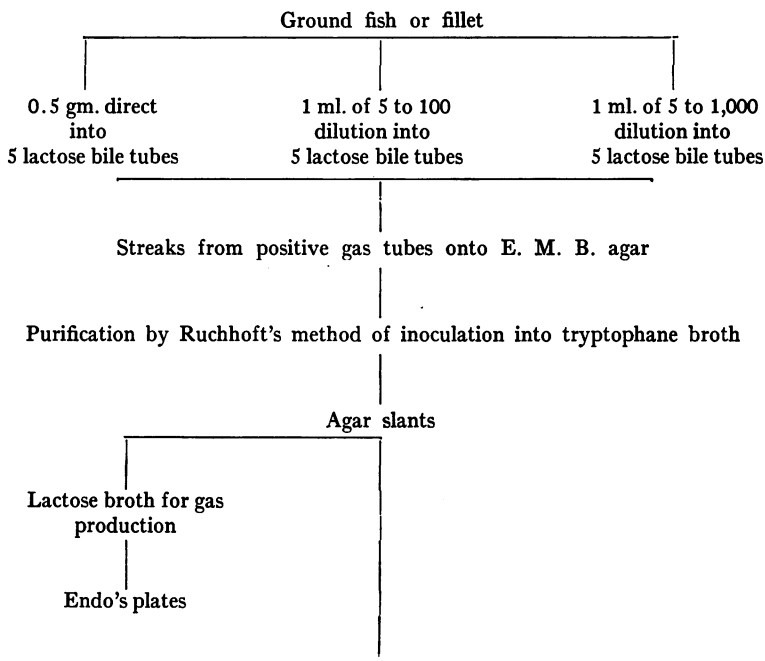
METHOD OF INVESTIGATION

Fish and fillets were purchased from retail stores. The fish were carefully filleted in the laboratory and both the laboratory and the store fillets were skinned. The flesh was then ground twice through sterile meat grinders into sterile pans, and 5 gm. portions were weighed into dilution bottles containing 99 ml. of sterile water for a 5 to 100 dilution. After being in a mechanical shaker for 10 minutes, 10 ml. were added to 90 ml. of sterile water to make a 5 to 1,000 dilution. Five tubes of lactose peptone brilliant green bile broth were inoculated with 0.5 gm. portions of the ground fish, 5 tubes with 1 ml. of the 5 to 100 dilution, and 5 tubes with 1 ml. of the

5 to 1,000 dilution. After incubation at 37° C. for 48 hours, the number of gas-positive fermentations was noted and streaks from these tubes were made on eosin-methylene-blue agar plates. E.M.B. agar plates were used because they are believed to be slightly more selective than Endo's agar. After 24 hours, incubation colonies most resembling *E. coli* were picked and inoculated into lactose broth fermentation tubes. Those which failed to referment were classified as false presumptives, and discarded. From the positive tubes streaks were made upon Endo's medium to determine colony characteristics and insure the purity of the culture. Agar slants were made and saline suspensions from these used to inoculate tryptophane broth, Clark and Lubs medium, sodium citrate, and uric acid tubes. Readings were made

FIGURE I

DIAGRAM OF PROCEDURE AND CLASSIFICATION OF ORGANISMS



Classification

	Endo's	*V.P.*	*M.R.*	*Na. citrate*	*Uric Acid*	*Indol*
Escherichia coli	T	—	+	—	—	+
Intermediates						
a. coli-like	At	—	+	—	—	±
" "	T	—	+	+	—	±
b. indeterminate	?	?	?	?	?	?
c. aerogenes-like	T	+	+	+	+	+
Aerobacter aerogenes	T	+	—	+	+	—

At—not typical for *E. coli*

Indeterminates—various combinations of reactions not included elsewhere in the chart.

at 24 hours for indol production, and at 48 hours for the other tests except the methyl red reaction, which was determined after 96 hours' incubation. An organism was considered to be of fecal type if it was a Gram-negative rod producing typical *E. coli* colonies on Endo's medium, producing indol, and was methyl red positive, Voges-Proskauer, sodium citrate, and uric acid negative.

Twenty fillets purchased at intervals from May to August yielded 203 cultures producing gas in brilliant green lactose peptone bile broth in the primary inoculations. After repurification 181 of these refermented lactose, and of this number 60 were typical, according to the previous definition, of fecal *Escherichia coli*. Four of the 20 fillets did not show evidence of any typical fecal *Escherichia coli*. Five eviscerated haddock purchased during the same period yielded 44 gas positive tubes; 40 refermented lactose, and 4 were typical of fecal *E. coli*.

Brilliant green lactose peptone bile broth was used instead of the usual lactose broth because it was believed that this medium was more selective for organisms of the Gram-negative, lactose-fermenting type. From 6 fillets, where duplicate tubes were set up, lactose broth yielded 46 gas positives from which 10 typical *E. coli* were isolated, and brilliant green lactose peptone bile broth gave 54 positive gas tubes from which 17 typical *E. coli* were isolated.

False presumptive tests from the two sets of broths were about equal in frequency of occurrence. These apparent false presumptives may have been due to either the loss of the gas-producing organism on transfer, or to a non-colon type of symbiosis in the original direct inoculation.

Brilliant green lactose peptone bile broth alone was used for the remainder of the fillets. Table I shows the distribution of positive gas tubes from the dilutions of the fillet and fish.

The majority of isolations and the fewest false presumptive tests were obtained from the primary inoculation of 0.5 gm. flesh. The number of samples run is not large enough to consider this difference conclusive. Positive 5 to 1,000 tubes were obtained from 11 of 17 fillets and from only 2 fish.

Table II shows the distribution according to the 6 criteria mentioned of all the Gram-negative lactose-fermenting organisms isolated.

Of the cultures isolated from fillets, 60, or 30 per cent, were typical of fecal *E. coli*. From whole fish only 4 typical *E. coli* were isolated and the proportion of intermediates was higher. Gas pro-

TABLE I

DISTRIBUTION OF POSITIVE GAS TUBES AND ESCHERICHIA COLI FROM FISH AND FILLETS

	Fillet Sample No.																				*Fish Sample No.*				
Dilution	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	1	2	3	4	5
Flesh, 0.5 gm.																									
Tubes positive	5	5	5	5	5	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Confirmed	5	5	4	5	5	2	5	4	5	4	4	2	5	5	5	5	5	4	4	5	5	5	3	5	5
E. coli found	4	5	1	0	1	0	2	2	4	2	1	1	2	3	0	0	3	2	1	1	0	1	0	3	0
5 to 100 dilution																									
Tubes positive	5	5	5	2	5	1	1	5	5	5	5	5	5	3	5	5	3	1	4	3	3	3	0	4	5
Confirmed	5	5	1	2	5	1	0	5	5	5	4	1	5	2	4	5	2	1	3	3	3	3	0	4	5
E. coli found	0	2	1	0	1	0	0	4	3	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0
5 to 1,000 dilution																									
Tubes positive	2	..	..	1	4	..	0	1	4	4	3	5	4	0	0	3	1	0	0	0	0	0	0	0	4
Confirmed	1	..	..	1	4	..	0	1	4	4	2	5	4	0	0	3	1	0	0	0	0	0	0	0	2
E. coli found	0	..	..	0	0	..	0	1	4	1	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0

TABLE II

CLASSIFICATION OF GRAM-NEGATIVE, LACTOSE-FERMENTING ORGANISMS ISOLATED FROM COMMERCIAL FILLETS AND FISH

	Flesh	*5 to 100 Dilution*	*5 to 1,000 Dilution*
Haddock Fillets			
Typical fecal *E. coli*	35	15	10
Intermediates—Coli-like	20	18	9
Indeterminate	26	23	8
Aerogenes-like	1	2	1
Typical *Aero. aerogenes*	4	7	2
False presumptives	9	12	1
Total number of cultures	95	77	31
Eviscerated Whole Haddock			
Typical fecal *E. coli*	4	0	0
Intermediates—Coli-like	4	3	0
Indeterminate	11	11	2
Aerogenes-like	2	1	0
Typical *Aero. aerogenes*	2	0	0
False presumptives	2	0	2
Total number of cultures	25	15	4

duction was seldom observed in dilutions of 5 to 1,000 of whole fish flesh. This would indicate that if treatment and handling were in any way related to the number of positive gas tubes, and to the type of organisms found, then the whole fish were considerably better in sanitary quality than were the fillets.

DISCUSSION

These experiments have demonstrated the presence of bacteria of the coli-aerogenes group on the flesh of commercial fillets and whole fish; and *E. coli* characteristic of mammalian feces was present on many of the samples examined, particularly on the fillets. Since the work of Gibbons, Fellers, and others has shown that *E. coli* is not usually found in, or on, marine fish from deep water, it follows that the fish and fillets most likely become contaminated with these organisms during handling. The process of making fillets involves the more extensive handling and consequently results in greater possibility of contamination; this is shown by the greater abundance of *E. coli* found on fillets than on whole fish.

The presence of *E. coli* in oysters and clams is used as an evidence of pollution, and if such pollution is sufficiently serious the products are condemned. The data presented indicate that many fillets, and some whole fish, purchased at random from public markets are contaminated, but a different use should probably be made of the information. The fact that fish are well cooked before they are eaten minimizes, and in most cases removes, any danger of illness resulting from this type of contamination.

A test for *E. coli* is probably better qualitative evidence of careless handling than a total bacterial count, and a test for this, and perhaps related organisms, might be used for educational purposes, among those engaged in bringing fish to the consumer, in the effort to improve the methods of handling and marketing.

The significance of members, other than *E. coli*, of the coli-aerogenes group, is a controversial subject. The *Standard Methods of Water Analysis* of the American Public Health Association considers the presence of members of the group to be sufficient evidence of pollution to justify condemning water. However, prominent investigators, particularly Levine,[11] Tonney-Noble,[12] and Koser,[13] insist that only *E. coli* are from feces and that other members of the group are of little, or no, sanitary significance. The authors of this paper are inclined to accept the latter view.

The investigation here reported does not represent a sufficient number of samples to make the results conclusive. It is the intent, rather, to offer this preliminary study as a suggestion to laboratories concerned with the studies of fishery products that the occurrence of *E. coli* on fish and fillets is worthy of wider study. It is hoped that methods may be devised for evaluating the sanitary quality of these fishery products.

SUMMARY

1. Twenty commercial haddock fillets and 5 eviscerated haddock were purchased from local retail markets and examined for the presence of members of the coli-aerogenes group of bacteria.

2. Typical *Escherichia coli* were found on 16 of the fillets, and on 2 of the eviscerated fish.

3. Lactose-fermenting organisms other than *Escherichia coli* were commonly encountered on both fillets and eviscerated fish. Because of this fact, gas production from lactose broth tubes taken alone is not sufficient evidence of contamination. Differential tests should be made to determine whether or not the responsible organism is *Escherichia coli*.

4. Further investigation is recommended to study the possibility of using the occurrence of *Escherichia coli* as an index of the sanitary quality of marine fish and fillets.

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