Detection and Incidence of *Escherichia coli* on Storage Pen Surfaces of Fishing Trawlers

A. ROSEN AND R. E. LEVIN

Department of Food Science and Technology, University of Massachusetts, Amherst, Massachusetts 01002

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Six methods for the detection and enumeration of *Escherichia coli* on the storage pen surfaces of commercial fishing trawlers and in harbor wash water were evaluated. *E. coli* was found consistently present in Boston harbor water used for washing vessel holds and was detected either in small numbers or not at all on storage pen surfaces. Violet Red Bile Agar as a primary enumeration medium was found ineffective for detection of coliforms because of the nonselective development of large numbers of other gram-negative organisms. The use of *E. coli* broth at 44.5 C for primary most-probable-number determinations, followed by confirmation of *E. coli* on Levine Eosine Methylene Blue Agar, appears to offer numerous advantages over more conventional methods of detecting *E. coli* for survey studies of the fishing industry, where coliform-like organisms result in many false-positive presumptives with other methods.

The common practice in the New England fishing industry of using raw, untreated harbor water for washing trawler fish storage pens is of sanitary and public health significance. Such water is usually associated with sewage pollution arising locally or from large-volume municipal sewage disposal in the same locale. The industry practice of briefly hand-scrubbing pen surfaces and rinsing with untreated harbor water was previously shown (4) to achieve no detectable reduction in the bacterial load.

This study is concerned with the enumeration of *Escherichia coli* on storage pen surfaces after washing with unchlorinated harbor water and with the examination of the various schemes available for detection and confirmation.

**MATERIALS AND METHODS**

**Sampling.** Alginate wool swabs were used with stainless-steel templates to obtain 1-square inch swab samples (ca. 2.54 by 2.54 cm). Immediately after all of the fish in the storage hold of a vessel were unloaded, three swab samples were obtained from different panels of the same pen and dissolved in 20 ml of 1% Calgon. Three swab samples were again obtained adjacent to the first areas sampled after the storage surfaces of the hold and pens had been hosed down and scrubbed with unchlorinated harbor water in the usual manner by the cleanup crew. Water samples were also collected from the hose used on board the vessels for washing the storage pens and from the water source itself, the harbor water adjacent to the vessels. Bacteriological tests were performed within 24 hr after samples were obtained.

**Bacteriological methods.** Decimal dilutions of dissolved swabs and water samples were made in Nutrient Broth containing 0.5% NaCl. The detection procedures were separated into six groups, each designated with a Roman numeral (Fig. 1). Difco media were used throughout. In method I, Lauryl Tryptose (LT) Broth was used for five-tube most-probable-number (MPN) presumptive determinations for coliforms. Tubes showing gas after 48 hr of incubation at 35 C were used to inoculate tubes of Brilliant Green Lactose Bile (BGB) Broth, followed by the transfer of resulting growth onto Levine Eosine Methylene Blue (EMB) Agar and incubation at 35 C for 24 hr; the combinations of these procedures served as the confirmed test. Typical colonies of *E. coli* were picked and transferred onto slants of Nutrient Agar (NA), from which the indole, methyl red, Voges-Proskauer, and citrate (IMViC) tests were performed.

Method II made use of five-tube MPN determinations by using tubes of BGB incubated for 48 hr at 35 C, followed by confirmation with EMB and the IMViC tests.

In method III, plates of EMB Agar were inoculated directly with 0.1 ml of diluted samples which were spread with a sterile glass rod and then incubated at 35 C for 24 hr. Colonies having the typical appearance of *E. coli* were picked for IMViC tests.

Method IV consisted first in transferring growth from all positive LT MPN tubes from method I to tubes of *Escherichia coli* (EC) Broth followed by incubation at 44.5 C for 48 hr. The growth from positive tubes was then streaked onto EMB Agar plates, and colonies typical of *E. coli* were transferred to NA slants from which the IMViC tests were performed.

In method V, pour plates of Violet Red Bile (VRB) Agar were used for initial enumeration. Coliform
colonies were picked after 18 to 24 hr of incubation, purified, and transferred to NA slants from which growth was transferred to BGB Broth tubes; resulting growth from positive tubes was then streaked onto EMB Agar plates. All coliform-like colonies picked from the original VRB plates were subjected to the IMViC tests.

In method VI, EC Broth was used for primary MPN enumeration of E. coli. Growth from all tubes showing gas within 48 hr at 44.5 C was streaked onto EMB plates, and colonies typical of E. coli were transferred to NA slants from which the IMViC tests were performed.

The IMViC tests were performed by the standard

\[ \text{Table 1. Incidence of coliforms and E. coli on fish storage pen surfaces of two commercial trawlers and in untreated harbor wash water}\]

| Method | Organisms | Sample a
| | | Hose water | Harbor water | Pen surface before washing | Pen surface after washing |
|--------|-----------|-------------|--------------|---------------------------|--------------------------|
| Vessel 1 | | | | | |
| I | Coliforms | 49 | 230 | 22 | 25 |
| E. coli | 0 | 1.2 | 0 | 1.3 |
| II | Coliforms | 33 | 109 | 27 | 1.3 |
| E. coli | 0.4 | 0.2 | 0 | 0 |
| III | Coliforms | 10 | 30 | 3.3 | 0 |
| E. coli | 10 | 0 | 0 | 0 |
| IV | Coliforms | 49 | 230 | 22 | 25 |
| E. coli | 3.3 | 35 | 0 | 1.3 |
| V | Coliforms | 80 | 0 | 1,320 | 211 |
| E. coli | 40 | 0 | 0 | 0 |

| Vessel 2 | | | | | |
| I | Coliforms | 79 | 490 | 320 | 220 |
| E. coli | 4 | 4 | 1.3 | 13 |
| II | Coliforms | 79 | 130 | 26 | 16 |
| E. coli | 0 | 6 | 6 | 1.3 |
| III | Coliforms | 100 | 1,600 | 0 | 0 |
| E. coli | 0 | 2 | 0 | 0 |
| IV | Coliforms | 79 | 490 | 320 | 0 |
| E. coli | 14 | 35 | 0 | 0 |
| V | Coliforms | 130 | 270 | 148,000 | 5,700,000 |
| E. coli | 0 | 0 | 0 | 0 |

\( a \) Counts are given as number of bacteria per square inch of surface or as number per milliliter of water sample.

\( b \) Counts from pen surfaces represent the mean of three 1-square inch areas obtained from different locations of the same pen.
methods of the American Public Health Association (1). Preliminary results from these six methods indicated that all cultures presumptively positive regardless of the nature of confirmatory results yielded only gram-negative organisms, and the Gram stain was eliminated from later studies. Where solid media were used for initial enumeration, at least five typical coliform colonies per plate were picked where possible for confirming studies.

RESULTS

Comparison of five methods for detection and enumeration of E. coli from pen surfaces and wash water. Two vessels were used for this study (Table 1). Method IV was found to be the method of choice for enumeration of E. coli in wash water. Only methods I, II, and IV successfully recovered E. coli from pen surfaces. The use of VRB Agar as the primary medium in method V proved highly unsatisfactory for detection of E. coli in both wash water and on pen surfaces because of the development of large numbers of red coliform-like colonies, some of which exhibited zones of precipitation typical of E. coli which failed to grow in BGB Broth or EMB Agar. Considerable difficulty was encountered with the use of EMB Agar as the primary medium in method III because of the extensive growth of organisms other than coliforms. With all five methods used, E. coli was detected in only low numbers on pen surfaces or not at all, which is most likely accounted for by the low, though consistent, incidence of E. coli in hose and harbor water (Table 1).

Incidence of coliforms and E. coli on pen surfaces and in wash water. Eleven vessels were examined between October 1967 and October 1968 by using method IV. E. coli was detected on only two vessels before washing and on three after washing. The highest number of E. coli detected was 2.6 per square inch. The samples of hose and harbor water all yielded the consistent presence of E. coli and were highest in harbor water (harbor water, 1 to 35 per ml; hose water, 0.5 to 22 per ml).

Effectiveness of EC Broth as a primary enumeration medium for E. coli. The effectiveness of EC Broth incubated at 44.5 C as the primary medium for MPN determinations for E. coli was compared to the conventional method with LT Broth at 35 C. Four trawlers were examined in this study (Table 2). E. coli was detected on two vessels before washing and on three after washing (before, 0.3 to 1 per square inch; after, 1 to 2.7 per square inch). The samples of hose and harbor water all yielded E. coli (harbor water, 5 to 35 per ml; hose water, 4 to 21 per ml). No significant loss in the final number of confirmed E. coli was encountered with the use of EC Broth at 44.5 C, which offered the advantages of (i) eliminating many false-positive presumptives otherwise obtained with LT Broth, (ii) eliminating 1 additional day of incubation, and (iii) reducing considerably the amount of media and effort involved in obtaining final confirmation and in determining the identity of organisms producing gas in positive presumptive tubes.

DISCUSSION

Various methods and media are presently in use for enumerating coliforms and E. coli in food products and water. To determine the MPN values of coliforms in water, the Canadian Department of Fisheries (2) recommends Lactose Broth followed by confirmation with BGB Broth; for estimating the density of E. coli on fish fillets, the suggested method is the direct inoculation of dilutions into MPN tubes of BGB Broth, followed by confirmation in EC Broth at 45 C. The American Public Health Association (1) recommends the enumeration of E. coli and coliforms in water by performing a

| Trawler | Sample | Presumptive | Presumptive | Confirmed | Confirmed |
|---------|--------|-------------|-------------|-----------|-----------|
|         | Hose water | 172 | 130 | 1.7 | 33 | 4.9 |
|         | Harbor water | 130 | 49 | 4.6 | 23 | 3.3 |
|         | Before washing | 2,140 | 12 | 1 | 3.5 | 1 |
|         | After washing | 8.5 | 0 | 0 | 0 | 0 |
|         | Hose water | 345 | 79 | 1.2 | 4 | 4 |
|         | Harbor water | 1,090 | 39 | 2.1 | 49 | 34.8 |
|         | Before washing | 184 | 0 | 0 | 1 | 0 |
|         | After washing | 13.5 | 2 | 1 | 11.5 | 1 |
|         | Hose water | 278 | 79 | 17.2 | 700 | 21 |
|         | Harbor water | 221 | 109 | 17.0 | 34.8 | 13 |
|         | Before washing | 316 | 0 | 0 | 0 | 0 |
|         | After washing | 318.7 | 4 | 2.7 | 0 | 0 |
|         | Hose water | 265 | 96 | 6.7 | 245.7 | 10 |
|         | Harbor water | 480.3 | 65.7 | 7.9 | 35.6 | 17 |
|         | Before washing | 879.8 | 4 | 0.3 | 1.5 | 0.3 |
|         | After washing | 413.6 | 2 | 1.2 | 3.8 | 0.3 |

*Performed in Lauryl Tryptose Broth at 35 C.*
*Performed in EC Broth at 44.5 C.*
*According to IMViC tests.*
presumptive test in LT Broth, followed by confirmation with BGB Broth or EMB Agar and completing the test with the Gram stain and observing for gas production in LT Broth. Our results indicate that the direct inoculation of diluted samples into EC Broth incubated at 45°C appears to be the method of choice for the enumeration of E. coli in fishery products and untreated harbor water used for washing vessels.

VRB Agar is recommended by some workers for selectively detecting coliforms from food products of nonmarine origin. Lewis and Angelotti (5) recommend VRB Agar as a primary solid enumeration medium for detecting coliforms in foods, followed by confirmation in BGB Broth; for E. coli, they recommend MPN determinations in LT Broth, followed by confirmation in EC Broth incubated at 44.5°C. Hartman (3) observed a great variation in the percentage of confirmed coliforms when various foods were tested by use of VRB Agar as the primary detection medium. Our results indicate that VRB Agar is ineffective as a selective medium for the direct enumeration of coliforms from fishery sources and that the term coliform has no significance in the fishing industry since many gram-negative organisms gave rise to false-positive presumptive tubes of LT Broth and BGB Broth and mimicked coliforms on VRB Agar.

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