Rate of Occurrence of False-Positive Results from Total Coliform Most-Probable-Number Analysis of Shellfish and Estuaries

DAVID HUSSONG, RITA R. COLWELL, AND RONALD M. WEINER*
Department of Microbiology, University of Maryland, College Park, Maryland 20742

The incidence of confirmed test, false-positive coliform most-probable-number results was compared with environmental parameters and was found to be inversely related to water temperature. It is concluded that the completed coliform test must be done when water temperatures drop below 15°C.

Shellfish harvested from estuarine waters are examined for total numbers of coliforms, along with water and sediment samples from the harvesting areas. The most-probable-number (MPN) analysis (1, 3, 13) is routinely employed and is carried through the presumptive, confirmed, or completed sequence of tests. The completed tests are not always done when the sanitary quality of water is being assessed, notably in the cases of bathing and potable waters (2). To establish a balance between efficiency and accuracy, the incidence of false-positive and false-negative results at each stage of the analysis should be known.

It has been well documented that the presumptive test alone may be of limited reliability (11), historically because of those noncoliforms which may be present and capable of fermenting lactose aerogenically (4, 5, 6, 8, 9, 10, 12). In the study reported here, Chesapeake Bay oysters and oyster beds were examined over a 2-year period. Two sites in Chesapeake Bay, Tolly Point and Eastern Bay, were sampled on a routine basis. These sites were selected because they are commercially important oyster harvest areas and, in addition, the water column of both areas is subject to very little fecal contamination (mean total coliform completed test MPN, 8/100 ml). At approximately 1-month intervals during 1977 and 1978, bottom water samples were collected at one meter above the sediment by means of the Niskin sampler (General Oceanics, Inc.). Sediment samples were collected by using a Petite Ponar grab (Wildlife Supply Co.), and oysters were harvested using a drag-type dredge. All samples were processed within 30 min of collection.

Six oysters, each of which weighed ca. 16 to 20 g, including meat and liquor, after shucking, were scrubbed, rinsed, and aseptically shucked. The oyster tissue was pooled and homogenized in a solution consisting of sterile 0.5% (wt/vol) peptone (Difco Laboratories) in a 1:2 dilution of oyster tissue. Sediment samples were suspended in an estuarine three salts solution (3). Salinity and temperature were measured at the time of collection of the bottom water samples.

A five-tube, total coliform MPN analysis of each of the water, sediment, and oyster samples was performed in duplicate, and the results were normalized for 100 ml (or 100 g) of sample, following procedures recommended by the American Public Health Association (1). Samples (10, 1.0, 0.1, and, for sediment suspension, 0.01 ml) were transferred to appropriate tubes. Lactose broth (Difco), brilliant green bile (2%) broth (Difco), and eosin methylene blue agar (Difco) were employed. Total viable counts (TVC) of aerobic heterotrophic bacteria were enumerated on 30% strength 2216E Marine agar (Difco) (7) plates prepared in triplicate. The TVC plates were incubated at 17 ± 2°C for ca. 15 days before counts were made.

Within each MPN test series, the number of positive results at each successive step (Table 1) was compared, and the proportion of positive presumptive tests which failed to be confirmed as total coliform-positive was defined as the false-positive percent (FP%). The calculation was done using the formula: FP% = [(P - C)/P] × 100, where P is positive results and C is confirmed (or completed) positive results. This comparison was made for the presumptive-confirmed and also the confirmed-completed test steps. For each sample, the false-positive percentages were, in turn, compared with data for total coliform MPN, TVC, salinity, and temperature. Correlation coefficients were obtained using the Biomedical Computer Programs (BMDP) statistical package on the University of Maryland UNIVAC 1108 computer, and compared with values for critical r (15).

Bottom water salinities ranged from 7.4 to 15.0%. TVC and temperature values are re-
TABLE 1. Numbers of positive reactions obtained for presumptive, confirmed, and completed coliform MPN tests

<table>
<thead>
<tr>
<th>Date</th>
<th>Temp (°C)*</th>
<th>Tolly Point</th>
<th>Eastern Bay</th>
<th>Sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of positive reactions*</td>
<td></td>
<td>No. of positive reactions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oyster</td>
<td>Bottom water</td>
<td>Oyster</td>
</tr>
<tr>
<td>24 October 1977</td>
<td>15</td>
<td>21, 20, 20</td>
<td>(1.2E3)</td>
<td>13</td>
</tr>
<tr>
<td>18 November 1977</td>
<td>11</td>
<td>18, 15, 15</td>
<td>(2.0E3)</td>
<td>13</td>
</tr>
<tr>
<td>20 December 1977</td>
<td>5</td>
<td>7, 4, 2</td>
<td>(2.0E3)</td>
<td>5</td>
</tr>
<tr>
<td>18 January 1978</td>
<td>1</td>
<td>2, 0, 0</td>
<td>(2.0E3)</td>
<td>1</td>
</tr>
<tr>
<td>28 March 1978</td>
<td>6</td>
<td>10, 9, —</td>
<td>(5.0E3)</td>
<td>8</td>
</tr>
<tr>
<td>18 April 1978</td>
<td>10</td>
<td>13, 13, 13</td>
<td>(4.0E3)</td>
<td>9</td>
</tr>
<tr>
<td>19 May 1978</td>
<td>9</td>
<td>14, 14, 13</td>
<td>(4.0E3)</td>
<td>9</td>
</tr>
<tr>
<td>21 July 1978</td>
<td>26</td>
<td>5, 3, 3</td>
<td>(4.0E3)</td>
<td>24</td>
</tr>
<tr>
<td>6 September 1978</td>
<td>26</td>
<td>9, 7, 7</td>
<td>(9.3E4)</td>
<td>27</td>
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<td>31 October 1978</td>
<td>14</td>
<td>11, 6, 6</td>
<td>(6.0E3)</td>
<td>15</td>
</tr>
</tbody>
</table>

*Temperature of water 1 to 2 m below surface.

**Positive results at each test level: first column is presumptive, second is confirmed, third is completed.
Initial observations were recorded for 15 MPN tube series done in duplicate. Number within parentheses is the TVC for the corresponding sample. See text for procedures. —, No data.

Reported in Table 1. Oyster total coliform MPN values were consistently low, averaging 81/100 g at Tolly Point and 34/100 g at Eastern Bay. Bottom water total coliform MPN values averaged ca. 12/100 ml and 3.2/100 ml, respectively. Sediment counts at Eastern Bay averaged 13/100 g.

The percent occurrence of false-positive presumptive and confirmed results are presented in Table 2 and Fig. 1, respectively. Overall, some parameters were not found to be correlated (probability, P < 0.80%). For example, the percent occurrence of false-positive confirmed results did not correlate with: (i) percent occurrence of false-positive presumptive tests (r = 0.068); (ii) TVC (r = 0.193); or (iii) salinity (r = —0.125). Some equivocal correlations (90% < P < 95%) were noted, and these included total coliform MPN with false-positive percentages, both presumptive (r = —0.269) and confirmed (r = —0.272), and with salinity (r = —0.113).

The most important and definitive relationship detected was that of false-positive and confirmed results and sample temperature (Fig. 1). Although some variations were recorded between the stations as well as for each sample type, it was clear, particularly with regard to sediments (Table 1), that the number of false-positives detected in the confirmation tests increased significantly when the water temperature fell below 10°C. In fact, this relationship was statistically validated for each sample type and station. To cite composite data, water temperatures were found to have a strong negative correlation with percentage of false-positive confirmed tests (—0.593 correlation, significant at

TABLE 2. Percentage of false-positive presumptive MPN results

<table>
<thead>
<tr>
<th>Date</th>
<th>% of false-positive results</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Oyster</td>
</tr>
<tr>
<td>24 October 1977</td>
<td>5</td>
</tr>
<tr>
<td>18 November 1977</td>
<td>17</td>
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<tr>
<td>20 December 1977</td>
<td>43</td>
</tr>
<tr>
<td>18 January 1978</td>
<td>100</td>
</tr>
<tr>
<td>28 March 1978</td>
<td>10</td>
</tr>
<tr>
<td>18 April 1978</td>
<td>0</td>
</tr>
<tr>
<td>19 May 1978</td>
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</tr>
<tr>
<td>6 September 1978</td>
<td>22</td>
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<tr>
<td>31 October 1978</td>
<td>45</td>
</tr>
</tbody>
</table>

—, No data.
the 99.9% confidence level; critical $r = 0.372$; $n - 3 = 45$ (Table 3). The temperature at which significant false-positive results begin to be observed may be related to changes in the composition of the bacterial population (14).

Based on the results of this study, it is concluded that, in the past, total coliform MPN (confirmed test) results for cold, estuarine water samples (i.e., $<15^\circ C$) were subject to error, and reported values may have been higher than was, in fact, the case. It is recommended that the total coliform MPN evaluation of estuarine water, shellfish, and sediment samples include the completed test whenever the temperature of the water falls below $15^\circ C$.

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**LITERATURE CITED**


