Survival of $R^+$ *Escherichia coli* in Sea Water

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The survival of *Escherichia coli* strains in sea water appears not to be affected by the possession of an $R^+$ factor. Sea water induces no detectable curing of $R^+$ *E. coli*.

Recent workers have demonstrated the existence of significant numbers of coliforms with transferable multiple drug resistance in fresh water and the sea (3, 10, 11) and have raised the question of the public health implications of this contamination. A serious analysis of the degree to which $R^+$-containing bacteria in sewage-contaminated water are a factor in the spread of resistance transfer factors in the swimming population would require accurate information on the distribution of these bacteria. Richmond (9) has shown that 95% of the $R^+$ bacteria in urban sewage originate from healthy humans and estimates by Datta (2), Moorhouse (7), and Linton et al. (5) suggest that between 30 and 70% of such humans carry $R^+$ bacteria. The ratio of fecal coliforms to $R^+$-carrying fecal coliforms in sewage should therefore be expected to remain fairly stable. Once this ratio is known for the domestic sewage from any population during any time period, it should be possible to estimate the number of $R^+$ factors in a body of water contaminated by such sewage from a series of standard fecal coliform counts.

The accuracy of this estimation would be influenced by any difference in survival rates between the $R^+$ fecal coliforms and the drug-sensitive fecal coliforms or by any significant curing of the cells of their $R^+$ factors. Garrod and O'Grady (4) have commented on the fact that antibiotic-resistant strains frequently have a reduced growth rate and Monner, Jonsson, and Boman (6) have shown this to be true for some classes of ampicillin-resistant *Escherichia coli*. Williams Smith has published data on the frequency of ampicillin-resistant coliforms in sewage-contaminated rivers (10) and sea water bathing areas (11) in England and Wales. He found higher frequencies of ampicillin resistance in coliforms isolated from fresh water than in those isolated from sea water. Further, he found 13 of 20 ampicillin-resistant strains from river water were capable of transferring their resistance, whereas only 25 of 76 strains isolated from sea water could do so. This work was undertaken to discover whether naturally occurring ampicillin-resistant and, in particular, $R^+$ ampicillin-resistant bacteria had an altered survival rate in sea water.

The numbers of presumptive fecal coliforms (PFC) and ampicillin-resistant presumptive fecal coliforms (ARPFC) were determined at each of five sites in Galway Bay. The antibiotic resistance pattern of 200 strains randomly chosen from the ARPFC isolated at each site was determined (i.e., a total of 1,000 strains) and some of these strains were tested for their ability to transfer their resistance.

PFC were counted by the Teepol Broth Membrane Filter technique (13). Ampicillin (Beecham Laboratories) was added to a final concentration of 15 $\mu$g/ml to the same medium to count ARPFC. Antibiotic resistance was determined on Maconkey no. 3 agar (Oxoid Ltd., London) with disks (Scientific Hospital Supplies, Liverpool) containing ampicillin, kanamycin, chloramphenicol, tetracycline, neomycin, and streptomycin. The method of Moorhouse and McKay (8) was used to demonstrate the ability of strains to transfer their resistances to *E. coli* 711, a naladixic acid-resistant, lactose-negative auxotroph (obtained from E. Moorhouse). Ampicillin was used as a selective marker.

Untreated sewage enters Galway Bay at a series of outfalls. Five sites were chosen. Site 1 was near the outfall; site 2 was 500 yards from the outfall. Sites 3, 4, and 5 were bathing areas approximately 1, 2, and 3 miles from the outfall. Samples were taken as recommended by the American Public Health Association (12) approximately 20 yards offshore at a 10-cm depth at various stages of the tide.

Table 1 shows the results from 152 samples that were taken from the Bay at the five sites. The covariance of the 152 paired counts of PFC's and ARPFC's was found to be 0.9447, which is significant at the 0.001% level, showing
that the ratio PFC/ARPF C does not vary significantly as the concentration of fecal coliforms drops from an average of 44,000/100 ml at site 1 to an average of 90/100 ml at site 4. The percentage of ampicillin-resistant strains isolated at each site which were capable of transferring all or part of their multiple antibiotic resistance to *E. coli* 711 did not alter significantly from site to site (*X^2^ = 6.40, *P* = 0.1712).

It can, therefore, be inferred that there is no significant change in the ratio of presumptive fecal coliforms and presumptive fecal coliforms containing R+ factors associated with ampicillin as the sewage moves in the sea from site 1 to the other sites several miles away.

Only 59% (23 out of 39 tested) of strains resistant to ampicillin alone could transfer this resistance, whereas 87% (236 out of 271) of strains resistant to two or more drugs could do so. Therefore, one would expect that any change in the frequency of R+ strains in the PFC population would be reflected in the percentage of single drug resistance in the ARPF C’s tested at each site. No significant fluctuation can be seen in this percentage (*X^2^ = 6.177, *P* = 0.1863) (see Table 1) which supports the hypothesis that PFC’s containing R+ factors coding for ampicillin survive at the same rate as other PFC’s.

That R+ factors coding for ampicillin resistance represent a special case is improbable, and therefore we suggest that these results demonstrate that R+ fecal coliforms are not cured in sea water nor do they have a detectable different survival rate in sewage-contaminated sea water to drug-sensitive fecal coliforms. It should therefore be possible to estimate the numbers of R+ fecal coliform bacteria in sea areas polluted with domestic sewage from a knowledge of the prevailing ratio of R+/total fecal coliforms in the local sewage and the standard coliform count in the sea.

### LITERATURE CITED


### TABLE 1. Number of presumptive fecal coliforms and ampicillin-resistant, presumptive fecal coliforms at sites in Galway Bay and the frequency of single ampicillin-resistant strains and strains capable of transferring all or part of their resistances amongst the ampicillin-resistant presumptive fecal coliforms isolated at these sites

<table>
<thead>
<tr>
<th>Determination</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Site 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of sub/sites</td>
<td>2</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>No. of samples</td>
<td>16</td>
<td>30</td>
<td>38</td>
<td>38</td>
<td>30</td>
</tr>
<tr>
<td>Log mean PFC/100 ml*</td>
<td>44,000</td>
<td>250</td>
<td>739</td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td>Log mean ARPF C/100 ml</td>
<td>9,000</td>
<td>33</td>
<td>75</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>No. of ARPF C resistant to ampicillin only*</td>
<td>21/200</td>
<td>34/200</td>
<td>28/200</td>
<td>22/200</td>
<td>20/200</td>
</tr>
<tr>
<td>No. of ARPF C tested for R+ transfer</td>
<td>80</td>
<td>35</td>
<td>85</td>
<td>73</td>
<td>35</td>
</tr>
<tr>
<td>No. of ARPF C showing R+ transfer (%)</td>
<td>62 (77)</td>
<td>30 (85.7)</td>
<td>70 (82.3)</td>
<td>67 (91.78)</td>
<td>28 (80)</td>
</tr>
</tbody>
</table>

* 200 Isolates were tested at each site.