EVALUATION OF DIFFERENT BACTERIOPHAGE GROUPS AS FAECAL INDICATORS IN CONTAMINATED NATURAL WATERS IN SOUTHERN ENGLAND

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Abstract—River and seawater affected by faecal discharges were analysed to evaluate the reliability of coliphages and F-specific RNA bacteriophages as indicators of the microbiological quality of waters. F-specific RNA bacteriophages showed no direct relationship with the levels of faecal pollution, and this group was never detected in samples with a low level of enteroviruses (1-10 pfu/101.). In contrast, coliphages were constantly detected in the same samples. The concentration of coliphages detected in the samples with 1-10 and > 10 pfu/101. of enteroviruses were similar and higher than the Economical European Community guide and imperative levels of faecal coliforms (100 and 2000 FC/100 ml, respectively). Therefore, coliphages would be considered as an optimal indicator of the microbiological quality of the natural waters.

Key words—coliphages, F-specific RNA bacteriophages, indicators, faecal pollution, water quality

INTRODUCTION

Faecal pollution of natural waters is usually evaluated by the analysis of different groups of indicator microorganisms. It has been shown that a number of these microorganisms relate to the level of pollution of natural waters by sewage discharges, such as total and faecal coliforms, faecal streptococci and sulphite-reducing clostridia.

Faecal (or thermotolerant) coliforms have been considered useful as indicators of the hygienic quality of waters (APHA, 1985). However, several studies have demonstrated some shortcomings of this group on the basis of: (i) low survival outside the intestinal tract, particularly in marine environments (Goyal et al., 1978; LaBelle et al., 1980; Borrego et al., 1983); (ii) uncertain definition as a group (Barrow, 1977; Dufour, 1977; White and Godfree, 1986); and (iii) low relationship with the presence of pathogenic bacteria (Morifigio et al., 1990) and viruses (Goyal, 1983; Geldenhuys and Pretorius, 1989; Merrett et al., 1989). For this reason, it is important to consider other indicators which may exhibit a closer relationship with the presence of pathogenic microorganisms in water, particularly those which may persist longer in the environment.

Several authors have proposed the use of coliphages (specific bacteriophages of Escherichia coli) as appropriate faecal indicators because of their constant presence in sewage and polluted waters (Vaughn and Metcalf, 1975; Dhillon et al., 1976; Kott, 1984; Borrego et al., 1987). Moreover, this group has been suggested as a potential indicator of viral pollution, due to its similar nature and survival characteristics in the aquatic environment (Simkova and Cervenka, 1981; Stetler, 1984; Grabow, 1986).

However, several studies have pointed out disadvantages of coliphages as indicators because: (i) enteric viruses have been detected in their absence ( Montgomery, 1982; Deetz et al., 1984); (ii) coliphages may replicate in the natural waters under certain conditions (Vaughn and Metcalf, 1975; Borrego et al., 1990); and (iii) because autochthonous coliphages have been detected in unpolluted waters (Seeley and Primrose, 1980). These disadvantages have led other authors to propose the use of different phage groups as models of enteric viruses, such as F-specific phages (Primrose et al., 1982) and RNA bacteriophages belonging to the E morphological group (Havelaar et al., 1986). These bacteriophages possess structures and sizes very similar to enteric viruses, and they infect only those bacterial hosts which produce F pili (Havelaar et al., 1986). Havelaar and Hogeboom (1984) have developed a method for the detection of F-specific bacteriophages using a Salmonella typhimurium strain as bacterial host. This strain is unable to produce F pili at temperatures lower than 30°C, and thus productive infection with F-specific phages can never occur in the normal conditions of the aquatic environment.

The aim of this study is to determine the relationship between different phage groups which have been proposed as indicators and the classical indicators of faecal pollution, and to compare their reliability as
indicators regarding the presence of enteric viruses in seawater and river water samples collected in Southern England.

**MATERIALS AND METHODS**

**Sampling zones**

Samples were collected from two different aquatic environments: (a) a fresh water environment where the samples were obtained from seven sampling stations on the River Thames; and (b) a marine environment where samples were collected from five sampling stations in Ramsgate (Kent, England).

Sampling was carried out weekly for 6 weeks in both areas studied, and a total of 43 samples were processed. Samples were collected in sterile plastic bottles and transported to the laboratory in isothermic containers at 4°C. They were analysed within 4 h of sampling.

**Bacterial analyses**

The membrane filtration technique (APHA, 1985) was used for the quantitative analysis of total coliforms (TC), faecal (thermotolerant) coliforms (FC) and faecal streptococci (FS). Culture media and incubation conditions were: TC, membrane lauryl sulphate broth (Oxoid Ltd) incubated at 37 ± 0.1°C for 18 h; FC, membrane lauryl sulphate broth (Oxoid Ltd) inoculated at 44 ± 0.1°C for 18 h; and FS, Slanetz–Barley medium (Oxoid Ltd) incubated at 37 ± 0.1°C for 48 h. In all cases, resuscitation of filtered organisms was allowed for 4 h at 30°C prior to incubation at restrictive temperatures.

**Virological analyses**

**Somatic coliphages (CP).** Specific bacteriophages to the strain *Escherichia coli C* (ATCC 13706 Nal') were enumerated by the double agar layer technique (Bell, 1976). Portions of 0.2 ml of the bacterial host culture in exponential growth and 0.1 ml of the water sample were added to tubes containing 3 ml of soft agar (0.7%) and maintained at 45°C in a thermostatic water bath. Then, the mixture was poured onto Luria agar plates and after solidification, they were inverted and incubated at 37°C for 18–20 h.

**F-specific RNA phages (RNA-FSP).** Before analysis for this phage group, samples were treated with chloroform 10% (vol/vol) for 20–30 min, to inactivate F-specific DNA phages. After this treatment, the detection and enumeration of these phages were carried out as described by Havelaar and Hogeboom (1984). 1 and 0.1 ml of the sample were added to tubes with 1% Tryptone-yeast extract-glucose agar (TYGA), and each was subsequently mixed with 1 ml of an exponential culture of *S. typhimurium* WG 49. The mixture was poured onto Luria agar plates and after solidification, they were inverted and incubated at 37°C for 18–20 h.

F-specific RNA phages were only detected in river water with levels than faecal coliforms at 100 and 47.4%, respectively. In samples with a moderate level of faecal pollution, only total coliforms and coliphages were detected in all the samples tested. In addition, both indicator groups showed higher titres than faecal coliforms in 100 and 87.5% of the samples, respectively. Salmonella somatic (SSP) and RNA-FSP phages were only detected in river water with percentages of 27.8 and 55.6%, respectively.

**Total coliforms, faecal streptococci and coliphages**

were always detected in all samples with a high level of faecal pollution (>1000 FC/100 ml). However only total coliforms and coliphages showed higher levels than faecal coliforms at 100 and 47.4%, respectively (Table 1). SSP and RNA-FSP bacteriophage groups were detected in less than 50% of these samples. RNA-FSP showed higher titres than faecal coliforms in only 26.3% of the samples (50% in river water and 0% in seawater) (Table 1).

Regression coefficient (r) values obtained from the correlation analyses between the concentration of faecal coliforms and those of the other microbial groups studied are shown in Table 2. In the samples with a moderate level of pollution (<1000 FC/100 ml) the highest correlation coefficients were obtained with total coliforms (r = 0.71) and coliphages (r = 0.63). All the correlations were significant except for faecal streptococci. At higher levels of faecal pollution (>1000 FC/100 ml), no significant relationships were obtained between the concen-

**RESULTS**

Table 1 shows the relationships between the concentrations of different microbial groups tested and the concentration of faecal coliforms in river water and seawater samples with a moderate and high level of faecal pollution (<1000 and >1000 FC/100 ml, respectively). In samples with a moderate level of faecal pollution, only total coliforms and coliphages were detected in all the samples tested. In addition, both indicator groups showed higher titres than faecal coliforms in 100 and 87.5% of the samples, respectively. Salmonella somatic (SSP) and RNA-FSP phages were only detected in river water with percentages of 27.8 and 55.6%, respectively.

![Image](image-url)
Table 2. Regression coefficients between the concentrations of faecal coliforms (FC) and those of other indicators

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>≤ 10² FC/100 ml (n = 24)</th>
<th>&gt; 10² FC/100 ml (n = 19)</th>
<th>Overall (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td>0.61</td>
</tr>
<tr>
<td>Faecal streptococci</td>
<td>0.17</td>
<td>P</td>
<td>0.52</td>
</tr>
<tr>
<td>Coliphages</td>
<td>0.63</td>
<td>&lt;0.001</td>
<td>0.41</td>
</tr>
<tr>
<td>Salmonella somatic phages</td>
<td>0.37</td>
<td>&lt;0.1</td>
<td>0.21</td>
</tr>
<tr>
<td>F-specific RNA phages</td>
<td>0.47</td>
<td>&lt;0.05</td>
<td>0.22</td>
</tr>
</tbody>
</table>

n = Number of samples; r = regression coefficient; P = degree of significance.
NS = No significance at 90% confidence level.

Table 3. Relationships between the concentrations of different phage groups and the presence of enteroviruses in the samples

<table>
<thead>
<tr>
<th>Phage groups</th>
<th>Absence of enteroviruses (n = 13)</th>
<th>Presence of enteroviruses (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–10⁴</td>
<td>&gt; 10⁴</td>
</tr>
<tr>
<td>Coliphages</td>
<td>258†</td>
<td>210†</td>
</tr>
<tr>
<td>Salmonella somatic phages</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>F-specific RNA phages</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

*Titre expressed as plaque forming units (pfu)/100 ml.
†Geometric mean of the concentrations (expressed as pfu/100 ml).

DISCUSSION

As indicators of faecal pollution coliphages have some disadvantages (Dhillon et al., 1976; Deetz et al., 1984; Kott, 1984; Borrego et al., 1987). For this reason, other investigators have proposed the use of other different phage groups as potential indicators of water quality (Primrose et al., 1982; Havelaar et al., 1986). In this study, coliphages were detected at higher numbers than faecal coliforms in most samples tested with a moderate level of pollution. This may be due in part to better survival in aquatic environments (Borrego et al., 1983; Cornax et al., 1991). Coliphages and total coliforms exhibited the best correlation with levels of faecal coliforms. These results agree with those obtained by those authors who have proposed coliphages as good indicators of the presence of pathogenic bacteria (Borrego et al., 1987; O'Keefe and Green, 1989; Morinigo et al., 1990) and of enteric viruses (Simkova and Cervenka, 1981; Stetler, 1984; Grabow et al., 1984). In contrast, O'Keefe and Green (1989) observed no significant correlation coefficients when the level of faecal pollution was low. This observation could be due to the fact that these authors employed water samples with a lower level of pollution.

In the present study, samples with higher titres of faecal streptococci than faecal coliforms were not found. This finding is not consistent with the results of studies in warmer waters, in which even at moderate levels of faecal pollution, the concentrations of faecal streptococci were higher than those of faecal coliforms (Vasconcelos and Swartz, 1976; Borrego et al., 1982). This difference could be attributed to the medium used for the enumeration of faecal streptococci (Geldreich, 1976; Beaudoin and Litsky, 1981), or the different survival characteristics or the members of the FS group in marine waters.

A differential detection rate of F-specific RNA bacteriophages was noted depending on the sampling source. From river water samples, the detection rates were higher than 50%, regardless of the faecal pollution level (55.6% for moderate and 70%
for high levels of pollution, respectively). In contrast, in seawater this group of phages was only detected from samples with concentrations higher than 1000 FC/100 ml (Table 1). This difference could be explained by a lower persistence of these phages in seawaters, or a higher dilution of pollution in the marine environment. However, in a previous study, Cornax et al. (1991) did not observe a significant difference in the survival rate between F-specific RNA bacteriophages and somatic coliphages. It is relevant to note that this study was carried out in the Mediterranean Sea which has different physical and chemical characteristics compared with the North Sea.

The low concentration of RNA-FSP bacteriophages, compared with the levels of coliphages obtained in this study using the same methodology, is an important shortcoming in their application as a universal indicator of enteric viruses. In addition, visualization of the plaques produced by these isometric phages can be difficult and requires practice (Kfir et al., 1990).

SSP and RNA-FSP bacteriophages did not show a direct relationship with the levels of faecal pollution, since an increase of the numbers of faecal coliforms did not correspond with a higher percentage of detection of these phages (Table 1). This finding agrees with that of Havelaar et al. (1990) who suggested that the use of RNA-FSP is more appropriate as a model of behaviour of viruses in water and wastewater treatment, than as a general indication of faecal pollution.

Coliphages were constantly detected in samples with low levels of enteroviruses (Table 3). This observation contrasts with the absence of SSP and RNA-FSP bacteriophages in samples with levels of 1–10 enteroviruses/101 l. (Table 3). Low rates of detection were obtained for these phages in all the samples, compared with the constant presence of detectable concentrations of coliphages (Table 4). This result leads us to conclude that RNA-FSP bacteriophages would not meet the requirements of an optimal indicator of water quality. In the samples with >10 pfu/101 l. of enteroviruses, the concentrations of coliphages were always higher than RNA-FSP bacteriophages, and exhibited a proportional relationship with the titres of enteroviruses.

The concentrations of coliphages detected in samples with 1–10 and >10 pfu/101 l. of enteroviruses were similar (210 pfu/100 ml) and higher (4450 pfu/100 ml) than the guide and imperative levels of faecal coliforms (100 and 2000 cfu/100 ml, respectively) adopted by the EEC (1975) for the quality of the bathing waters. Thus, in any review of the technical standards of the European Community Bathing Water Directive it might be appropriate to consider coliphages as candidates for inclusion in regulations either as supplementary indicators to the faecal pathogenic bacteria or as potential surrogates for enteric viruses. In contrast it is unlikely that, the reliability and sensitivity of SSP and RNA-FSP bacteriophages will be appropriate for indexing the quality of recreational waters.

Acknowledgements—The authors wish to thank the University of Malaga and the Director of the Robens Institute (University of Surrey) for permission to publish this research. Thanks are also due to Michaela Merret-Jones for assistance with field and laboratory analyses, to the National Rivers Authority for laboratory facilities for bacteriological analysis of seawater samples and for funding the analyses of enteric viruses, to the Water Research Centre and Department of the Environment for funding bacteriological analysis of seawaters and to Thames Clearwater for funding the bacteriological analysis of river water samples.

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Bacteriophages as indicators


