Bacteriophage as an Alternative Indicator for Microbiological Pollution at Marine Beaches

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Bacteriophage as an Alternative Indicator for Microbiological Pollution at Marine Beaches
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INTRODUCTION

The US EPA recommends detection of Enterococci group bacteria as an indicator for pollution in marine recreational waters. In Georgia, this indicator has been used for beach monitoring since 2004. Studies show that Enterococci are not associated with certain waterborne pathogens such as enteric viruses. Bacteriophages have been proposed as an alternative indicator for fecal pollution in water. These viruses are relatively easy to enumerate and requires less intensive technical training.

METHODS

Monthly samples were collected from four sites at Saint Andrews and Clam Creek Beaches on Jekyll Island from January-March 2016. Environmental factors were measured in situ using YSI Multiprobe. Samples were transported to the lab on ice and processed upon collection.

Bacteriophage enumeration: Phage were cultured according to EPA Method 1602: 100 mL of sample were inoculated with log-phase host E. coli cells on single layer agar with 2x TSB. Plates were incubated at 37°C for 18-24 hrs. Plaque formation was counted on each plate and results were reported as PFU/100 mL.

Enterococci enumeration: Water samples (10, 10, 100 mL) were filtered aseptically using sterile 0.45 µm cellulose nitrate filters. Filters were transferred to mEI agar plates and incubated at 410.5°C for 24 hours described by USEPA Method 1600. Colonies with blue halo regardless of the colony color were considered positive for Enterococci. All results were calculated and reported as CFU/100 mL.

RESULTS

Table 1. Environmental data for Jekyll Island (mean±SD).

<table>
<thead>
<tr>
<th></th>
<th>Precipitation (in)</th>
<th>Air Temperature (°C)</th>
<th>Water Temperature (°C)</th>
<th>Salinity (ppt)</th>
<th>pH</th>
<th>Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>0.05</td>
<td>16.6±8.5</td>
<td>14.6±4.6</td>
<td>19.2±6.1</td>
<td>7.4±0.7</td>
<td>61.9±51.9</td>
</tr>
<tr>
<td>February</td>
<td>0.05</td>
<td>16.3±9.0</td>
<td>15.7±4.0</td>
<td>20.1±4.5</td>
<td>7.5±0.4</td>
<td>20.8±8.1</td>
</tr>
<tr>
<td>March</td>
<td>0.05</td>
<td>20.5±4.5</td>
<td>17.2±4.1</td>
<td>21.7±3.8</td>
<td>7.6±0.2</td>
<td>22.6±15.3</td>
</tr>
</tbody>
</table>

Figure 1. Study area.

Figure 2. Bacteriophage and Enterococci mean concentration (PFU/100mL) at Saint Andrews Beach.

Figure 3. Bacteriophage and Enterococci mean concentration (PFU/100mL) at Clam Creek Beach.

CONCLUSIONS

Enterococci significantly correlated with somatic coliphage (r²=0.657, p=0.002).

Two beaches significantly differ from each other in terms of indicator levels (p<0.001).

Colder temperature seems to be a driver for somatic coliphage occurrence (r²=0.621, p=0.003). Summer sampling results will prove more information on the environmental factors and their relation with coliphage distribution.

US EPA is currently developing guideline values for monitoring coliphage contaminated recreational waters. One major milestone will be assessing the risk of gastrointestinal disease by testing beaches for these alternative indicators.

Ongoing studies include detecting enteric viruses and their relation with the occurrence of these viruses.

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REFERENCES


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