Prevalence and Occurrence of Zoonotic Bacterial Pathogens in Surface Waters Determined by Quantitative PCR


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Summary
The prevalence and concentrations of Campylobacter jejuni, Salmonella spp. and enterohaemorrhagic E. coli (EHEC) were investigated in surface waters in Brisbane, Australia using quantitative PCR (qPCR). Water samples were collected from Brisbane City Botanic Gardens (CBG) Pond, and two urban tidal creeks. Of the 32 water samples collected, 8 (25%), 1 (3%), 9 (28%), 14 (44%), and 15 (47%) were positive for C. jejuni mapA, Salmonella invA, EHEC O157 LPS, EHEC VT1, and EHEC VT2 genes, respectively. The high prevalence, and concentrations of potential zoonotic pathogens along with the concentrations of one or more fecal indicators in surface water samples indicate a poor level of microbial quality of surface water, and could represent a significant health risk to users.

Objective
The study investigated the prevalence and concentrations of various zoonotic pathogens in surface waters in Brisbane, Australia using PCR/quantitative PCR (qPCR). Secondly, the correlation between traditional fecal indicator bacteria (i.e., E. coli and enterococci) and the selected zoonotic bacterial pathogens that are also commonly found in human sewage were investigated.

Methodology
•Surface water samples were collected from Brisbane City Botanic Gardens (CBG) Pond, and two creeks (i.e., Oxley Creek and Blunder Creek) in Brisbane, Australia. Four sites (OC1-OC4), one site (BC1), and three sites (CBGP1-CBGP3) were selected in Oxley Creek, Blunder Creek and CBG ponds (not shown in the map).
•Membrane filtration method was used for the isolation of E. coli and enterococci. For DNA isolation, 500mL aliquots were filtered through membranes and DNA was extracted directly on the membrane using the QiAgen Blood & Tissue kit.

Results
•To detect the presence of inhibitors, surface water samples (n = 3) were spiked with 10³ gene copies of S. Typhimurium DNA containing the invA gene. The qPCR CT values were compared to those obtained from the same concentrations of DNA that was used to spike 500-ml of distilled water. For the spiked distilled water, the mean CT value for Salmonella invA gene was 21.6 ± 0.4. For surface water samples, the mean CT values for undiluted DNA, ten-fold, and 1000-fold are shown in the Table below. The results indicated that the undiluted DNA extracted from surface water samples contained PCR inhibitory substances. Ten to 100 fold dilution of DNA is required to remove the effects of PCR inhibitory substances from surface water samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Threshold cycle (Ct) value for the qPCR</th>
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<tbody>
<tr>
<td>Undiluted DNA</td>
<td>10-fold dilution</td>
</tr>
<tr>
<td>Surface water 1</td>
<td>37.6 ± 2.6</td>
</tr>
<tr>
<td>Surface water 2</td>
<td>34.6 ± 6.1</td>
</tr>
<tr>
<td>Surface water 3</td>
<td>31.3 ± 6.5</td>
</tr>
<tr>
<td>Mean Ct values</td>
<td>34.5 ± 3.1</td>
</tr>
</tbody>
</table>
•Of the 12 samples tested from the CBG Pond, five (42%) were positive for C. jejuni mapA gene. Quantitative PCR detected 3.0 X 10² to 7.0 X 10³ gene copies/100mL of C. jejuni mapA gene in these positively identified samples. Of the 12 samples tested from the CBG Pond, only one (8%) was positive for Salmonella invA gene, and the concentration was 1.2 X 10⁵ gene copies/100ml. However, the Salmonella invA could not be detected in any samples from the Oxley Creek or Blunder Creek. Among the 12 samples tested from the CBG Pond, five (42%), three (25%), and five (42%) were positive for EHEC O157 LPS, VT1 and VT2 genes, respectively.
•Overall, of the 32 samples tested, eight (25%), one (3%), nine (28%), 14 (44%) and 15 (47%) were positive for C. jejuni mapA gene, Salmonella invA gene, E. coli O157 LPS, VT1, and VT2 genes, respectively.
•Binary logistic regressions were used to identify whether any correlation existed between the concentrations of fecal indicators and the presence/absence results for potential target pathogens. The presence/absence of the potential pathogens did not correlate with either E. coli or enterococci concentrations.

Conclusions
•The high prevalence and concentrations of potential zoonotic pathogens along with the concentrations of one or more fecal indicators in surface water samples indicate a poor level of microbial quality of surface water especially after rainfall events, and could represent a significant health risk to users. This underlines the need to undertake appropriate mitigation measures to protect public health risks.
•This study also indicated a poor correlation between fecal indicators and potential zoonotic pathogens tested. Therefore, testing fecal indicators alone may not be adequate to assess the microbiological quality of surface water and consequent health risks. The need to undertake a suite of tests to assess the microbiological quality is recommended.
•The study undertaken was limited in terms of the geographical area. Additionally, the results derived were based on four sampling episodes. It is recommended that more widespread sampling is undertaken to determine the geographical and temporal stability of the methods adopted and to assess the prevalence of the detected pathogens outside the study area within this region.