

## Development and Application of Microbial Source Tracking Tools for Emerging and Re-emerging Infectious Risk Management in Water Environment

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### [Abstract]

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Actual measurements were taken of the fluctuations in indicator microorganisms such as *Escherichia coli* and phages, as well as enteric viruses, not only during clear weather but also after sewage overflow events during rainy weather in Tokyo Bay. In water areas predominantly treated with simple chlorination, infectious phages were detected in the absence of *Escherichia coli*, indicating a potential risk of infection by viruses. This suggests the possibility of infection risk from viruses even when the water has been treated with simple chlorination.

Novel genetic markers specific to wastewater-associated *Escherichia coli* were explored for microbial source tracking (MST). Ultimately, two wastewater-associated *Escherichia coli* genetic markers, namely W\_nqrC and W\_clsA\_2, were identified. PCR primers were also designed for the detection of these two genetic markers. It was found that when these genetic markers were used in combination, the sensitivity was approximately 25%, and the specificity was approximately 99%. These genetic markers can also be detected using colony PCR, making them useful for determining whether the detected *Escherichia coli* in routine water quality testing is of wastewater origin or not.

The contamination status of *Escherichia coli* and MST markers, such as in deer and wild boar gathering areas known as wallows are investigated. In addition to measuring *Escherichia coli* using culture methods and real-time PCR, MST markers targeting multiple viruses, *Bacteroidales*, and mitochondrial DNA, as well as measuring bacterial 16S rRNA and environmental DNA, were employed to reveal the overall picture of microbial contamination in the wallows, including the activity patterns of wildlife captured by automated cameras. The MST markers were detected even when *Escherichia coli* was not detected, indicating different behavior compared to *Escherichia coli*.

### [References]

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