Antibiotic Resistance in Water Environments: Gene Transfer Potential and Mechanisms

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[Abstract]

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The increasing use of antibiotics has led to the emergence of antibiotic-resistant bacteria (ARB), posing a global health threat. However, there is little information on the presence of this indicator ARB in the water environments, especially river water. The present study aimed to (1) investigate the antibiotic-resistant bacteria isolated from river flowing in a Tohoku are in Japan by detecting antibiotic resistance genes (ARGs) as well as antibiotic susceptibility tests. The isolates of ESBL-producing *E. coli* from river water were further analyzed for source tracking using molecular typing analysis, (2) Quantify the potential for horizontal gene transfer of antibiotic-resistant bacteria released into the environment.

For the 108 isolates of ESBL-producing *E. coli*, the possession of the β-lactamase genes (21 genes) was examined using mono-plex PCR analysis. Seventeen of 21 β-lactamase genes were detected in the isolates and the most frequently detected gene was $bla_{\text{CTX-M group-1}}$ possessed by 65 isolates. It is noteworthy that the detected β-lactamase genes included not only bla_{IMP} (9 isolates), an endemic carbapenemase in Japan, but also bla_{KPC} (7 isolates), $bla_{\text{OXA-48}}$ (3 isolates), bla_{VIM} (9 isolates), and bla_{NDM} (5 isolates) which are considered epidemic carbapenemase in foreign countries because of few reports at clinical facilities in Japan. Among seven sampling stations in the two rivers, the highest number (n=15) of β-lactamase genes were detected in the isolates from the station receiving treated wastewater from a municipal wastewater treatment plant. This result implies that healthy people living in the city and patients clinically reported must harbour ESBL-producing *E. coli*. The results of MLST analysis showed that ESBL-producing *E. coli* type ST131, which is prevalent worldwide, was dominant among the isolates and was detected in spatially distant rivers in the Tohoku region.

The conjugative transfer of antibiotic resistance ARGs from a model bacteria, enterococci and *E. coli* in water environment were evaluated by in-vitro experiment. The transfer frequency of *vanA* by filter mating were 10^{-3} to 10^{-7} per recipient to three different enterococcus strains. In the activated sludge condition, the vancomycin resistance was transferred to OG1RF only and its frequency was about 10^{-7}. On the other hand, when Enterobacteriaceae harboring $bla_{\text{CTX-M}}$ were used, conjugative transfer was confirmed under any simulating environments (10^{-4} to 10^{-8}). Gram-negative bacteria were shown to have a higher potential for the transmission of ARGs compared to Gram-positive bacteria. Transmission of resistance genes in the environment was also considered sufficient, suggesting that intensive treatment is necessary, especially in areas where bacterial density is assumed to be high.