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# Dissemination of antibiotic resistance by environmental bacteriophages in a constructed wetland

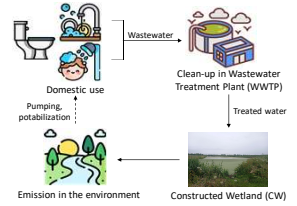
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## CONTEXT

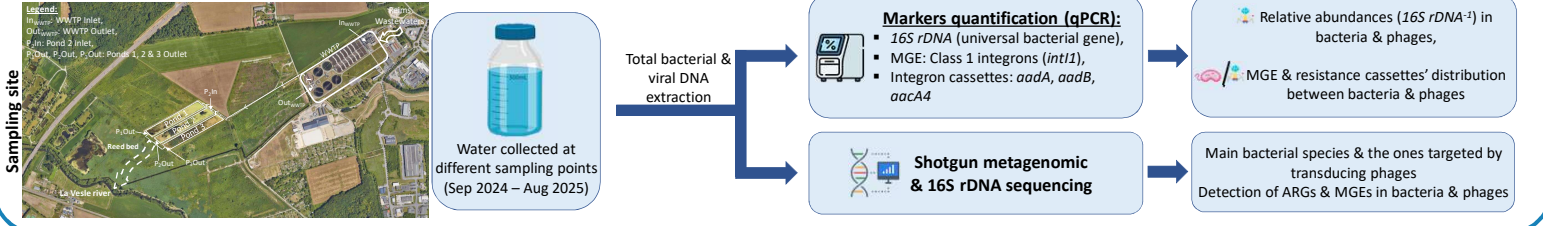
Constructed wetlands (CW), a nature-based solution in wastewater treatment are increasingly being used. Indeed, after being treated in a Wastewater Treatment Plant (WWTP) and before its discharge in the final environment, the water can have its physical & chemical properties improved flowing through a CW. Nevertheless, WWTP are not designed to reduce antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), resistant bacteria, nor bacteriophages. Their fate while flowing through a CW actually remains poorly understood and analyzing it appears to be essential.

Also, recent studies demonstrated the presence of bacterial ARGs & MGEs in phage capsids, resulting from an accidental capture during phage assembly. This event can further allow environmental phages to transfer these previously encapsidated genetic determinants through transduction to bacteria, participating in the dissemination of antibiotic resistance (AR) in the environment.

This study aims to determine the dissemination of AR in bacteria and phages in a CW constituted of three ponds (2 ha each) planted with different vegetables, making it a large-scale pilot structure. Observing possible transducing bacteriophage species changes through the WWTP-CW system and determine AR determinants abundances could allow a better understanding of AR dissemination in the environment by transducing bacteriophages.

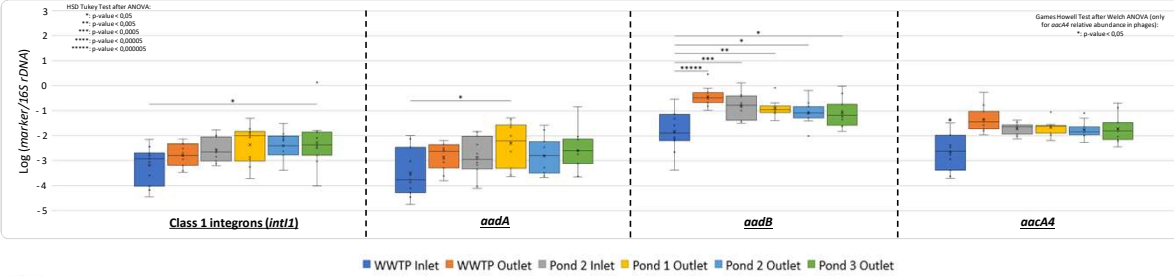


## METHODS



## RESULTS

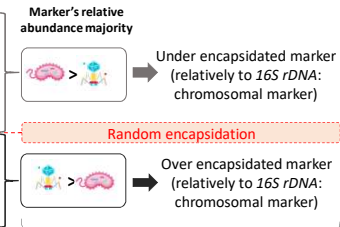
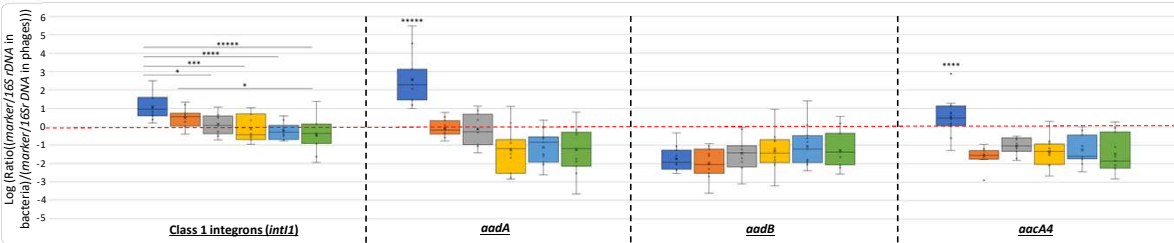
### Markers' relative abundances in phages



- No change in *int1* & *aadA* relative abundances between WWTP inlet and pond outlets,
- Increase between WWTP inlet and pond outlets (*aadB* & *aacA4*),
- No specific tendency of AR determinant decrease in the system

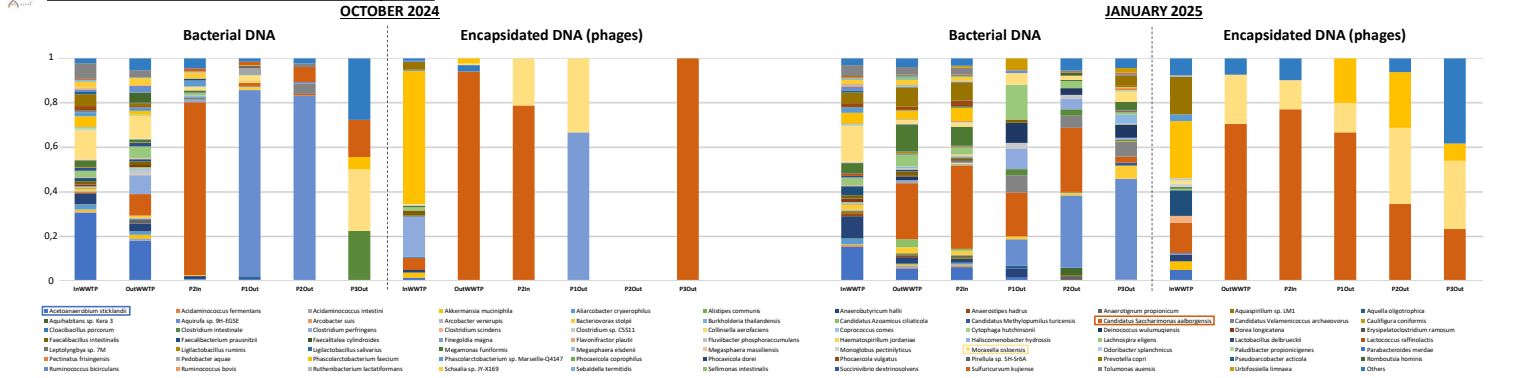
Shotgun metagenomic analysis shows that ARGs are rarely detected in bacteriophages, but are detected in bacteria, particularly at the WWTP inlet.

### Distribution of MGE & resistance cassettes between bacteria & phages



Increase of transducing bacteriophages proportion while the water flows through the WWTP-CW system

### Taxonomic classification of bacterial species



- The bacterial species most frequently detected at the WWTP inlet are *Moraxella osloensis* and *Acetoaerobium sticklandii*, less abundant at other sites.
- The bacterial taxonomic profile differs between October 2024 and January 2025.

- The bacterial species detected in phage DNA result from the encapsidation of 16S rDNA by phages during phage production,
- Few bacterial species were detected in encapsidated DNA, particularly at the WWTP outlet and in the ponds (+ similar bacterial 16S rDNA profiles in January 2025)

- The species found in encapsidated DNA are also present in bacteria (not at the same abundances)
- Fewer bacterial species found in encapsidated DNA compared to bacteria.

## CONCLUSION & PERSPECTIVES

- If the encapsidation of markers is accidental as frequently implied in the literature, the relative abundance of markers in bacteriophages should be of 1 because every marker has the same chance of being encapsidated as 16S rDNA. It is not the case here where, for the 3 resistance cassettes, the ratio is clearly < 1, meaning these markers are over-encapsidated compared to their abundance in bacteria. These data shows no effect of the CW on antibiotic resistant determinants inside bacteriophages with no change in the relative abundance of the tested markers when the water arrives in the CW system.
- Shotgun metagenomic analysis shows a significant decrease in ARGs amounts at the WWTP outlet and in the ponds compared to the WWTP inlet (data not shown).
- A clear variation in bacterial communities is observed between the different sites of the WWTP-CW system. The difference in taxonomic bacterial communities between October 2024 and January 2025 may be due to different environmental parameters during these months.
- Changes of the markers' ratios in bacteria/phages could be explained by a decrease in specific bacterial species, which could carry these cassettes, whereas no change in transducing bacteriophage communities is observed.
- The effectiveness of the WWTP-CW ecosystem in reducing the prevalence of antibiotic resistance and mitigating its spread remains difficult to conclude → Further analysis and studies are required.