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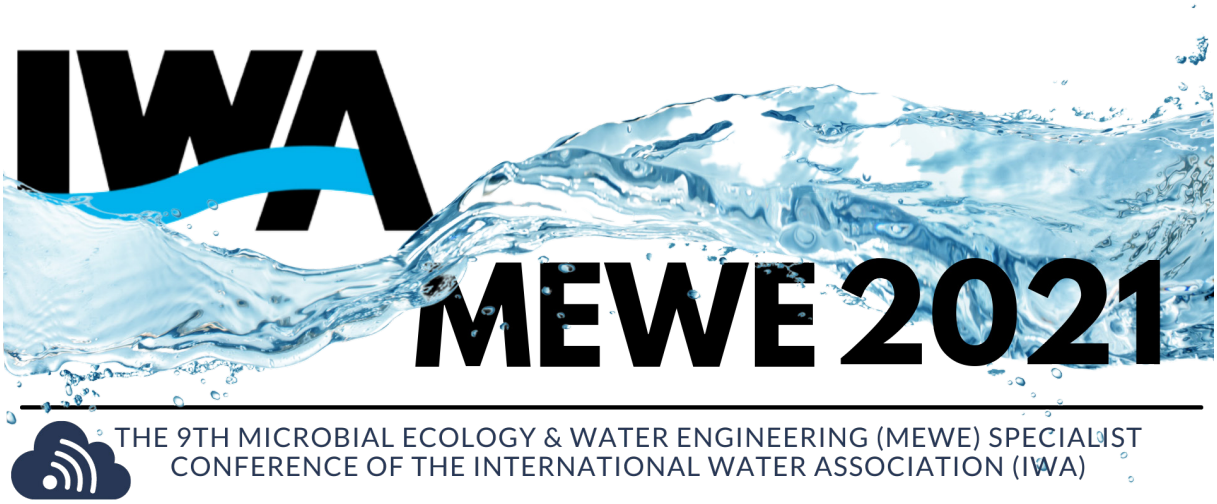

Distribution and abundance of plasmid-specific bacteriophages in wastewater systems

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Context. Plasmid-specific bacteriophages are viruses that specifically infect bacteria carrying conjugal plasmids, typically using the conjugal pilus as receptor. As pili differ across plasmid classes, the infection range of plasmid specific phages has specificity. Wastewater has been used as isolation source for such phages, however only the distribution and ecology of F-specific RNA phages have been described to date because it is a water quality parameter (as proxy for RNA-viruses from fecal sources).

Gap. The source, fate, and abundance of plasmid-specific phages are largely uncharacterized and there is a lack of experimental platforms to study them.

Aim. We aimed to (i) provide an experimental platform to quantify the abundance of plasmid-specific phages for several conjugal plasmid classes and (ii) describe the distribution of such phages in wastewater systems and (iii) identify what controls their distribution.

Methods. We introduced four model plasmids, belonging to different plasmid incompatibility groups (IncP1, IncN, IncH, IncF) into an avirulent *Salmonella enterica* serovar Typhimurium, which displays a low background abundance of somatic phages in wastewater. These strains were used in double layer agar assays with water samples from contrasting sources (hospital wastewater, influent and effluent of municipal wastewater treatment plants in Sweden and Denmark).

Findings. Depending on the location and plasmid class, phage abundance ranged from below detection limit to about 5 10^3 pfu/mL. Hospital wastewater contained significantly more IncP-, but less IncF-, specific phages than domestic wastewaters, potentially reflecting differences in

plasmid loads. The comparison between influent and effluent of WWTP revealed a reduction in phage density by 1.5 log, but no significant removal from primary clarification.

Utilization. First, fate data for viruses in wastewater can inform wastewater-based epidemiology. Second, these data help understand phage-plasmid interactions, especially relevant as plasmids can disseminate AMR, and may suggest novel ways to control AMR in wastewater systems.

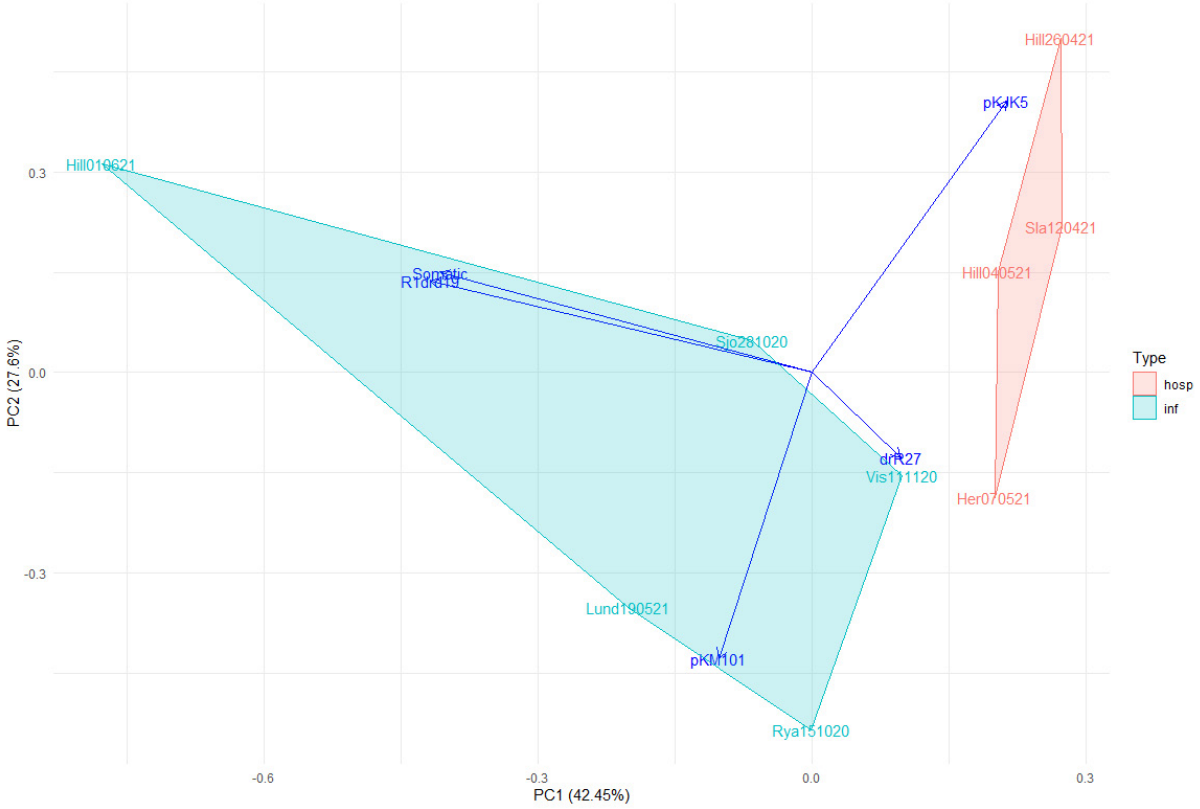

Figure 1: A principal component analysis of the composition of plasmid-specific phages highlights difference between hospital wastewater samples (in red) and WWTP influent samples (in green). The former are characterized by higher density of pKJK5 (an IncP plasmid)-specific phages and the latter by higher density of R1-specific phages (IncF).

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