

Persistence of Antimicrobial Resistance and Human Fecal Biomarkers in Decentralized Wastewater Effluent: Implications for Environmental Monitoring and Microbial Source Tracking

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Introduction

- Decentralized wastewater treatment (DWT) effluent can carry microbes, fecal pathogens, and genetic material into receiving environments, creating potential downstream exposure risks
- It's currently unclear how DWT system type/design affects persistence of antimicrobial resistance (AMR) genes and common human fecal biomarkers (HFBs) from these sources

Genetic Targets

- β-lactam AMR genes:** *bla*TEM, *bla*OXA-48, *bla*CTX-M, KPC
- HFBs:** Pepper Mild Mottle Virus (PMMoV), crAssphage, HF183, BacHuman
- Why β-lactam AMR? Clinically relevant, confer resistance to widely used antibiotics (penicillins, carbapenems, and cephalosporins); resistance genes are often enriched in biological treatment

Objectives

- Quantify the abundance of β-lactam AMR genes and HFBs in treated effluent from a range of DWT systems
- Assess how system type influences direct input for downstream AMR contribution
- Identify the most reliable and persistent HFBs from these systems for downstream applications of microbial source tracking in environmental samples

Anticipated Outcomes

- This study will clarify how DWT systems affect the persistence of AMR and HFBs, specific to each treatment method
- Findings might guide environmental monitoring programs and microbial source tracking efforts
- Results can support public health risk prevention for communities that are heavily reliant on decentralized and onsite treatment

References

Ahmed, W., Bivins, A., Payyappat, S., Cassidy, M., Harrison, N., & Besley, C. (2022). Distribution of human fecal marker genes and their association with pathogenic viruses in untreated wastewater determined using quantitative PCR. *Water Research*, 226, 119093.

Chettleburgh, C., Ma, S. X., Swinwood-Sky, M., McDougall, H., Kireina, D., Taggar, G., McBean, E., Parreira, V., Goodridge, L., & Habash, M. (2023). Evaluation of four human-associated fecal biomarkers in wastewater in Southern Ontario. *Science of The Total Environment*, 904, 166542.

Mattioli, M. C., Benedict, K. M., Murphy, J., Kahler, A., Kline, K. E., Longenberger, A., Mitchell, P. K., Watkins, S., Berger, P., Shanks, O. C., Barrett, C. E., Barclay, L., Hall, A. J., Hill, V., & Weltman, A. (2021). Identifying septic pollution exposure routes during a waterborne norovirus outbreak—A new application for human-associated microbial source tracking qPCR. *Journal of Microbiological Methods*, 180, 106091.

Sidhu, A. S., Mikolajczyk, F. N., & Fisher, J. C. (2023). Antimicrobial Resistance Linked to Septic System Contamination in the Indiana Lake Michigan Watershed. *Antibiotics*, 12(3), 569.

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Methodology

Sampling Process

Treated Effluent (grab samples) collected from variable treatment scenarios:

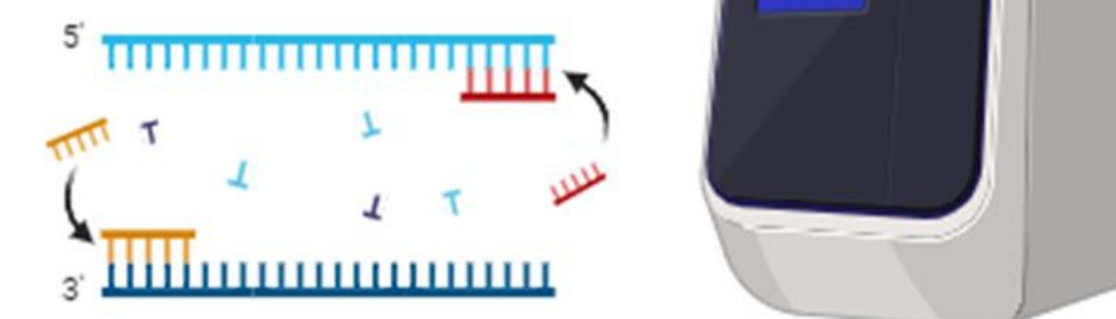
- Conventional Septic Systems
- Aerobic Treatment Units/Home Aeration Units
- Package Treatment Plants with Extended Aeration
- Constructed Wetlands
- Sewer Treatment Lagoons
- Alternative/Combination Systems

Influent samples are also collected to calculate removal of AMR in these systems

Laboratory Workflow

Microbial:

- Filter Concentration
- DNA Extraction (FastDNA Spin Kit)
- qPCR



Physicochemical Data:

- pH
- Temperature
- COD
- NH₃
- Suspended Solids
- Turbidity
- Conductivity
- Weather

Data Processing:

Data cleaning and visualization conducted in RStudio.

Practical Challenges

Site access restraints

- Topography/terrain, systems below ground
- Private property and permissions

Low-flow onsite systems

- "Effluent" samples are unique to the system type (e.g., to a drainfield versus a surface discharge)

Aging of failing systems

- Systems struggling to meet NPDES discharge limits may be less likely to allow sampling, despite being the systems of concern

Key Takeaways

Most systems achieved substantial removal of β-lactam AMR (approximately 1 to 4 log removal)

- Systems with effluent polishing or extended treatment likely enhance removal
- Lesser removal observed for a package plant system noted to have general treatment performance challenges
- Influent concentration varies with target; *bla*TEM highly abundant across most systems

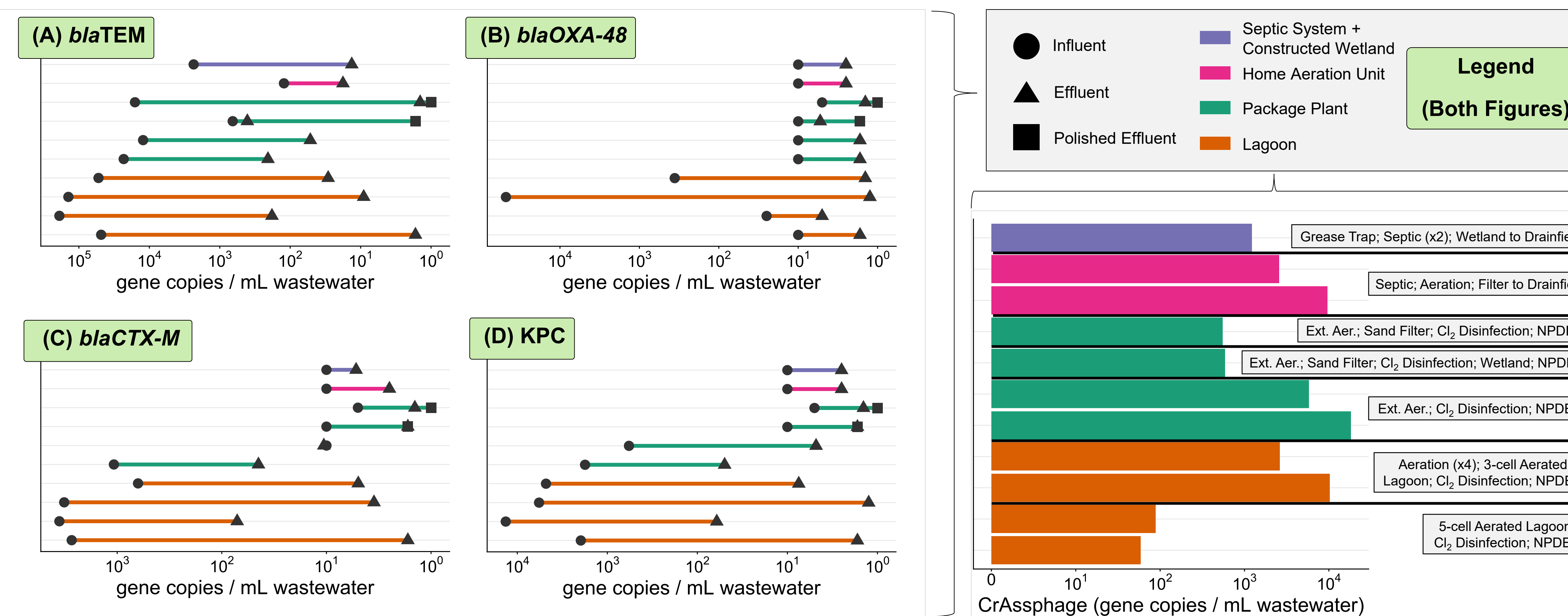
CrAssphage persistent through various treatment mechanisms; abundant in most effluents thus far

- Often resilient to Cl₂ Disinfection

Conclusion / Future Work

- Continued sampling for greater variety of onsite and decentralized treatment mechanisms
- Addition of other HFBs (HF183, PMMoV, and BacHuman) to compare with persistence of crAssphage

Preliminary Results / Findings



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