Aquatic Pollutants

REWA

Reduction and assessment of antimicrobial resistance and emerging pollutants in natural-based water treatment systems

D4.3 Guidelines to mitigate AMR: a Guidance Document on Microbiome-Directed Tools for Bench-Scale Assessment of Risks and Benefits of Water Treatment Processes

December 2024 (revised August 2026)

Prepared by: Kristian Koefoed Brandt

Executive Summary

This guidance document outlines a framework for assessing the risks and benefits of emerging water treatment technologies designed to remove pathogens, antimicrobial-resistant microorganisms, antibiotic resistance genes, and other biological contaminants of emerging concern from water systems. Driven in part by the revised European Union Urban Wastewater Treatment Directive, which introduces stronger requirements for surveillance and mitigation of antimicrobial resistance (AMR) dissemination, the document emphasizes the need to assess emerging water treatment technologies early, even before pilot or full-scale implementation. The guidance recommends standardized bench-scale testing using replicated microcosms that closely mimic real operational conditions. Key evaluation elements include treatment intensity, exposure duration, and post-treatment regrowth of surviving microorganisms. Particular attention should be given to the potential selection and proliferation of resistant bacteria. A polyphasic assessment approach is recommended, combining microbiome-directed methods with conventional ecotoxicological tests to evaluate impacts on microbial community structure, function, and antibiotic susceptibility. Overall, the document provides a conceptual foundation for systematic microbiome-based evaluation of emerging water treatment technologies aimed at mitigating AMR risks.

Table of contents

1. Introduction

The European Union has recently strengthened its regulatory framework to address the interconnected challenges of wastewater pollution, pharmaceutical residues, and antimicrobial resistance (AMR) as outlined in the revised Urban Wastewater Treatment Directive (DIRECTIVE (EU) 2024/3019 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL, 2024; https://eur-lex.europa.eu/eli/dir/2024/3019). The new framework also introduces systematic AMR surveillance in wastewater. Wastewater treatment plants will increasingly be used as monitoring points for antimicrobial-resistant bacteria and resistance genes, supporting a One Health approach that links human, animal, and environmental health. This surveillance will provide early warning of emerging resistance trends and help guide public health interventions across Europe (Kardos et al., 2025). The directive will imply a need for more advanced treatment requirements to remove micropollutants and microorganisms associated with human pathogenicity or AMR from urban wastewater before discharge into the environment (Gao et al., 2025; Kardos et al., 2025).

The REWA project was initiated to address the urgent need to develop novel, efficient, and safe water treatment technologies. According to its project description, the main goal of the project was *'the strategic development and implementation of sustainable and cost-effective technologies for the removal of contaminants of emerging concern (CECs), metals, pathogens including antimicrobial resistant bacteria and antibiotic resistance genes (ARGs) from water'*. I propose that risks and benefits associated with such technologies be assessed as early as possible during technology development, before implementation of water treatment processes. Many new water treatment technologies (including several REWA technologies) involve materials and associated chemical processes meant to inactivate and kill microorganisms. No consensus currently exists on how to test these new processes before pilot- or full-scale implementation. In this guidance document on Microbiome-Directed Tools for Bench-Scale Assessment of Risks and Benefits of Water Treatment Processess, I provide general considerations and specific recommendations on how to assess potential risks and benefits associated with emerging water treatment technologies for the control of biological contaminants such as pathogenic microorganisms and antimicrobial resistance (AMR).

This project was funded by the European Commission, the Academy of Finland (Finland), the Ministry of Health (Israel), Innovationsfonden Denmark (Denmark), and the Water Research Commission (South Africa) under the 2020 Aquatic Pollutants Joint call of the Aquatic Pollutants ERA-NET Cofund (GA No 869178). This ERA-NET initiative is an integral part of the activities developed by the Water, Oceans, and Antimicrobial Resistance (AMR) Joint Programming Initiatives (JPIs).

2. General considerations

I argue that emerging water technologies for the control of microbiological contaminants (i.e. pathogenic microorganisms, microorganisms resistant to antimicrobials and toxin-producing microorganisms) should be evaluated for their effectiveness and associated risks already at the bench scale during technology development prior to pilot- or full-scale implementation. In REWA, we have primarily focused on bacterial contaminants, and the following guidelines will reflect this focus, but most of the recommendations will also be relevant for other groups of microbiological contaminants. Microbial community assembly processes ultimately control the environmental fates of microbial contaminants over spatiotemporal scales (Nemergut et al., 2013). These processes include diversification (i.e. generation of new genetic variation for instance via adaptive mutations, horizontal gene transfer or genetic recombination), selection (i.e. 'survival of the fittest' associated with changed relative abundance of individual taxa or in relative abundance of individual bacterial strains belonging to the same species), drift (i.e. stochastic events associated with changed relative abundance of individual taxa or in relative abundance of individual bacterial strains belonging to the same species), and dispersal (i.e. movement of microorganisms in space and over time). I argue that any emerging water treatment technology should be tested with reference to these ecological and evolutionary processes. Hence, bacteria can rapidly evolve resistance to treatments aimed at killing them through adaptive mutations (Baym et al., 2016), and once resistance exists in a bacterial community, it can spread to other species via horizontal gene transfer (Ghaly and Gillings, 2022). Thus, water treatment technologies that kill most bacteria may select for, or co-select, antibiotic-resistant bacteria (Jia et al., 2022). In the absence of competitors, these resistant 'survivor bacteria' may subsequently grow to high densities and pose a public health risk. In the following section, I provide more specific recommendations for designing experimental systems to test emerging water treatment technologies already at the bench-scale development stage. These recommendations are based in part on experimental research carried out during the REWA project and in part on my personal expert opinion ((Mohammadzadeh et al., 2025; Xu et al., 2025; Mohammadzadeh et al., 2026)).

3. General recommendations towards standardized bench-scale assessment of emerging water treatment technologies for elimination of microbiological contaminants

3.1. Experimental systems.

I recommend a strictly controlled, replicated microcosm approach that mimics the expected operational conditions of the full-scale technology as closely as possible. Hence, microcosms should be constructed based on representative water samples that reflect the eventual application of the technology (e.g., drinking water samples for drinking water treatment technologies and wastewater treatment effluents for tertiary wastewater treatment technologies).

3.2. Water treatment intensity and duration.

Water treatment intensity and duration should be part of the experimental design to assess the treatment's potency in terms of both benefits and risks. The immediate effect of water treatment intensity and duration should be tested against a no-treatment control.

3.3. Regrowth of 'resistant survivor bacteria' and ecological connectivity.

In order to follow the potential regrowth of 'resistant survivor bacteria' surviving the water treatment (see above), it is imperative that the experimental setup includes sufficient post-treatment time for cellular repair and regrowth to happen. The regrowth conditions should mimic the expected operational conditions of the full-scale technology as closely as possible. Hence, I, for instance, recommend performing regrowth experiments in treated water mixed with representative water samples similar to the aquatic recipients of the treated water upon full-scale implementation. Environmental 'gradients' between wastewater and aquatic recipients will generally reduce the risk of selection and regrowth of wastewater-derived microorganisms, e.g., due to competitive exclusion of freshwater bacteria in seawater.

3.4. Endpoint selection for ecotoxicological assessment.

A plethora of microbiome-directed methods can be used to assess impacts of wastewater treatments on water quality, but only a few methods have been standardized for risk assessment (Brandt et al., 2015). The methods used in REWA are described in more detail in Deliverables 4.1 and 4.2 (i.e. D4.1 and D4.2; (Xu et al., 2025)). Specifically, I recommend a polyphasic approach with complementary endpoints. Hence, microbiome-directed tests should be used to complement single-species tests (e.g. whole-cell bacterial bioreporters). I further recommend that microbiome-directed approaches should not only target microbial community function or structure, but also antibiotic susceptibility.

4. Conclusions and perspectives

There is an urgent need for a more systematic framework to test the risks and benefits of emerging water treatment technologies for controlling microbiological contaminants such as pathogenic microorganisms or AMR. This report provides a conceptual starting point for microbiome-directed approaches in this field.

5. References

Brandt, K.K., Amézquita, A., Backhaus, T., Boxall, A., Coors, A., Heberer, T., Lawrence, J.R., Lazorchak, J., Schönfeld, J., Snape, J.R., Zhu, Y.G., Topp, E., 2015. Ecotoxicological assessment of antibiotics: A call for improved consideration of microorganisms. Environment International 85, 189-205.

Gao, R., Gao, S.-H., Li, J., Su, Y., Huang, F., Liang, B., Fan, L., Guo, J., Wang, A., 2025. Emerging Technologies for the Control of Biological Contaminants in Water Treatment: A Critical Review. Engineering 48, 20.

Ghaly, T.M., Gillings, M.R., 2022. New perspectives on mobile genetic elements: A paradigm shift for managing the antibiotic resistance crisis. Philosophical Transactions of the Royal Society B: Biological Sciences 377.

Jia, S., Gao, X., Zhang, Y., Shi, P., Wang, C., Zhou, Q., Ye, L., Zhang, X.-X., 2022. Tertiary Wastewater Treatment Processes Can Be a Double-Edged Sword for Water Quality Improvement in View of Mitigating Antimicrobial Resistance and Pathogenicity. Environmental Science & Technology.

Kardos, M.K., Patziger, M., Jolánkai, Z., Clement, A., 2025. The new urban wastewater treatment directive from the perspective of the receiving rivers' quality. Environmental Sciences Europe 37, 10.

Mohammadzadeh, M., Bello, A., Lassen, S.B., Brandt, K.K., Risteelä, S., Leiviskä, T., 2025. Pilot-scale adsorption of pharmaceuticals from municipal wastewater effluent using low-cost magnetite-pine bark: regeneration/enumeration of viable bacteria with a study on their biotoxicity. Environmental Research, 120774.

Mohammadzadeh, M., Lassen, S.B., Jensen, M.K.S., Strobel, B.W., Brandt, K.K., Leiviskä, T., 2026. Ciprofloxacin adsorption to magnetite-pine bark biosorbents as affected by preconditioning with distinct microbiomes. Environmental Science and Pollution Research.

Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and Processes of Microbial Community Assembly. Microbiology and Molecular Biology Reviews 77, 342-356.

Xu, J., Jensen, M.K.S., Lassen, S.B., Brandt, K.K., Dechesne, A., Smets, B.F., 2025. Aeromonas isolation reveals this genus's contribution to antimicrobial resistance fluxes across the wastewater–treated water–river interface. Journal of Applied Microbiology 136.