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Metagenomic analysis of urban wastewater resistome and mobilome: Implications for Reclaimed Water Safety in Agriculture.

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Abstract

The reuse of reclaimed wastewater for agricultural irrigation is a promising solution in regions experiencing water scarcity, yet it raises concerns about the persistence of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs). This study employed metagenomic analyses to characterize the resistome and mobilome in influent, effluent, and sludge samples from three urban wastewater treatment plants (WWTPs) in Barcelona, Spain. Weekly samples were collected over three weeks and analyzed using high-throughput DNA sequencing and UHPLC-qTOF to quantify ARGs, MGEs, and antibiotic concentrations. The results revealed high levels of ARGs across all WWTPs, with ARG removal efficiencies ranging from 2.34 to 2.43 log reductions and antibiotic removals between 50–99%. Despite treatment, effluents retained moderate levels of ARGs, some of which were associated with human activity, high mobility, and pathogenicity. These findings suggest potential risks related to the use of reclaimed water in agriculture and underscore the need for robust risk assessment and management strategies. Structural equation modelling and source tracking further elucidated factors influencing ARG persistence. This study contributes critical insights for improving water reuse safety and informing policy on wastewater treatment standards.

Keywords: Reclaimed wastewater, antibiotic resistance, metagenomics.

1. Introduction

The use of reclaimed wastewater for agricultural irrigation has become an increasingly important practice, particularly in areas facing water scarcity[1]. This approach not only provides a sustainable water source but also contributes to the conservation of freshwater resources. Reclaimed wastewater, especially when subjected to advanced treatment processes, can be a valuable resource for irrigation, allowing for the continued growth of crops while mitigating water shortages. However, despite its potential benefits, the practice raises concerns regarding the presence of contaminants that may persist even after treatment, such as pathogenic microorganisms, pharmaceuticals, and antibiotic resistance genes (ARGs)[2]. These contaminants can be introduced into the soil and accumulate in crops, presenting risks to both environmental health and food safety.

2. Objectives

We aimed to i) examine the removal efficiency of resistome and antibiotic load across the three different WWTPs and decipher the contributing factors; ii) to analyse the abundance of clinically relevant β-lactam, as well as the presence of β-lactam-resistant Gram-negative bacteria of clinical importance in the WWTP.

3. Material and Methods

Influent, effluent, and sludge samples were collected from three municipal WWTPs in Barcelona, Spain, once per week over three consecutive weeks in February 2024. These WWTPs treat both domestic wastewater and pre-treated industrial water from the metropolitan area. Metagenomic analyses were performed on all sample types to characterize the resistome and mobilome. DNA was extracted from each sample, and high-throughput sequencing was used to identify ARGs, MGEs, and the overall bacterial community. Additionally, the antibiotic load in the samples was measured using UHPLC-qTOF.

4. Results and conclusions

The results showed that Spanish WWTPs exhibited high levels of ARGs, with an efficiency of 2.34–2.43 log reduction in ARGs and a 50–99% removal of antibiotics (Figure 1). Effluent samples were ranked at moderate levels (Level 2 and Level 3) in the exposure prioritization scheme based on total ARG abundance. Notably, ARGs associated with anthropogenic activities, high mobility, and potential pathogenicity were found to be present and even enriched in the reclaimed water, highlighting the need for enhanced risk management. Furthermore, source tracking of the effluent resistome and structural equation modelling were conducted to investigate the differences in ARG abundance and diversity across the various sample types. The findings of this study, along with the recommendation for a comprehensive exposure assessment, will support decision-making in resistome management at WWTPs, aiming to reduce ARG and antibiotic-resistant bacteria (ARB) contamination in receiving environments.

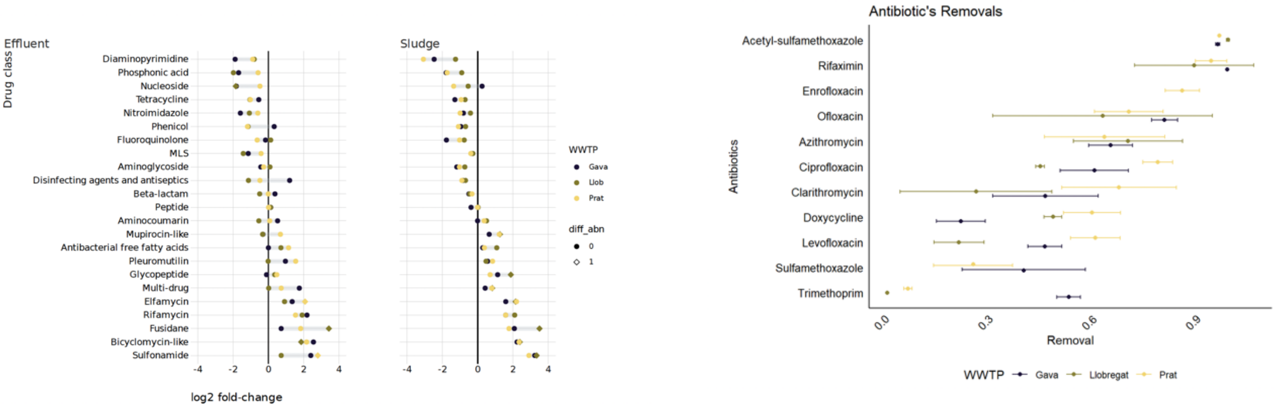

Figure 1. A) Removal efficiency of ARGs by target drug in the Gava, Llobregat and Prat WWTPs. Negative fold-change, removal; positive-fold change, enrichment. B) Antibiotic removal by antibiotic family in the Gava, Llobregat and Prat WWTPs.

5. References

1- Ofori S., Puškáčová A., Růžičková I. y Wanner J. (2021), Treated wastewater reuse for irrigation: Pros and cons, Science of The Total Environment, 760, 144026.

2- Subirats J., Di Cesare A., Varela Della Giustina S., Fiorentino A., Eckert E.M., Rodriguez-Mozaz S., Borrego C.M. y Corno G. (2019), High-quality treated wastewater causes remarkable changes in natural microbial communities and intI1 gene abundance, Water Research, 167, 114895.