

# Biomarkers for monitoring antibiotic resistance in aquatic environments



CATOLICA

CBQF · CENTRO DE BIOTECNOLOGIA E QUÍMICA FINA LABORATÓRIO ASSOCIADO

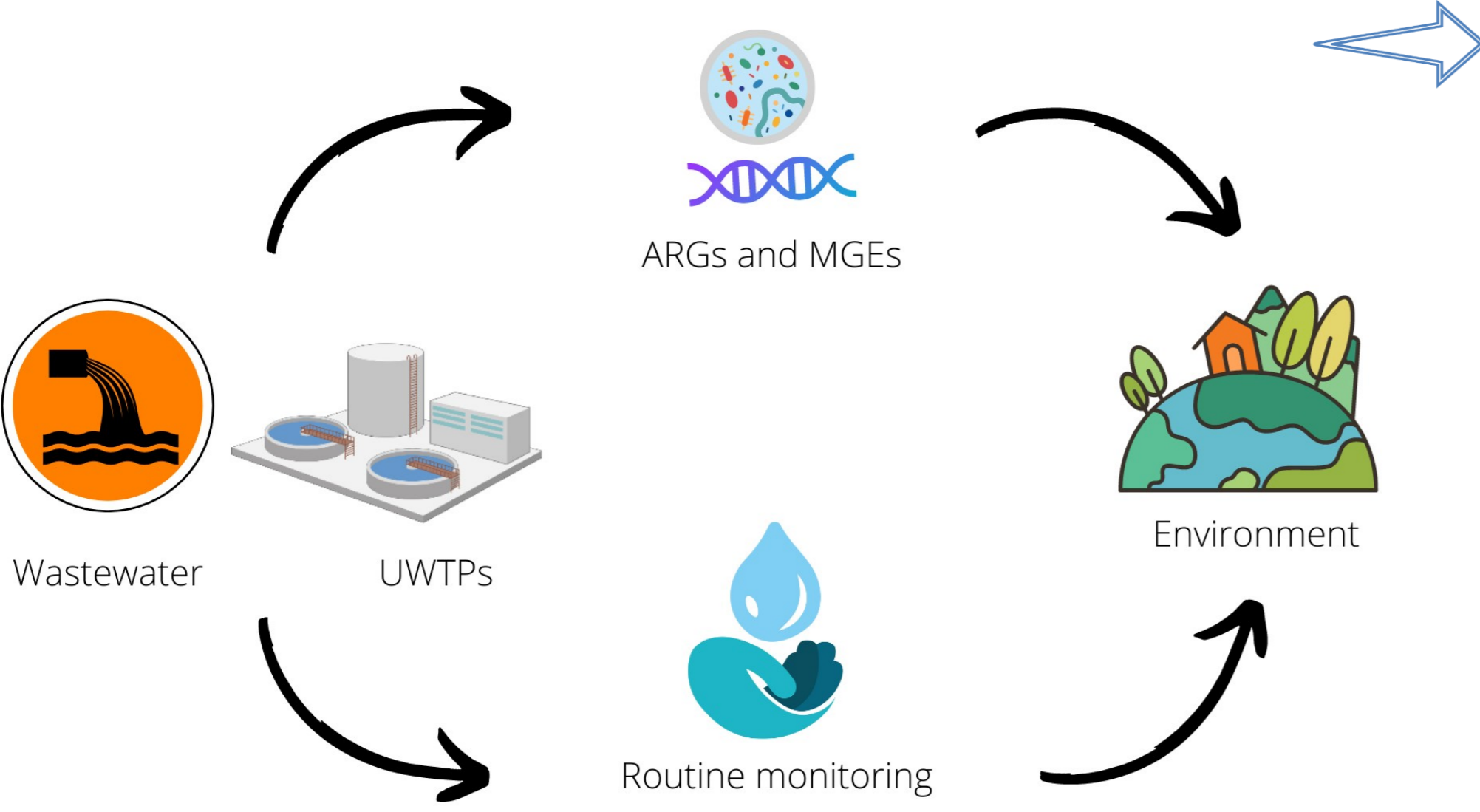
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PORTO

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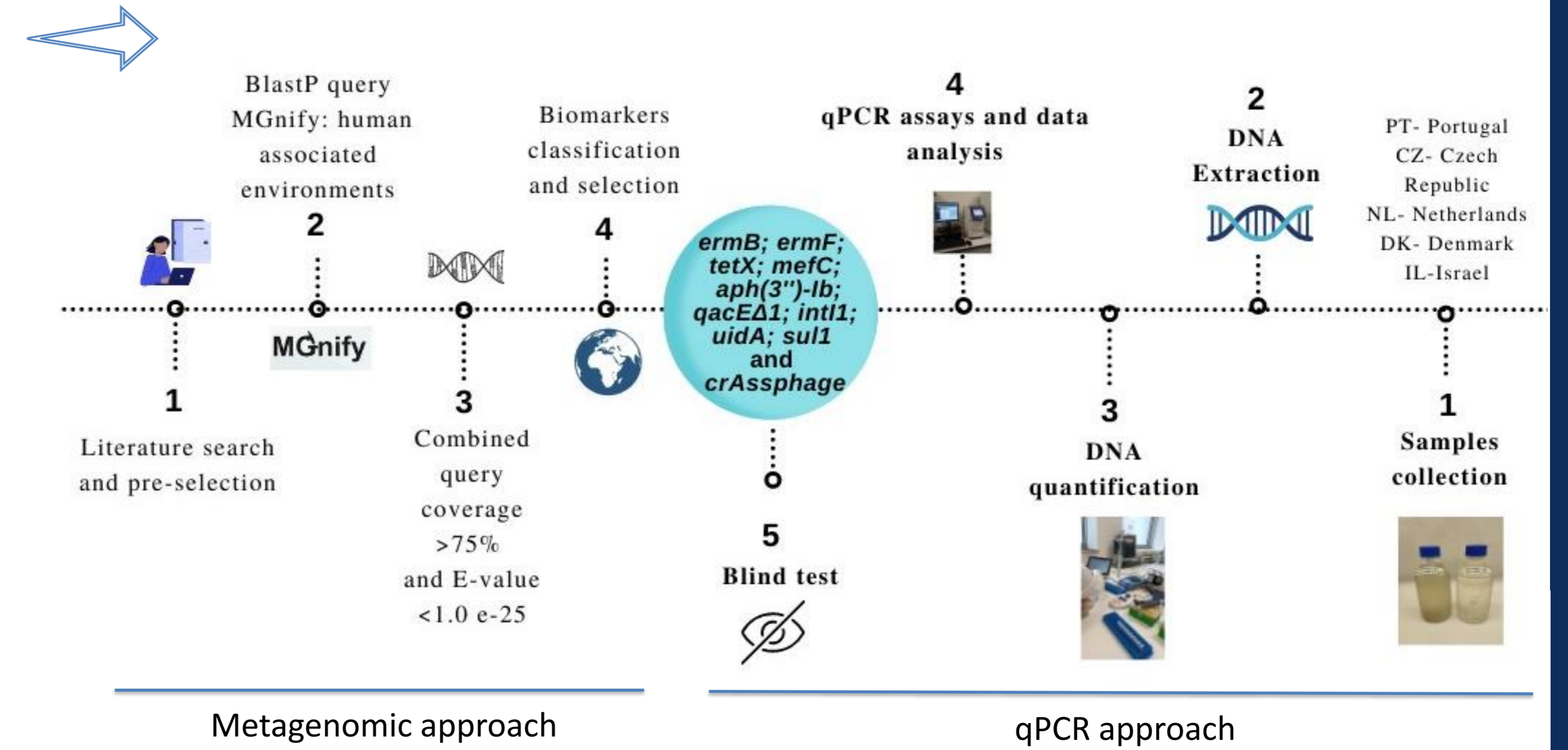
## Introduction



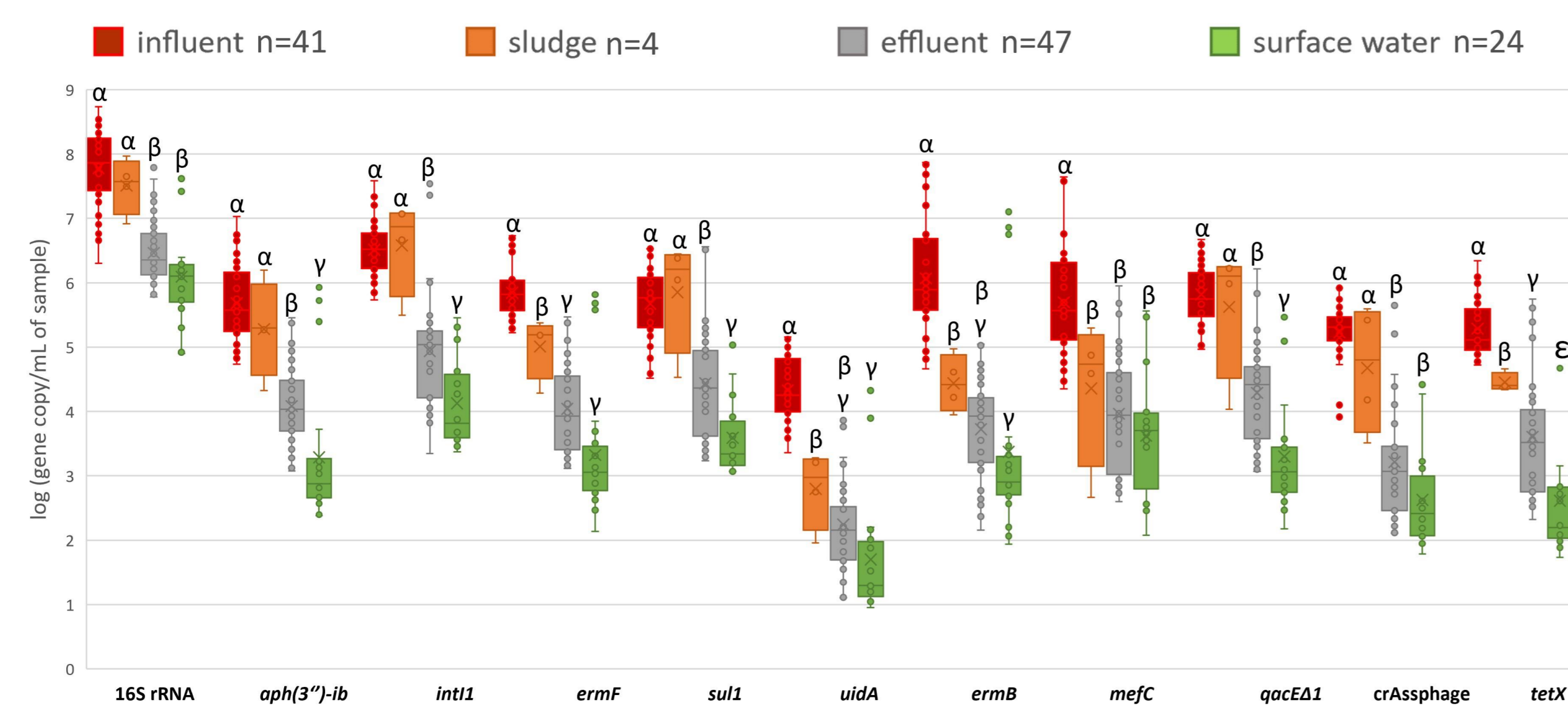
## Objectives

- ✓ Identify appropriate biomarkers, whose detection and quantification could indicate anthropogenic sources of contamination;
- ✓ Track the spread of antibiotic resistance in (waste)water;
- ✓ Facilitate regular antibiotic resistance monitoring for regulatory purposes.

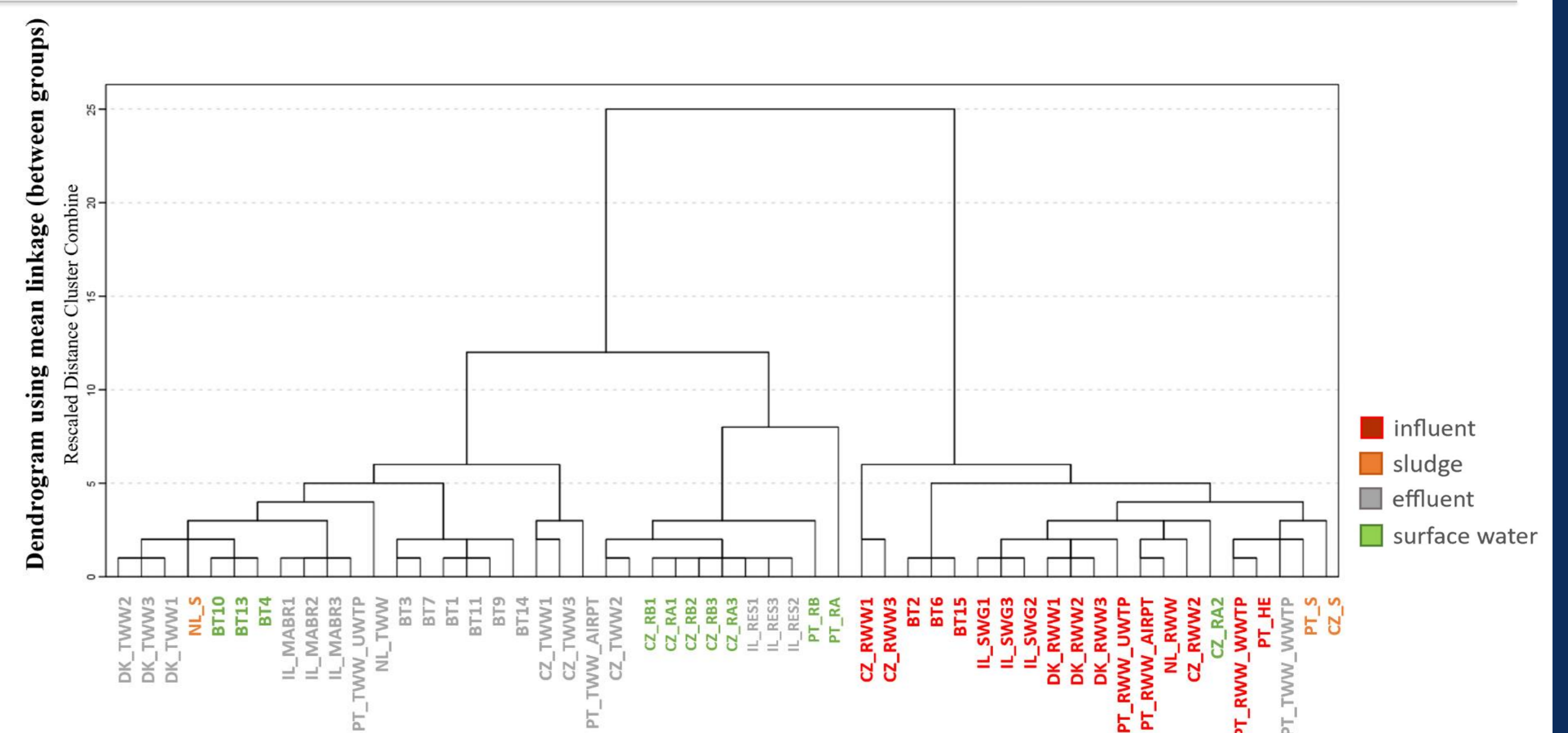
## Methods



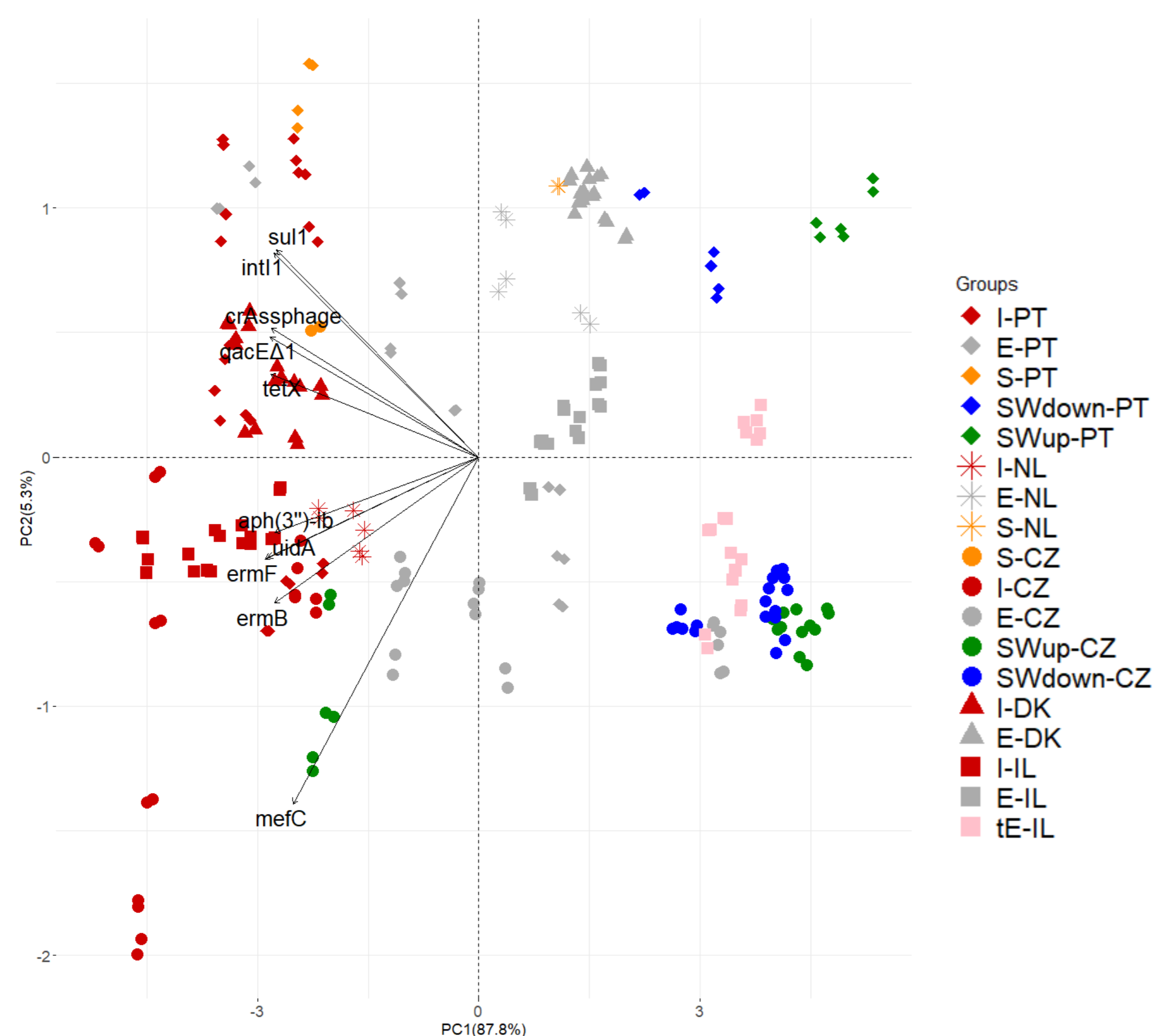
## Results



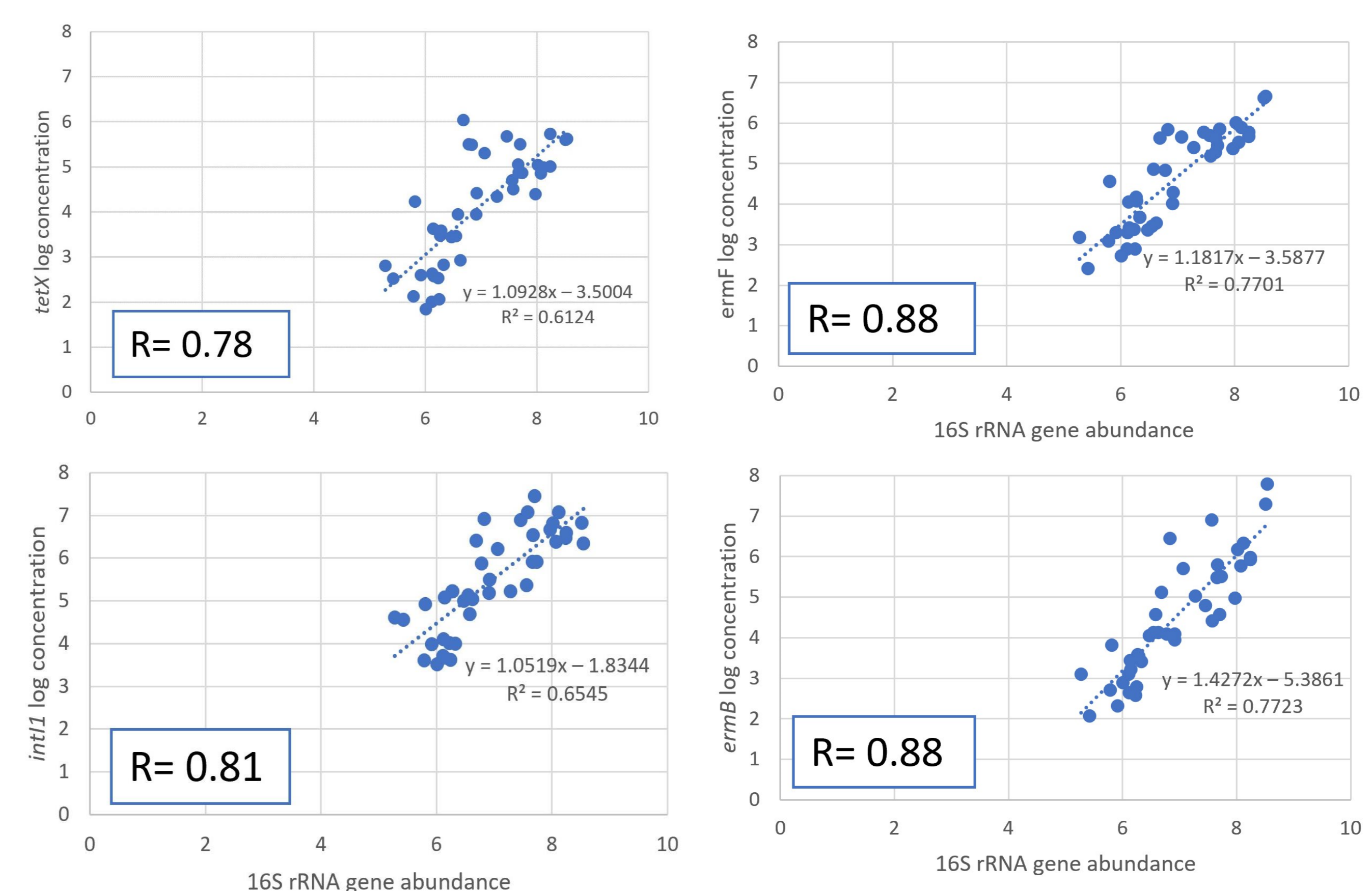
**Figure 1:** Gene abundance (log (gene copy/mL of sample)) in wastewater and surface water. α, β, γ and ε indicate significantly ( $p < 0.05$ ) different Tukey's groups comparing the genotype of samples: influent, sludge, effluent, and surface water.



**Figure 3:** Biomarkers abundance hierarchical cluster analysis. Euclidean distance using mean linkage between average samples from influent, sludge, effluent and surface water and blind test samples (BT1-15) from influent, effluent (secondary and UV treatment), surface water (river) for all biomarkers (*int1*, *sul1*, *ermB*, *ermF*, *aph(3'')-Ib*, *uidA*, *qacEΔ1*, *tetX*, *mefC* and *crAssphage*).



**Figure 2:** Principal Component Analysis (PCA) based on biomarker distribution in the different types of sample: influent (I), effluent (E), sludge (S) and surface water (SW up and down) samples, based on the quantification by qPCR of the ten biomarkers (*int1*, *sul1*, *ermB*, *ermF*, *aph(3'')-Ib*, *uidA*, *qacEΔ1*, *tetX*, *crAssphage* and *mefC*), for the five countries in the study (NL = The Netherlands, CZ = Czech Republic, DK = Denmark, IL=Israel and PT= Portugal).



**Figure 4:** Pearson's correlation coefficient (R) between the concentration (log (gene copy/mL of sample)) of the biomarkers *int1*, *ermB*, *ermF*, *tetX* and the 16S rRNA gene abundance (log (gene copy/ 16S rRNA copy number)).

## Conclusions

- ✓ The tested biomarkers were found to discriminate between different types of sample, allowing the assessment of the efficacy of wastewater treatment or the impact of discharges from WWTPs or other sources on the aquatic environment.
- ✓ The selection of suitable biomarkers that can typify different water sources and levels of contamination with ARGs and MGEs, together with harmonised qPCR procedures, may facilitate regular and integrated regulatory requirements for antibiotic resistance monitoring in wastewater and related aquatic environments.

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