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LAIST LABORATÓRIO DE ANÁLISES DO IST TÉCNICO LISBOA

Linking Wastewater Microbial Signatures to Environmental Drivers and Public Health Risk

Doctoral Program in Bioengineering and Biosciences

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Background

Wastewater-based surveillance (WBS) allows the monitoring of pathogen circulation in the population through the analysis of microbial markers in wastewater. This approach provides information on community health, **complementing** traditional clinical surveillance.

However, results can be affected by several factors, including differences in sampling methods, losses during sample processing and the presence of inhibitors. These factors complicate data interpretation and comparison between studies.

To account for this variability, different normalization approaches have been proposed, but their performance varies across studies. A better understanding of the factors influencing microbial signals is therefore needed to improve the reliability of WBS data.

Main objectives

Identify the main sources of methodological variability affecting microbial detection and quantification in wastewater

Evaluate the influence of environmental factors (temperature, precipitation and wastewater flow) on wastewater microbial signatures, as well as the suitability of microbial normalization markers

Investigate the relationship between microbial and environmental data and evaluate their relevance to public health

Methodology

1. **Environmental sampling and monitoring**
Comparison of different sampling strategies (grab, composite and passive sampling), combined with the monitoring of environmental parameters

2. **Method development and optimization**
Comparison of concentration and nucleic acid (DNA/RNA) extraction methods, followed by the implementation of qPCR, dPCR and NGS

3. **Microbial characterization**
Detection and quantification of selected bacteria, viruses and fungi of public health relevance, including viability assays

4. **Normalization markers**
Comparison of commonly used normalization markers with potential new candidates

5. **Data analysis and risk assessment**
Analysis of microbial and environmental data to identify correlations and evaluate their relevance to public health

Expected outcomes

Better understanding of the variability introduced during wastewater sampling and analysis

Identification of normalization markers that improve the comparability of microbial concentrations across different conditions

More reliable interpretation of wastewater microbial data and its relevance to public health surveillance

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