Wastewater monitoring for antimicrobial resistance using NGS and digital PCR

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Introduction

Wastewater is complex, containing a variety of PCR-inhibitory substances such as humic acids, heavy metals and complex organic matter. This challenges anyone detecting and monitoring microbial pathogens and antimicrobial resistance (AMR) markers.

In this study, we examined wastewater samples collected from various treatment plants across Germany for the presence of microbial pathogens and AMR markers. To compare single-host microbial profiles with population-level microbial profiles, individual stool samples were analyzed alongside wastewater. To address the inhibitory nature of these samples, DNA and RNA were extracted using kits with Inhibitor Removal Technology® (IRT), which enhances nucleic acid purity by eliminating PCR inhibitors and thereby improves the sensitivity, accuracy and reproducibility of downstream analyses. The extraction efficiency and target gene content were then evaluated using next-generation sequencing (NGS) and digital PCR (dPCR).

Materials and methods

Three wastewater samples from different treatment plants were concentrated by centrifugation, and six individual stool samples were collected and processed in the same way. DNA was extracted with the QIAamp PowerFecal Pro and AllPrep PowerViral DNA/RNA kits. AMR abundance was assessed by NGS using QIAseq xHYB AMR Panel and by dPCR using dPCR Microbial DNA Detection Assays on the QIAcuity Digital PCR System. NGS data were analyzed in the QIAGEN CLC Genomics Workbench with the Microbial Genomics Module, whereas dPCR data were processed in the QIAcuity Software Suite.

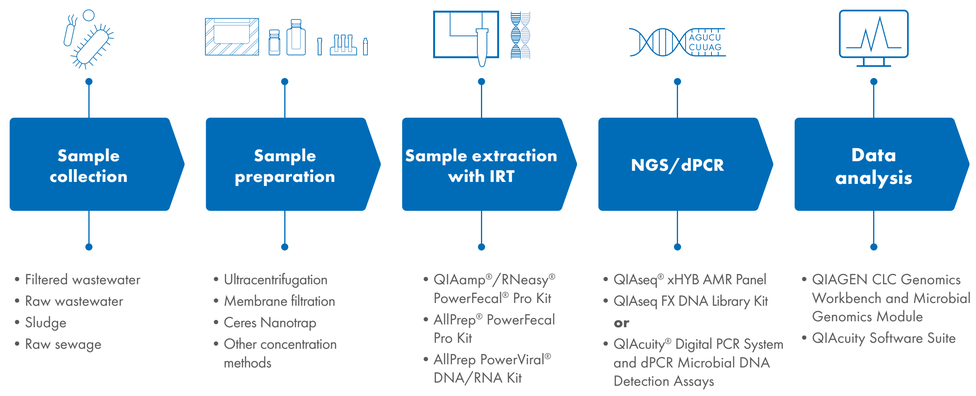

Figure 1. The recommended workflow for wastewater monitoring for AMR using NGS and digital PCR.

NGS confirms higher AMR diversity in wastewater compared to stool samples

To assess AMR abundance, three wastewater and six stool samples were extracted using the AllPrep PowerViral and QIAamp PowerFecal Pro kits, respectively, and analyzed by NGS with the QIAseq xHYB AMR Panel.

The resulting operational taxonomic unit (OTU) tables revealed a notably higher diversity of AMR markers in the wastewater samples compared to the stool samples. This is consistent with the composite nature of wastewater, which integrates microbial and resistance profiles from large populations as opposed to a single host.

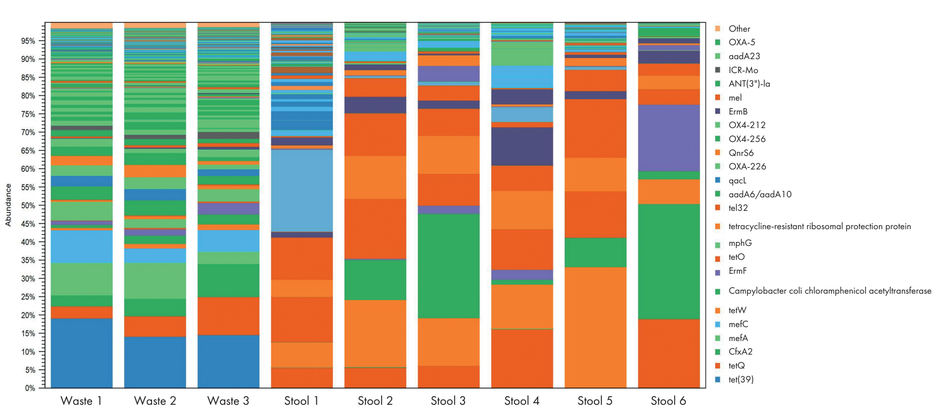

Figure 2. OTU tables comparing the abundance of AMR markers in the analyzed wastewater and stool samples.

dPCR shows high levels of multiple AMR genes in wastewater compared to stool samples

To quantify specific AMR markers, dPCR Microbial DNA Detection Assays were performed on the QIAcuity Digital PCR System using three samples each of wastewater and stool.

Wastewater samples showed high levels of multiple AMR genes, confirming the broader diversity observed by sequencing. In contrast, stool samples were dominated by Erythromycin resistance and showed high bacterial load, indicated by strong 16S rRNA gene (Pan Bacteria 1) signals. These results reinforce the utility of wastewater for comprehensive, population-level AMR surveillance.

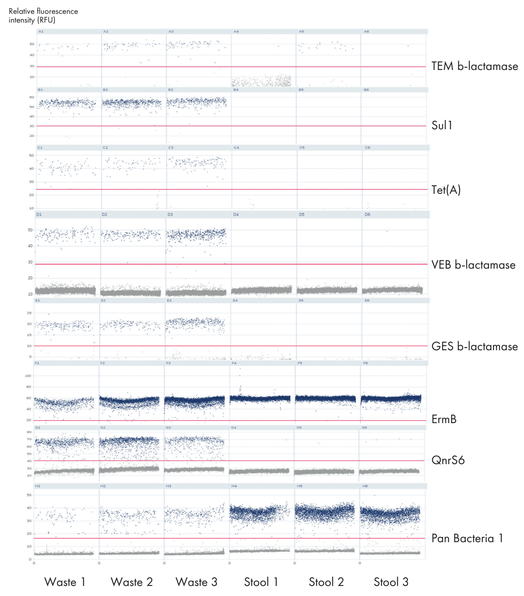

Figure 3. QIAcuity dPCR 1D scatter plots showing the levels of 7 different AMR markers and 16S rRNA Pan Bacteria 1 in the analyzed wastewater and stool samples.

dPCR concordant with NGS results

dPCR assay results were compared with AMR profiles obtained from the QIAseq xHYB AMR Panel. A high degree of concordance was observed between the two methods, demonstrating consistent quantification of key AMR markers and validating the reliability of both approaches for AMR detection.

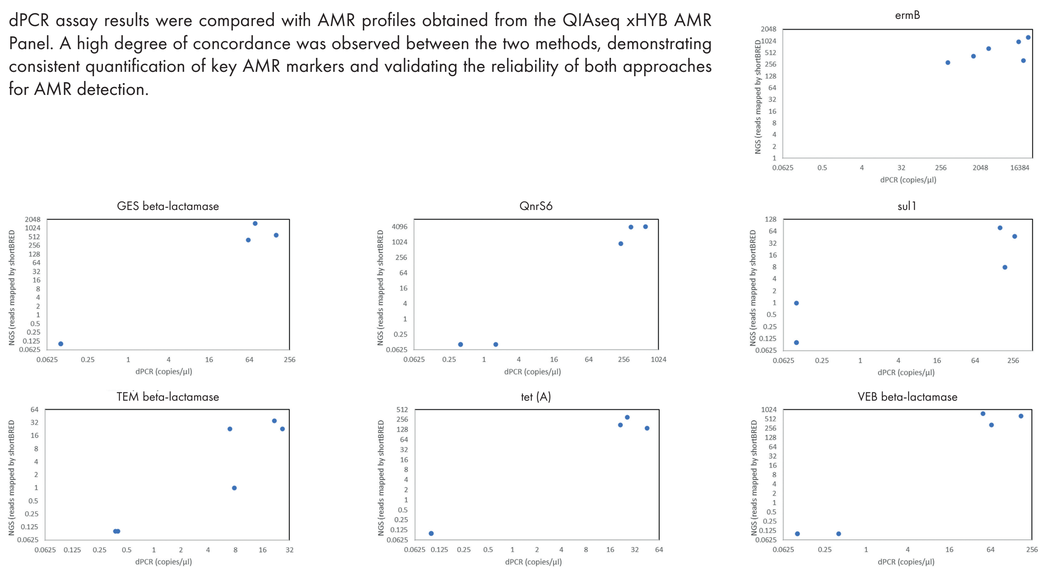

Figure 4. Two-dimensional scatter plots showing concordance between dPCR and NGS for each analyzed AMR marker. In these plots, each dot represents either a stool sample or a wastewater sample. In some graphs, not all six dots may be visible because certain AMR markers were absent from the stool samples.

Conclusions

A nucleic acid extraction kit using IRT combined with the QIAseq xHYB AMR Panel or dPCR Microbial DNA Detection Assays effectively monitored AMR markers from inhibitor-rich wastewater samples.

- Results demonstrate reliable detection of AMR markers with the QIAseq xHYB AMR Panel and highly sensitive, absolute quantification via dPCR.
- Strong concordance between dPCR and NGS results confirmed the robustness and complementarity of both methods for AMR profiling.

Together, these tools provide a comprehensive approach for monitoring AMR dynamics in individual (stool) and community-level (wastewater) samples. The described workflows can be adapted for broader pathogen detection, enhancing public health surveillance strategies.

Sample to Insight