Development and application of Multiplex qPCR for Antibiotic Resistance Genes in the Water Cycle

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Objectives

Develop, Evaluate and Apply Multiplex qPCR assays to detect ARG and dissemination in environmental water systems with higher accuracy than NGS and a higher throughput than regular qPCR, to obtain an accurate picture of ARG in the urban water cycle.

Methods

Six genes were included in the study: 16S rRNA, bla_{SHV}, IntI, qnrS, SulI and TetB. The selection was based on the available literature and the clinical relevance. Multiplex Q-PCR assays were composed by combining M1: internal control, SulI and qnrS and M2: 16S rRNA, bla_{SHV}, TetB and IntI. Compatible probes were designed and the Multiplex qPCR assays were evaluated using composite controls with varying, predetermined concentrations of ARG as well as with spiked environmental samples.

After development the Multiplex qPCR assays were applied to different surface water samples collected along the river Rhine catchment (NL, DE and CH), starting from relative undefiled conditions upstream in Switzerland to more anthropogenically polluted area's in Germany and the Netherlands. Furthermore wastewater samples from a Dutch hospital were investigated for the presence of ARG, the standard communal treatment was compared to on-site waste water treatment with a Pharmafilter. The ARG concentrations measured were corrected by the factor of loss of genetic material of the internal control added before the DNA extraction process. All qPCR experiments were done in triplicate.

Results

Multiplex qPCR

Accurate quantification of genes in composite and spiked environmental test samples could be achieved and exact quantification of ARG is therefore possible with the developed Multiplex qPCR assays. ARGs could accurately be identified and quantified through the developed multiplexes, even in test samples with concentrations of ARGs varying as far as 100-fold. The high throughput and accuracy of the multiplex qPCRs efficient quantification system is of additional value as the concentration of the significant spectrum of ARG in environmental samples is often unknown and large concentration disparities between different ARG are to be expected.

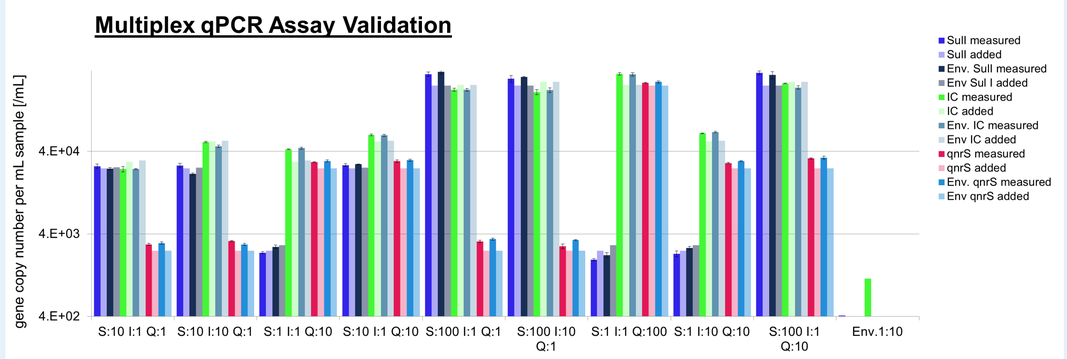

Figure 1: Validation of Multiplex qPCR Assay M1 using varying plasmid concentrations and spiked environmental samples

ARG Distribution along the Rhine

SulI and IntI were the most abundant ARG found. IntI concentrations varied across the Rhine with 3.3E+02 (Diepoldsau, CH) up to 3.6E+06 (Utrecht, NL). SulI concentrations were more stable with variotions from 1.2E+03 to 1.0E+04. TetB and bla_{SHV} could be detected in the area between the Swiss-German border. A correlation between the concentration of IntI and the concentration of other ARGs could be observed.

Overall, the expected increase of ARGs across the Rhine could not be observed. On the contrary, a decrease of both, total ARG concentrations and ARG concentrations normalized to the 16S rRNA gene, could be observed in the highly urbanized German 'Ruhr Gebiet' area at 686km and 739 km.

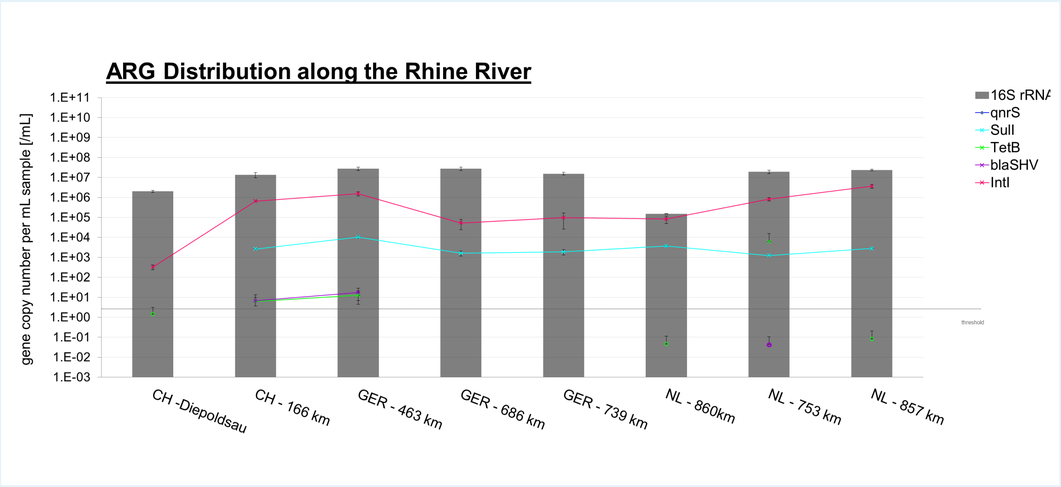

Figure 2: ARG and 16S rRNA Concentrations along the River Rhine from sampling campaign with samples taken at the same day, distances from origin of the Rhine at Lake Constance in km

ARG Concentration in Hospital and Municipal WW and the Advantages of local WW Treatment

High concentrations of ARGs could be detected in hospital WW. Further, a broader range of ARG could be detected in hospital WW when compared to municipal WW, with the Quinolone resistance genes (qnrS) being present only in hospital effluent.

The local treatment of hospital WW (with Pharmafilter installations) reduced the ARG concentrations by 3 to 5 log units, reducing the ARG concentrations to below ARG concentrations in communal WW effluent, both in total numbers and normalized to 16S rRNA.

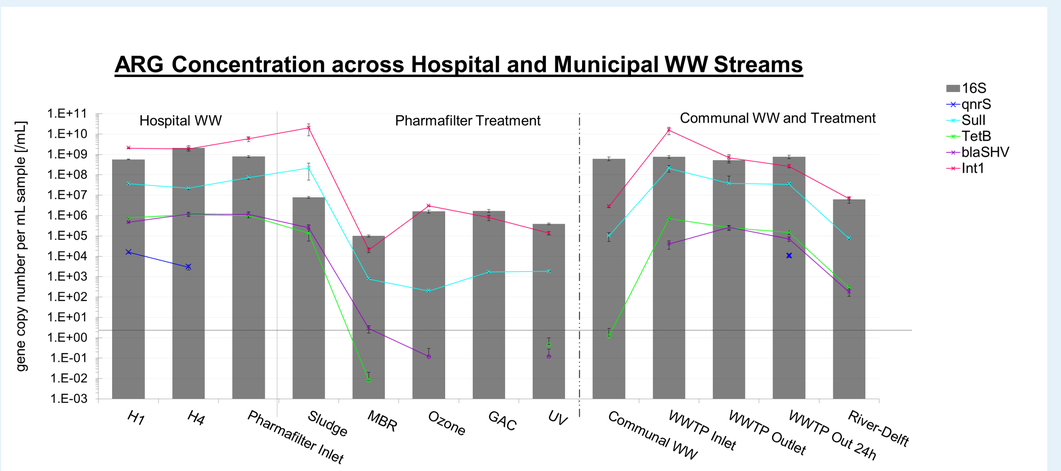

Figure 3: ARG and 16S rRNA Concentrations in Hospital Samples and after subsequent communal or local WW treatment (steps); Samples taken after the following steps: H1 –Dermatology, Oncology, Prenatal Care ; H4-,ER, Blood Sampling; MBR – Membrane Bioreactor; GAC – Granulated Activated Carbon; UV – UV-Treatment; WW(TP) – Waste Water (Treatment Plant)

Conclusions

- **Multiplex qPCR assays are a powerful tool to screen for AR and ARGs in environmental samples and obtain accurate results.**
- **The ARG concentration does not steadily increase along the course of the river Rhine.**
- **Local treatment of high-risk waste water effluents (e.g. hospital WW) might be beneficial in terms of ARG reduction.**

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