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INTER-LABORATORY CALIBRATION OF QUANTITATIVE ANALYSES OF ANTIBIOTIC RESISTANCE GENES

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Backgrounds

Antibiotic resistant bacteria and antibiotic resistance genes (ARGs) are major human-health threats, widely distributed in the environment. Quantitative PCR (qPCR) is a standard approach to detect and quantify ARGs in environmental compartments. However, the comparison of gene quantification reported by different laboratories is challenging since data are predominantly obtained under non-harmonized conditions, using different qPCR protocols.

Objectives

The aim of this study was to develop and calibrate standardized qPCR procedures for quantification of key ARGs, analyzing the same samples with common protocols and distinct equipment, reagents batches and operators.

Methods

Treated wastewater from three European countries were processed immediately after collection and transported to the laboratory for total DNA extraction. DNA extracts from each sample were pooled and aliquots were distributed by five partners involved in the calibration procedure. The genes 16S rRNA, *vanA*, bla_{TEM}, *qnrS*, *sul1*, $bla_{CTXM-32}$ and *intI*1 were analyzed using harmonized qPCR protocols and the constructed pNORM1 plasmid, which contains fragments of the seven targeted genes, was used for generating standard curves.

Conclusions

The 16S rRNA gene was the most abundant, followed by *sul1*, *intI*1, *qnrS* and bla_{TEM}. Quantifications made by different partners were reproducible and inter-laboratory variation was < 20%. The notorious exception was for the *qnrS* gene, and therefore protocol improvement is recommended. The genes $bla_{CTXM-32}$ and *vanA* were below the limit of quantification in most or all of the samples analyzed. The inter-laboratory calibration is an adequate approach to reliably assess ARG abundance and environmental contamination in different environments and geographic locations.