Concentration Levels of Resistance Genes in Wastewater and Receiving Environment

Brief description of the dataset

This dataset comprises of weekly concentrations of resistance *qnrS* gene in South West UK.

Monitoring and analytical information

The study area and sampling points

Wastewater influent and effluent were collected for 7 consecutive days running from Wednesday to Tuesday between June and October 2015 from five major WWTPs contributing to one river catchment in the South-West UK and covering an area of approximately 2,000 km^2 and the population of ~1.5 million (this constitutes >75% of the overall population in the catchment). All WWTPs use conventional sedimentation following secondary treatment (except for sequencing batch reactors (SBRs) that used decantation following settling *in-situ*). Respective wastewater and river water samples were collected on the same days. Selected WWTPs utilise different treatment technologies: activated sludge (AS) and trickling filters (TF). Influent wastewater samples were collected between screening and primary sedimentation. Digested sludge was also collected at WWTP B and E over three consecutive days. River water was collected from upstream and downstream of the effluent discharge point at varying distances depending on accessibility. River water was not collected for Site E as the WWTP discharges directly to the estuary.

Influent wastewater was collected as volume proportional 24 h composites with average sub-sample collection frequencies of approximately 15 minutes using an ISCO 3700 autosampler. Sub-samples (80 mL) were cooled to 4˚C (samplers were packed with ice) during collection to limit biological activity and pooled after 24 h (Petrie et al. 2016). Effluent wastewater samples were collected using time proportional approach due to the limited variation of this matrix over 15-minute intervals as discussed elsewhere. River waters (8 L) were collected as grab samples. All samples were transported to the laboratory on ice for further processing. Sample preparation and analysis

qnrS gene quantification using dPCR

DNA extraction and quantification

1 mL of unfiltered wastewater samples were centrifuged in sterilised micro-centrifuge tubes for 5 minutes at 3000 g. The supernatant was discarded and the remaining cell pellet was re-suspended in 200 µL phosphate buffered saline (PBS). 5 µL lysozyme were then added, followed by an incubation at 37 °C for 15 minutes. 200 µL of binding buffer and 40 µL proteinase K were added and incubated at 70 °C for 10 minutes. DNA extraction was performed in accordance with manufacturer's instructions (High Pure PCR Template Preparation Kit, Roche, Germany). Briefly, 100 µL of isopropanol alcohol was added. The samples were then transferred to a filter tube assembled inside a collecting tube and centrifuged for a minute at 8000 g. The supernatant was discarded and the filter tube assembled in a new collecting tube. 500 µL of inhibitor buffer and 500 µL of washing buffer were respectively added after cycles of centrifugation at 8000 g. The supernatant was finally discarded before centrifugation for 10 min at 9000 g. The filter tube was then assembled into a sterilised micro-centrifuge tube. 200 µL of elution buffer pre-warmed to 60 °C were used. Samples were centrifuged at 8000 g for a minute. The resulting DNA samples in the micro-centrifuge tubes were stored at -20 °C. To determine the success of the DNA extraction method, DNA was quantified by using a Thermofisher Nanodrop instrument, that was first calibrated and blanked using pure water (Castrignano et al. 2020).

qnrS gene quantification using dPCR

A QuantStudio 3D Digital PCR system was used with a QuantStudio 3D PCR V2 kit (Life Technologies, Thermo Fisher Scientific). PCR reaction mixtures were prepared with 7.3 µL Master Mix V2, 0.7 µL *qnrS* TaqMan Assay (20 X primer/ probe mix), 1.5 µL nuclease free water and 6.0 µl DNA sample. 14.5 µL of this mixture were loaded onto the digital PCR load blades and distributed in high density nanofluidic PCR chips that were loaded onto a GeneAmp PCR 9700 system.

The program was run using thermal cycling conditions. Temperature was first ramped to 95 °C and held for 10 min. It was then lowered to 60 °C for 2 min before increasing to 98 °C for 30 seconds. This cycle between 60 °C and 98 °C was repeated 40 times to allow for efficient gene amplification. The system was then lowered being to 60 °C and held for 2 min, before cooling to room temperature. After cooling, each chip was processed using the QuantStudio 3D Digital PCR system. AnalysisSuite™ software was used to get quantification of the targeted gene and statistical analysis of the results (Castrignano et al. 2020).

Format of the dataset

Concentrations of qnrS gene in influent and effluent wastewater during the monitoring week in all the sites are in copies in µl.

References

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