Quantification of Antibiotic Resistance Genes in a Managed Aquifer Recharge System

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Introduction

Managed Aquifer Recharge (MAR) offers a sustainable strategy to augment water availability in arid regions[1,2]. Groundwater resources are recharged with treated wastewater effluent, which may potentially retain contaminants. Antibiotic Resistance Genes (ARGs) which encode bacteria to withstand antibacterial treatments[3] may pass through wastewater treatment[4], before proceeding into an MAR facility. Antibiotic resistance may then proliferate in groundwater bodies prior to pumping for agricultural irrigation or drinking water treatment [3].

Quantitative concentrations of ARGs in recharge water are needed to assess risks to human and environmental health. In the absence of data characterizing ARGs, safe treatment target levels have yet to been established. Safe and sustainable operation of MAR may be guided by assessing current conditions captured in samples obtained from a MAR facility to then determine health-based target removal values.

Research Questions

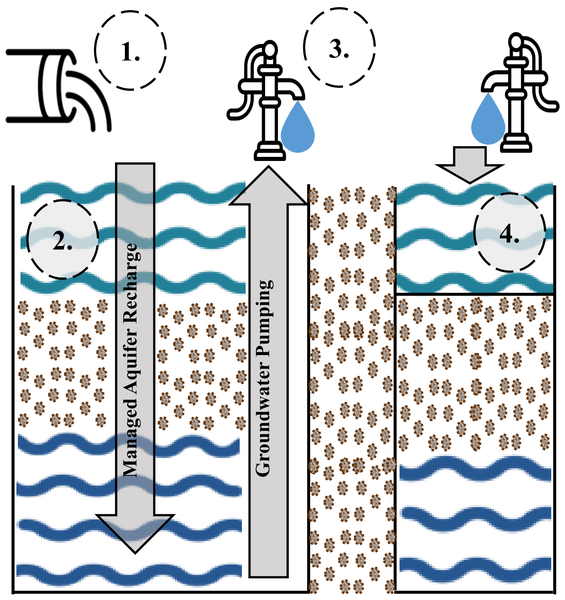
To assess antibiotic resistance in an MAR system,

1. Concentrations of ARGs in a water recharge system must be determined
2. ARB and ARG data will be interpreted to inform safe treatment and processing of sustainable groundwater recharge

Site Sampling

Water grab samples were collected in triplicate from four representative stages at the Gilbert Riparian Preserve MAR site

1. Treated wastewater effluent
2. Ponded wastewater effluent in a recharge basin
3. Pumped groundwater from the recharged aquifer
4. Ponded groundwater from an independent aquifer



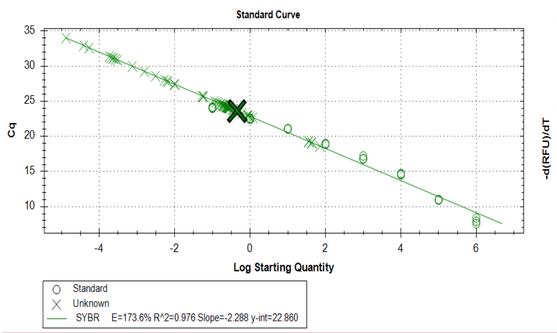
Methodology

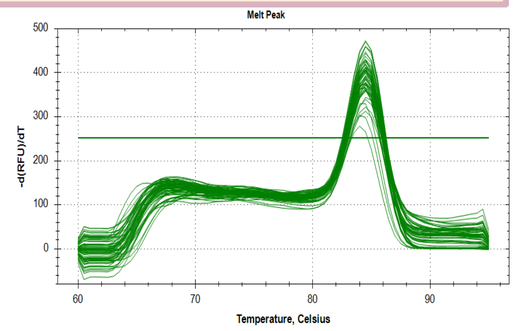
Water samples were filtered through 0.22µm membranes. Filter membranes were then processed with a series of solutions from a DNA extraction kit to isolate bacterial DNA present in the water samples.

Quantitative polymerase chain reaction (qPCR) was used to determine the number of copies of the *sul1* gene present in samples. *sul1* was selected as a target ARG following previous evidence of high environmental detections[5]. The *sul1* ARG encodes resistance to sulfonamide class-antibiotics[6] and is a serious threat to the treatment of clinically important infections[7].

Results

A standard curve was produced using known *sul1* gene concentration standards. With an R^2 value near 0.98, but Efficiency (E) value higher than 100%, further optimization may be performed before quantification of *sul1* gene copies. A single clear melt peak informs there is no sample contamination or reaction by-products that could interfere with quantification.





Discussion

Optimization of qPCR conditions will allow for further refinement and accurate quantification of the *sul1* gene in the representative samples. To obtain the concentration, the number of gene copies per unit of sample volume is needed. Here, the presumptive presence of the gene in all MAR stages indicates quantification is the next step as the *sul1* gene is abundant enough to be quantified.

References

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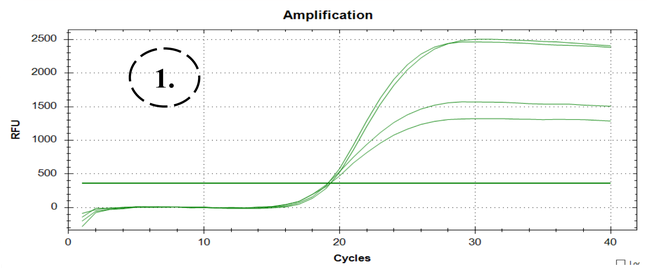
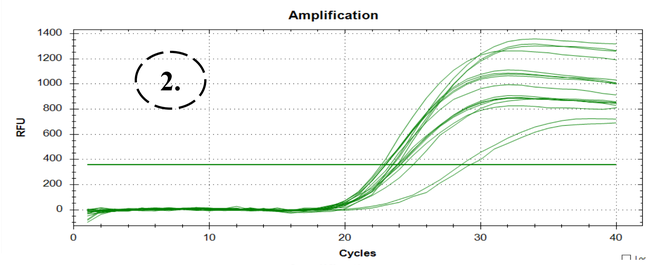
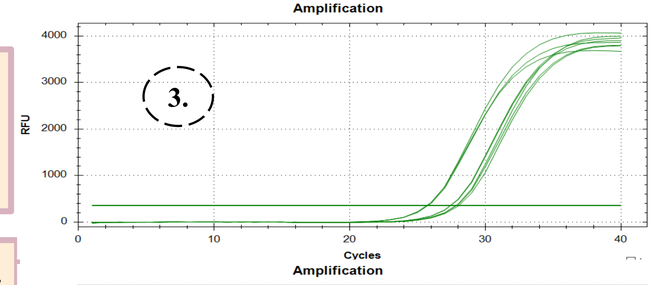
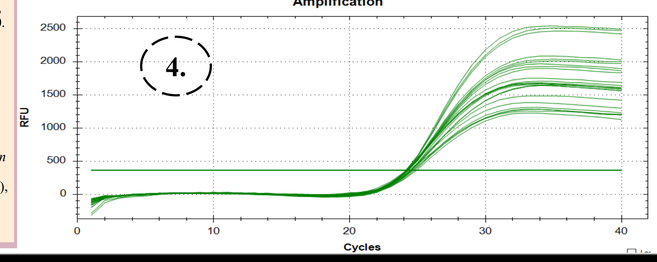
Results

Amplification curves illustrate clear amplification of qPCR reactions. Amplification prior to about 30 cycles may be presumed as a positive *sul1* gene detection, with quantification possible using the standard curve. The four sample types are displayed below.









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