



Antimicrobial Resistance Genes (ARGs) in Wastewater Sources



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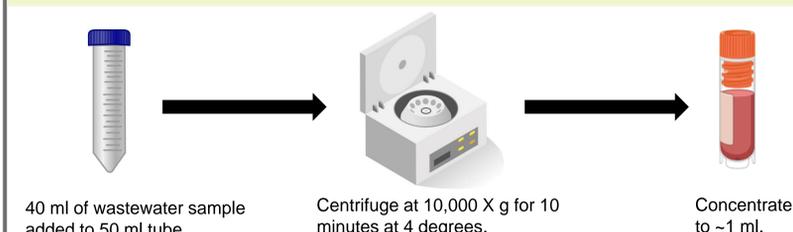
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INTRODUCTION

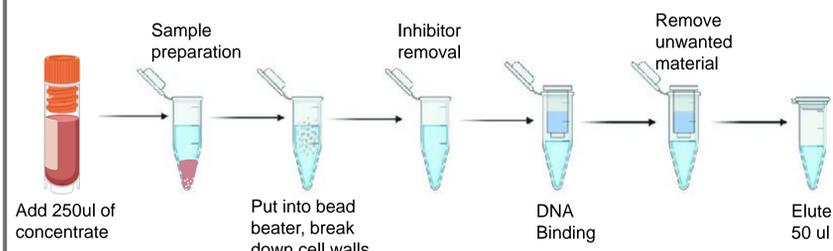
- Wastewater monitoring can provide non-invasive insight into the presence of ARGs to understand and suggest mitigation strategies for water reuse.
- An urban and a rural wastewater treatment plant were chosen for their mixing and prevalence of ARGs for whole communities (Lai, Muziasari et al. 2021).
- An urban community sewershed site and a rural hospital sewershed were chosen to look closer at each community, as well as at more vulnerable populations.
- qPCR screening was used to provide semi-quantitative results for an array of 90 ARG targets, providing presence/absence of what ARGs are in a sample.
- Using the screening results for each sample, ddPCR provides quantitative gene concentrations.

METHODS

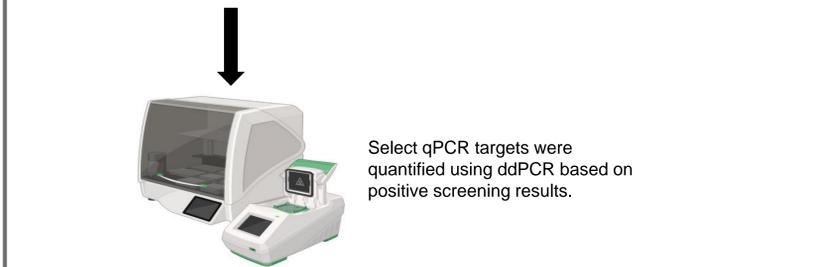
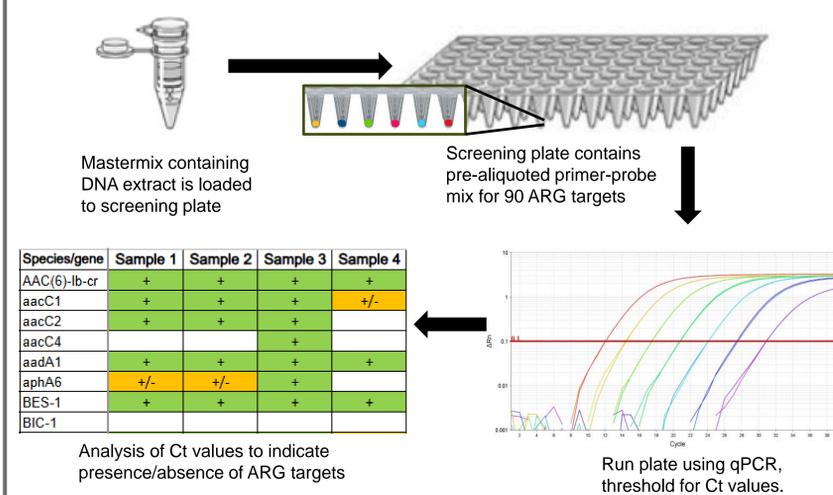
Concentration



DNA Extraction



qPCR Screening and ddPCR Quantification



RESULTS

Sites tested for ARGs											
Site Code	UW	WW1	HW	WW2							
Urban/Rural	Urban	Urban	Rural	Rural							
Site Type	Community	WWTP	Hospital	WWTP							
# of samples	n = 2	n = 3	n = 2	n = 2							
Average ddPCR ARG Concentrations by sampling location (GC/100ml, log10)											
Location	CMY-2	SHV	CTM-X	TEM	KPC	NDM	OXA-48	VIM	int1	tetW	mcr-1
WW1	6.43	6.30	6.30	7.13	7.43	ND	5.80	5.60	9.81	7.68	ND
UW	6.59	6.43	6.71	7.27	7.08	3.29	6.02	4.56	9.82	9.49	3.00
WW2	4.67	5.55	4.95	7.03	6.32	ND	3.98	6.68	9.93	7.40	ND
HW	5.97	7.14	6.66	7.38	8.40	ND	7.12	4.26	9.44	9.41	ND

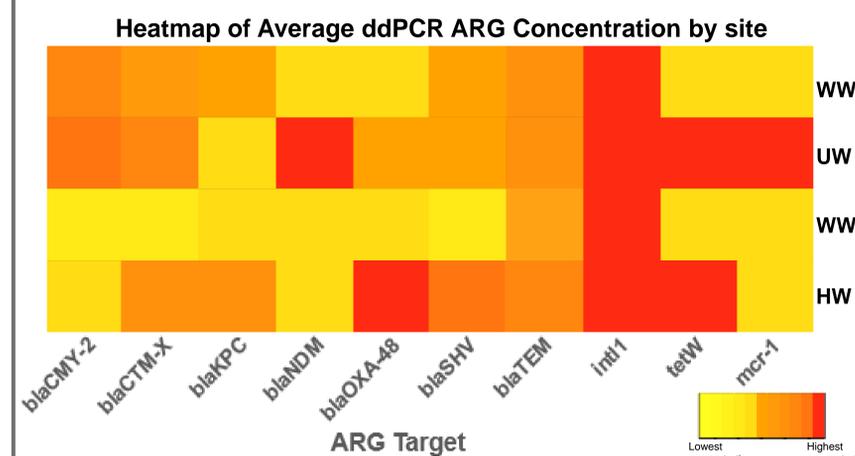


Figure 1. Heat map comparing average ARG concentrations in (GC/100ml) between all sites. Corresponding colors are relative to each column, to easily see the differences in concentrations by gene target.

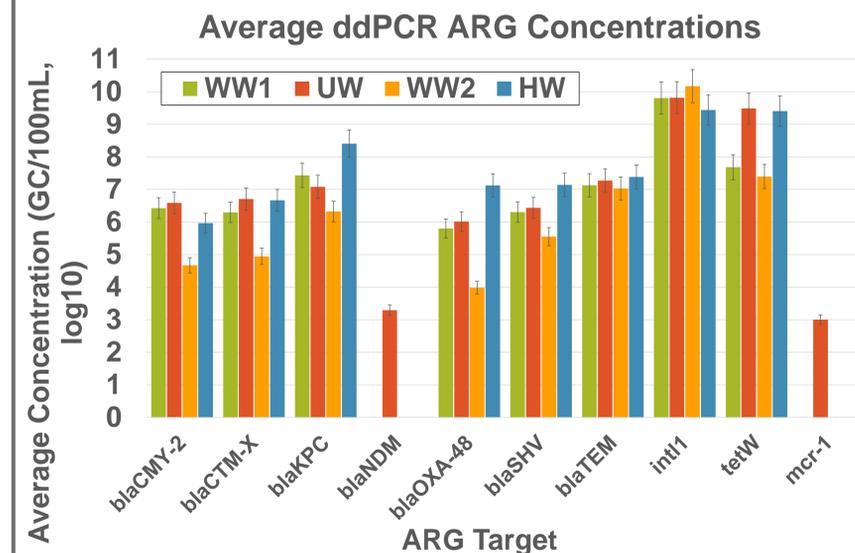


Figure 2. Bar graph of average ARG concentrations between all sites with standard error bars.

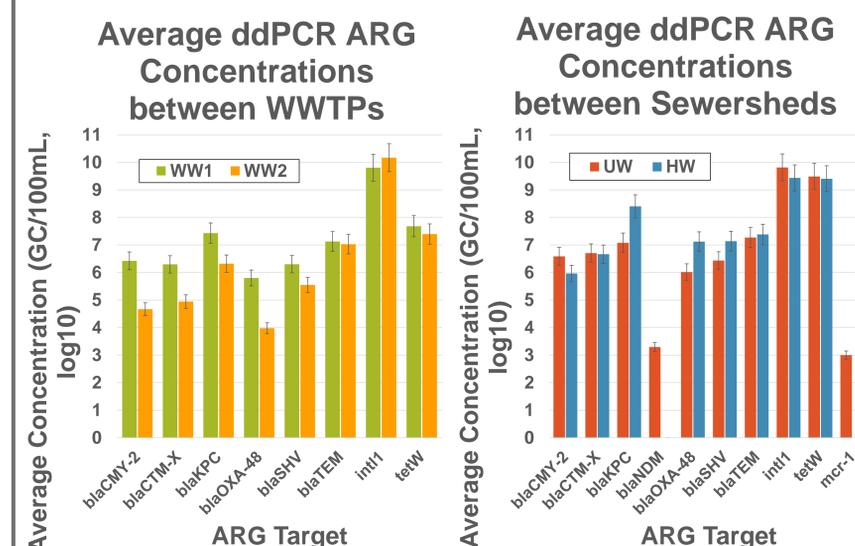


Figure 3. Bar graph of average ARG concentrations between WW1 and WW2 with standard error bars.

DISCUSSION

- Through the qPCR screening, 71 of the 90 ARG targets were positively detected in at least one of the sites tested. From this, more focused targets could be quantified through ddPCR. Beta-lactamases were mainly chosen and quantified because of the wide use of beta-lactam antibiotics.
- bla*NDM and *mcr-1*, both considered superbug genes resistant to last-resort antibiotics such as carbapenems and colistin, were detected at UW (1.97×10^3 and 1.00×10^3 , respectively). These genes have been found in *E. coli* and *Salmonella* and can be spread through contaminated food and water.
- On average, WW1 was found to have higher ARG concentrations than WW2, notably in *bla*CMY-2 (2.66×10^6 vs 4.68×10^4) and *bla*OXA-48 (6.35×10^5 vs 9.54×10^3).
- bla*TEM and *tetW* are comparable between the community and hospital sewershed sites (10^7 and 10^9 , respectively) and between treatment plants (10^7 and 10^7 , respectively). Tetracycline and penicillin are the most used livestock antibiotics in the United States (Kim et al., 2018) which may account for their similar concentrations between the rural and urban sites despite population differences.
- int1* had the highest concentrations of all the sites. It is used as a general indicator of antibiotic resistance and is not itself an ARG, but an “assembly platform” within bacterial DNA that allows them to acquire and integrate gene cassettes via HGF, which can encode for antibiotic resistance.

CONCLUSIONS

- qPCR screening for ARGs before further analysis saves time and resources.
- Positive results from the urban sewershed and hospital sites indicate that domestic wastewater is a major source of ARGs of concern.
- Wastewater treatment plants can provide a site for monitoring ARGs in large communities with enough sensitivity for PCR methods.

FURTHER RESEARCH

Further testing is currently being done to compare influent and effluent concentrations at the urban wastewater treatment plant to identify possible environmental threats. Testing may also be needed to identify bacteria that these genes are associated with.

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[1]: Lai, Foon Yin. “Profiles of environmental antibiotic resistomes in the urban aquatic recipients of Sweden using high-throughput quantitative PCR analysis.” *Environmental Pollution*, vol. 287, 15 Oct. 2021, <https://doi.org/10.1016/j.envpol.2021.117651>.
 [2]: Kim, Chandik. “A review of analytical procedures for the simultaneous determination of medically important veterinary antibiotics in environmental water: Sample preparation, liquid chromatography, and mass spectrometry.” *Journal of Environmental Management*, vol. 217, 1 July 2018, pp. 629–645, <https://doi.org/10.1016/j.jenvman.2018.04.006>.