Effects of Disinfection on Antibiotic Resistance in Wastewater

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Introduction

- Because of their genetic plasticity, bacteria can acquire resistance to antibiotics.
- Wastewater treatment has the potential to control the spread of antibiotic resistance (AR) into the environment.
- Current infrastructure may not be sufficient in controlling AR bacteria (ARB) and genes (ARG), due to horizontal gene transfer.
- Ultraviolet (UV) irradiation, chlorination, and ozonation are three common types of wastewater disinfection that could control AR.

Antibiotics

- Chemical
- Affinity for molecular site
- Ruled by chemical mechanisms and metabolic pathways

Disinfection

- Chemical or physical
- No affinity (tolerance varies)
- Ruled by probability, mechanisms of disinfection, and other variables

Objective

To determine whether disinfection is an adequate method of controlling antibiotic resistance within wastewater treatment and, if so, which disinfection method is best.

Methods

- Two wastewater samples, one before and one after disinfection, were taken at three different wastewater treatment facilities (WWTF) in El Paso, TX, each with a different disinfection method.
- Membrane filtration followed by cultivation on agar plates treated with five different antibiotics was used to quantify ARB in the water samples.

Table 1: qPCR genes tested

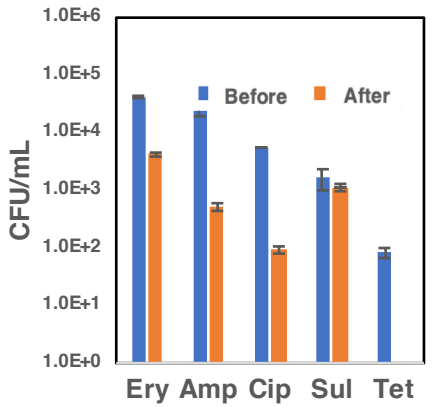
	Gene	Resistant to
1	16s	Nothing (total bacteria count)
2	tetW	tetracycline
3	qnrA	ciprofloxacin
4	qnrB	
5	qnrS	
6	sul1	sulfamethoxazole
7	ermB	erythromycin
8	blaTEM	ampicillin
9	intl1	Not AR (integrase)

- The DNA of each sample was extracted.
- The amount of ARG was measured using real-time quantitative polymerase chain reaction (qPCR) with primers for the 9 genes in Table 1.

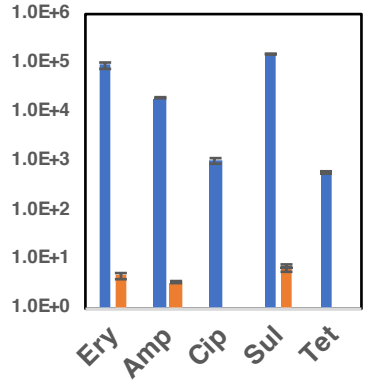
Results

- Most of the plate count data showed less colony forming units (CFU) after disinfection than before, no matter the type of disinfection.

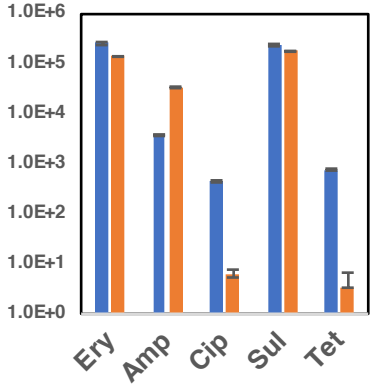
Plant 1 – UV Disinfection



Plant 2 - Chlorine Disinfection

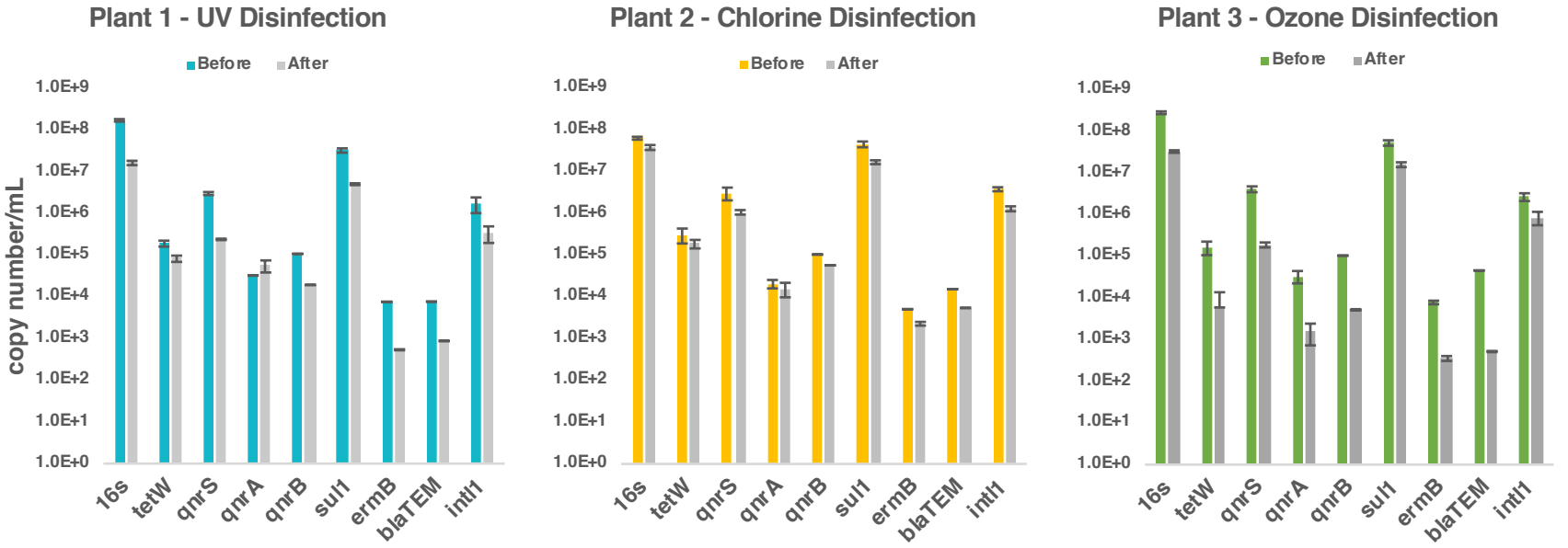


Plant 3 – Ozone Disinfection

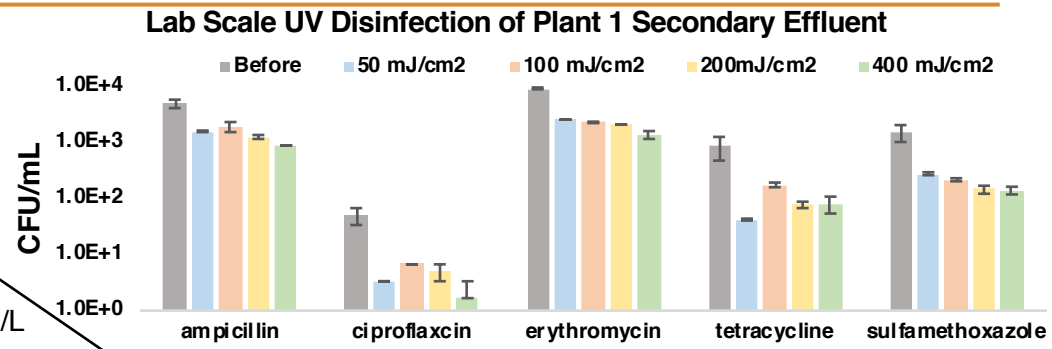


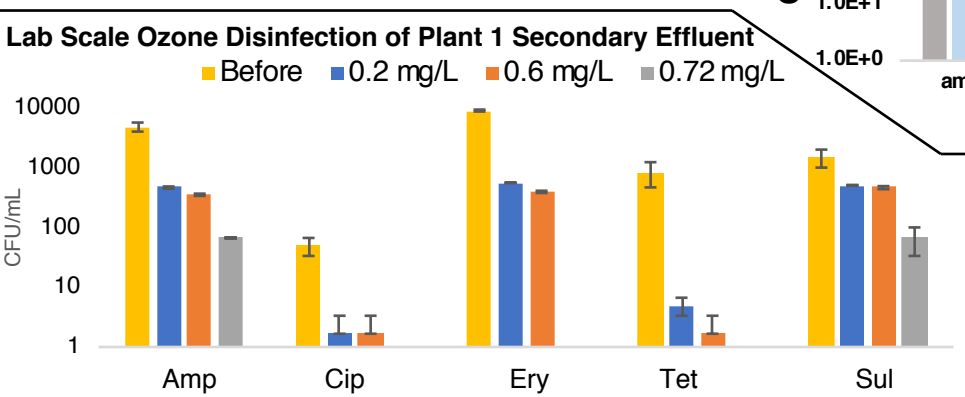
- Based on this data, chlorination is most effective and ozone is least effective at ARB log removal.
- These colony data are biased as most bacteria are unculturable in a lab setting.

- For the qPCR data, most ARG are lower in concentration after disinfection than before, with ozone as the most effective and chlorine as the least effective.
- However, just because the AR gene is present does not mean it is being expressed or that the added primer attached to the right gene. The qPCR detects extracellular DNA and ARG in dead cells as well.



- Lab scale tests of UV show the effects of different doses on AR.
- As UV dose increased, ARB decreased.
- Not a huge difference between highest and lowest doses.





- Lab scale tests of ozone show the effects of different doses on AR.
- The lowest dose removes AR substantially; however, the highest dose completely removed some ARB.

Conclusion

- For ARB removal in WWTF, chlorine is the most effective.
- For ARG removal in WWTF, ozone is the most effective.
- For lab scale study, the higher the dose the better the ARB removal.
- Overall, ozone seems to work best. AR could be controlled by disinfection with optimal dose and contact time, but more data are needed.
- We need to be smarter about antibiotic use & include AR consideration in WWTF design.

Future Work & Questions

- In which methods may ARG be released into the environment?
- Does quality of water have a big effect on AR survival? Which constituents of water are most influential?
- What other methods could control ARB and ARG? Is nanotechnology (antimicrobial nanoparticles), absorption (biochar/GAC), coagulation, or filtration better than disinfection alone?
- How does our DNA data differ with the expression of genes (RNA only)?

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