# The Effect of Different Disinfection Techniques on the Prevalence of Antibiotic Resistance in Wastewater Treatment

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## Introduction

The genetic plasticity of bacteria allows pathogenic bacterial strains to acquire an ability to survive against a wide variety of environmental threats, including antibiotics. Thousands of people die every year from antibiotic resistant (AR) bacterial infections; however, this number could increase to millions by 2050 if no action is taken[1]. Wastewater treatment has a unique role in the prevalence of AR strains as waste is collected and treated here before it is released into the environment. Current wastewater infrastructure may be insufficient treatment of antibiotic resistant bacteria (ARB) and genes (ARGs), as the high density of bacteria in wastewater promotes horizontal gene transfer. Disinfection is the only stage in the traditional treatment process that has the potential to control AR. Ultraviolet (UV) irradiation, chlorination, and ozonation are three common disinfection methods used by wastewater treatment facilities to inactivate bacteria. Their ability to control ARB and ARG is widely unknown, as several controlled studies of AR after these disinfection techniques have had contradictory results.

## 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

1. Sampling

Two wastewater samples, one before and one after disinfection, were taken at three different wastewater treatment facilities in El Paso, TX. Each of the facilities disinfected wastewater with either UV irradiation, chlorination, or ozonation.

Table 1: ARGs and antibiotics tested

| | Gene | Resistant to |
|---|---|---|
| **1** | 16s | Nothing (total bacteria count) |
| **2** | tetW | Tetracycline (Tet) |
| **3** | qnrA | Ciprofloxacin (Cip) |
| **4** | qnrB | |
| **5** | qnrS | |
| **6** | sul1 | Sulfamethoxazole (Sul) |
| **7** | ermB | Erythromycin (Ery) |
| **8** | blaTEM | Ampicillin (Amp) |
| **9** | intI1 | Not AR (integrase) |

2. Quantification of ARB by heterotrophic plate count

EPA standard membrane filtration method was used to enumerate the ARB in each sample. The 0.22 μm pore-size membranes were placed on agar plates treated with five different antibiotics to quantify ARB. The antibiotics used are shown in Table 1.

3. Quantification of ARGs by qPCR

The DNA contained in each sample was extracted using a Qiagen DNeasy PowerWater Kit. The amount of ARGs was then measured using a real-time quantitative polymerase chain reaction (qPCR) machine with the extracted DNA. Primers for each of the genes were added, as shown in Table 1.

4. Lab-scale study

Disinfection dosage was investigated by treating the "before disinfection" samples with several different doses of each disinfection method and the ARB and ARGs quantification processes were repeated.

[1]Liu, S.-S., Qu, H.-M., Yang, D., Hu, H., Liu, W.-L., Qiu, Z.-G., Hou, A.-M., Guo, J., Li, J.-W., Shen, Z.-Q., Jin, M., Chlorine disinfection increases both intracellular and extracellular antibiotic resistance genes in a full-scale wastewater treatment plant, *Water Research* (2018), doi: 10.1016/j.watres.2018.02.036

## Results

1. *ARB and ARGs in Wastewater Treatment Facilities (WWTFs)*

For the heterotrophic plate count (HPC) data, most of the plates had fewer colony forming units (CFU) after disinfection than before, no matter the type of disinfection. Chlorine is the most effective, reaching 4 log removal, while ozone is the least effective (Figure 1). Ozone treatment could only achieve a half log removal for some ARB. For Tet and Cip ARB, all the disinfection methods work well. These data can only represent a small portion of ARB in the sample because most bacteria are unculturable in a lab setting.[2]

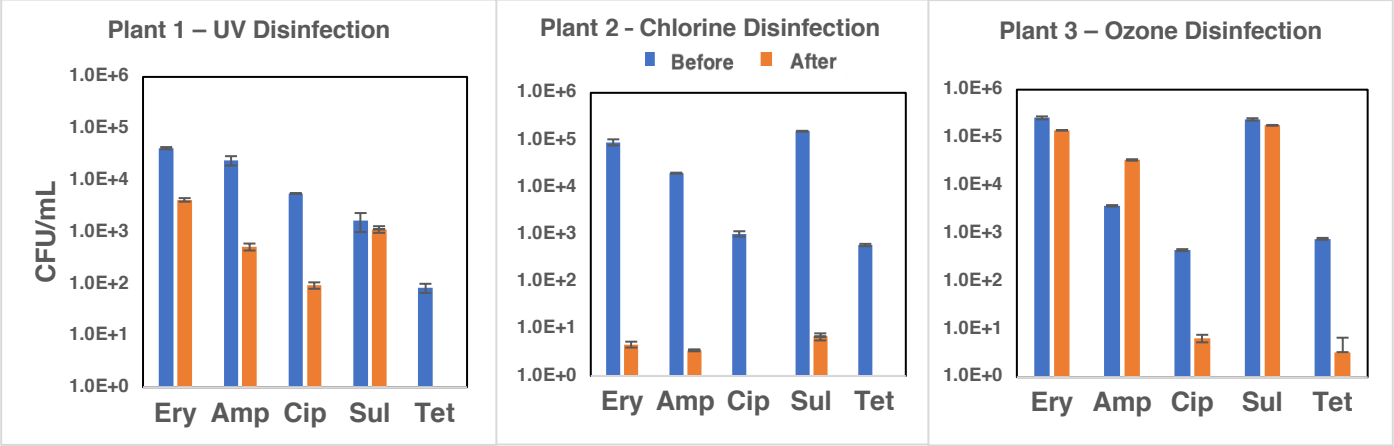


Figure 1: ARB concentration in wastewater samples before and after disinfection

For the qPCR data, our data follow a similar trend between each wastewater treatment facility based on the output (Figure 2). Most ARG are lower in concentration after disinfection than before. Ozone seems to remove the best, as several ARG are reduced at least one or one and a half log. Chlorine seems to be least effective at ARG removal, averaging only a half log removal, which contrasts with our HPC data. However, just because an AR gene is present in the cell does not mean it will get expressed and the added primers may bind to genes that are not AR. Moreover, qPCR can detect AR genes in dead cells.

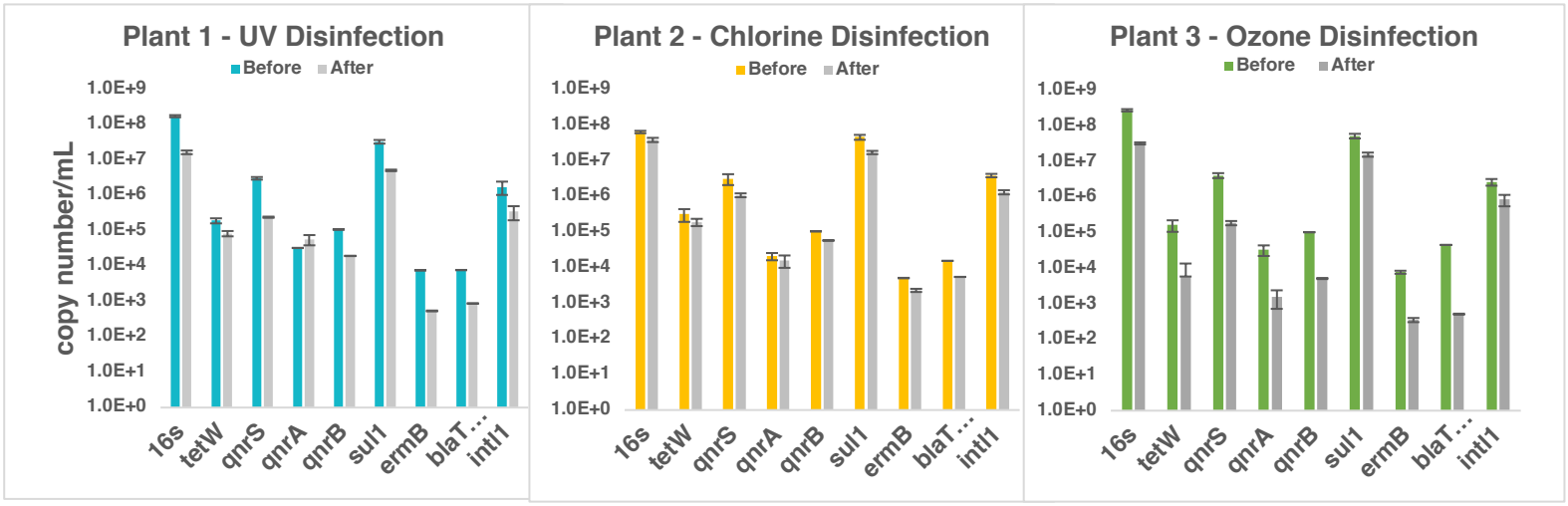


Figure 2: ARGs concentration in wastewater samples before and after disinfection

[2]Staley JT and Konopka A., Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annual Review of Microbiology,* (1985) 39: 321–346 doi: 10.1146/annurev.mi.39.100185.001541 [doi]

*2. Lab-scale study*

Lab scale tests of UV and ozone treatment were also performed to look at the effects of different doses of each method on AR. As the dose of UV treatment increased, ARB decreased overall (Figure 3). After about 200 mJ/cm$^2$, increased UV dose tended to not enhance log removal of ARB notably.

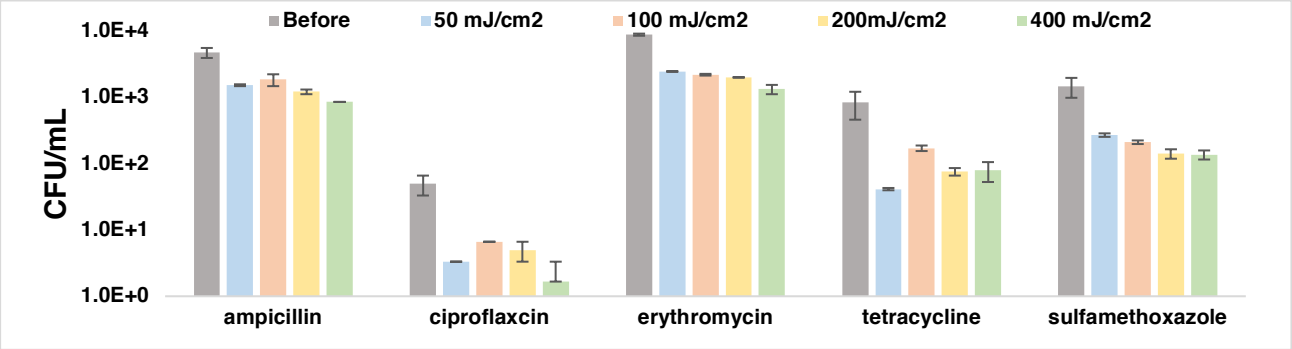


Figure 3: ARB concentration in secondary effluent of Plant 1 before and after lab-scale UV disinfection at different doses

Figure 4 shows the lab-scale test results for ozonation. Based on these results, the lowest dose does remove some AR. However, the highest dose completely removed AR in some cases. It is difficult to determine if this would be a realistic dose at WWTFs.

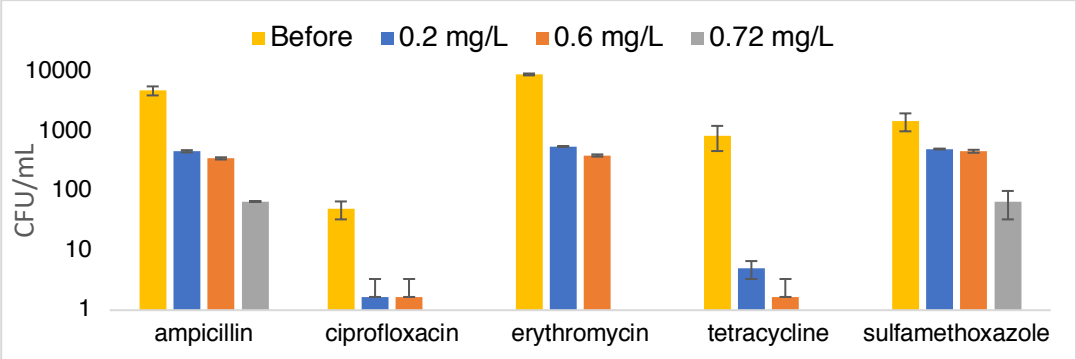


Figure 4: ARB concentration in secondary effluent of Plant 1 before and after lab-scale ozone disinfection at different doses with a contact time of 30 minutes

**Conclusion & Future Work:**

For ARB removal, all disinfection methods show effectiveness against AR; however, chlorine is the most effective in full-scale treatment plants.

For ARG removal, all disinfection methods show adequate levels of ARG removal although few genes increased in prevalence. Ozone is the most effective disinfection approach in ARG removal.

For lab scale ARB removal, the higher the dose, the better the ARB removal.

Antibiotic resistance could be controlled by disinfection with optimal dosage, but more data are needed. The combination of disinfection with other methods including nanotechnology (antimicrobial nanoparticles), absorption (biochar/GAC), coagulation, and filtration may lead to better control of ARB and ARG.