

Are Antibiotic-Resistant Bacteria a New Challenge to Disinfection?

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Why are Antibiotic-Resistant Bacteria of Concern?

Every year over 60,000 tons of antibiotics are consumed in the world to raise livestock, not to mention the quantity for human health use. Some antibiotics contained in the livestock will ultimately transfer to humans through food. The antibiotics that are either degraded or not degraded are finally disposed of and discharged to the environment. This causes bacteria to become resistant to the antibiotics, sometimes they even multiply in the presence of antibiotics. The bacteria with resistance to one or more kinds of antibiotics are known as antibiotic resistant bacteria (ARB). (Pruden et al., 2006)

The most serious concern with antibiotic resistance is that some bacteria have become resistant to almost all of the easily available antibiotics. These bacteria are able to cause serious disease. This is a major public health problem. Important examples are methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and multi-drug-resistant *Mycobacterium tuberculosis* (MDR-TB).

Antibiotic resistance genes (ARGs) carried by bacteria in these environments can be transferred to closely related human pathogens by some transfer mechanisms, such as horizontal gene transfer (HGT), which may greatly decrease the antibiotic therapy effect on human infectious diseases. HGT is an essential step for competitive bacterial survival in the environment and is also considered to be one of three major drivers for ARGs transfer. The three major mechanisms by which bacteria transfer genes horizontally are conjugation within bacteria, natural transformation, and transduction, with conjugative transfer within bacteria being the most studied.

So far, many ARB and ARGs have been found in various environments, such as sewage, treated drinking water, river water, ground water and soil. (Bouki et al., 2013; Rizzo et al., 2013) Thus, ARB and ARGs are considered as emerging contaminants. A global strategy has been proposed by the World Health Organization to contain antibiotic resistance regarding its potential threat to both public health and the environment (Yuan et al., 2014).

Wastewater Treatment Plants as an Incubator for ARB

Effluents from wastewater treatment plants (WWTPs) are considered to be hotspots for antibiotics allowing ARGs and ARB to spread into the environment. Because of the high microbial biomass and abundant nutrients, as well as various antimicrobial agents, wastewater represents a favorable habitat for both the survival of ARB

and the transfer of antibiotic resistance, spreading resistant bacteria into subsequent aquatic and terrestrial environments. Various ARB, including multiple antibiotic resistant (MAR) bacteria, have been detected in a large number of WWTPs. And *Escherichia coli* and *Enterococci* are considered as the principal MAR species. Although most ARB can be partially removed through common wastewater treatment processes, there are still large numbers that survive in the effluent, since WWTPs are not designed for the removal of these new pollutants (Guo et al., 2013).

The biological treatment process creates an environment potentially suitable for resistance development and spread because bacteria are continuously mixed with antibiotics at sub-inhibitory concentrations. Guo et al. (2015b) documented the amplification of bacterial resistance to erythromycin in activated sludge.

The ARGs acquisition rate by HGT can possibly be affected by various environmental conditions, such as temperature, pH, antibiotic concentration, etc. It is not clearly known what are the fates of ARB and ARGs in WWTPs. There is a lack of knowledge about how wastewater treatments impact the dissemination of ARB & ARGs.

Differences in UV Treatment of Normal Bacteria, ARB

Whether UV treatment will lead to the microbial selectivity of ARB or not is a question. Limited systematic studies have been carried out until now. In previous research, we have tested *E. coli* with the plasmid embedded aminobenzyl penicillin resistance gene. Little difference was found between amp-resistant *E. coli* and normal *E. coli*, concerning their UV fluence response curves (Guo et al., 2012).

In a more recent publication, to understand the effect of UV disinfection on antibiotic resistant bacteria, both total heterotrophic bacteria and antibiotic resistant bacteria (including cephalosporin-, ciprofloxacin-, erythromycin-, gentamicin-, vancomycin-, sulfadiazine-, rifampicin-, tetracycline- and chloramphenicol-resistant bacteria) were examined in secondary effluent samples from a municipal wastewater treatment plant. The results showed that UV disinfection results in effective inactivation for total heterotrophic bacteria, as well as all antibiotic resistant bacteria. After UV treatment at a fluence of 5 mJ/cm², the log reductions of nine types of antibiotic resistant bacteria varied from 1.0 to 2.4 (Guo et al., 2013). The findings suggest that UV disinfection could eliminate antibiotic resistance in wastewater treatment effluents and thus ensure public health security.

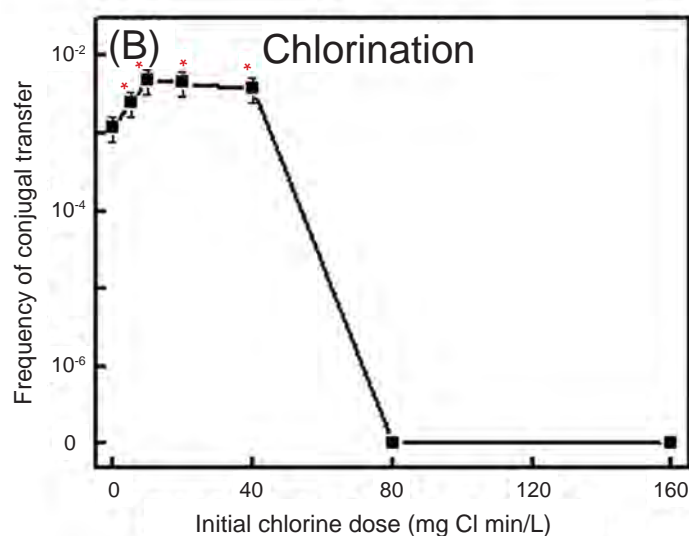
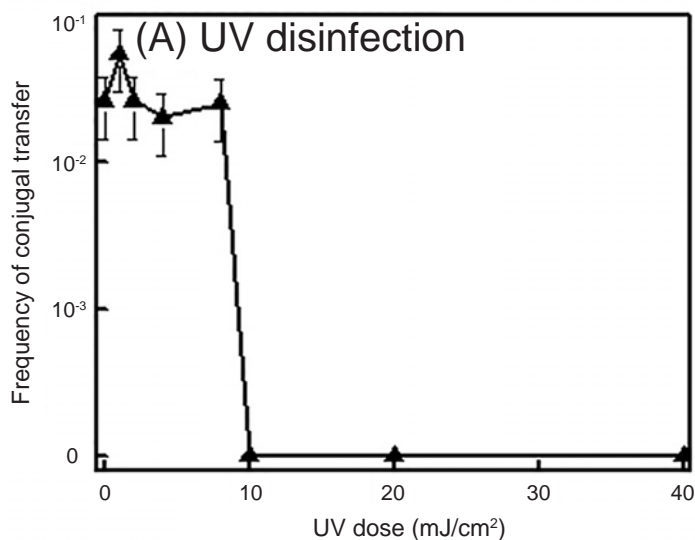


Figure 1. Effect of UV disinfection (A) and chlorination (B) on the frequency of conjugative transfer. Significant differences between the frequency with 5–40 mg active Cl min/L chlorine added and the control (no chlorine added) were tested with the student test, * $p < 0.05$. The error bars indicated the standard deviation of triplicated detection results (Guo et al., 2015a).

Interestingly, the experimental results indicated that UV disinfection led to enrichment of bacteria with resistance to sulfadiazine, vancomycin, rifampicin, tetracycline and chloramphenicol, while the proportions of cephalixin-, erythromycin-, gentamicin- and ciprofloxacin-resistant bacteria in the wastewater decreased (Guo et al., 2013). Since heterotrophic bacteria are a mixed cluster of bacteria, there are many complicated impact factors among them. So it is difficult to make an absolute decision. The results might reveal the microbial selectivity of UV disinfection for antibiotic resistant bacteria. It needs more well-designed experiments to verify.

Besides, ARG transfer is also impacted by UV irradiation. It was reported that low UV doses (up to 8 mJ/cm²) had little influence on the frequency of conjugative transfer, while the frequency of ARG transfers was greatly suppressed by high doses of UV (>10 mJ/cm²) (Guo et al., 2015a). This is good news for UV disinfection that ARGs transfer can be controlled through UV exposure as long as the UV dose is higher to a certain extent. By comparison, low chlorine doses (up to 40 mg Cl min/L) significantly promoted the frequency of conjugative transfer by 2- to 5-fold (Fig. 1). The generated chloramine stimulated the bacteria and improved the cell permeability which helps transfer (Guo et al., 2015a).

Treatment Strategies that Decrease Problem of ARB

Existing water treatment processes are not designed for ARB removal, although we have investigated that operation of activated sludge treatment can influence the release of ARB (Guo et al., 2015b; Yuan et al., 2015). Thus, the most effective technology is still disinfection. But previously, attention has not been paid to ARB when doing research about disinfection. So ARB becomes a new challenge for disinfection. Besides the well-known shortcomings of chlorination, chlorine disinfection also has a potential

of increasing ARG transfer if adequate doses are not applied. In this case, UV disinfection is highly recommended to decrease the problem of ARB. The detailed research is still needed to reveal the differences of UV treatment on ARB and normal bacteria. ■

References

- Bouki C., Venieri, D., Diamadopoulos E., 2013. *Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review*. *Ecotoxicol. Environ. Saf.* 91(1), 1–9.
- Guo, M.-T., Q.-B. Yuan and J. Yang. 2015a. *Effect of ultraviolet disinfection and chlorination on horizontal transfer of antibiotic resistance genes in municipal wastewater*. *Environmental Science and Technology*, 49(9): 5771–5778.
- Guo, M.-T., Q.-B. Yuan and J. Yang. 2015b. *Insights into the amplification of bacterial resistance to erythromycin in activated sludge*. *Chemosphere*, 136: 79–85.
- Guo, M.-T., Q.-B. Yuan, and J. Yang. 2013. *Microbial selectivity of UV treatment on antibiotic-resistant heterotrophic bacteria in secondary effluents of a municipal wastewater treatment plant*. *Water Research*, 47(16): 6388–6394.
- Guo, M.-T., J. Huang, H. Hu, W. Liu, and J. Yang, J. 2012. *UV Inactivation and characteristics after photoreactivation of Escherichia coli with Plasmid: health safety concern about UV disinfection*. *Water Research*, 46(13): 4031–4036.
- Pruden, A., Pei, R.T., Storteboom, H., Carlson, K.H., 2006. *Antibiotic resistance genes as emerging contaminants: studies in Northern Colorado*. *Environ. Sci. Technol.* 40(23), 7445–7450.
- Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M.C., Michael, I., Fatta-Kassinos, D., 2013. *Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review*. *Sci Total Environ.* 447C, 345–360.
- Yuan, Q.B., M.-T. Guo and J. Yang, 2015. *The sludge loading rate regulates the growth and release of heterotrophic bacteria resistant to six types of antibiotics in wastewater activated sludge*. *Environmental Science: Processes and Impacts*, 17: 206–212.