Title: Development and Removal of Antibiotic Resistance Genes
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Antibiotics have been widely used treat bacterial diseases since 1940s. However, the benefits offered by antibiotics have gradually faded due to the increased occurrence and frequencies of antibiotic resistance. The widespread use of antibiotics has driven selection for resistance in bacteria and is becoming a global problem for human health and the environment. Antibiotic resistance is exacerbated by the ability of bacteria to share their antibiotic resistance genes (ARGs) with other bacteria via horizontal gene transfer (HGT). Many existing studies on HGT of ARGs focused on antibiotic concentrations at or above the minimal inhibitory concentration (MIC), which is the lowest concentration of an antibiotic that prevents visible growth of a bacteria culture. However, knowledge on the development of antibiotic resistance under different stressors at sub-MIC levels is still limited. Carbon nanotubes (CNTs) have been widely used in environmental, agricultural and biomedical applications due to their unique physical and chemical characteristics, which have resulted in the occurrence of CNTs in the natural environment, but limited studies have been done to evaluate the effects of CNTs on the spread of ARGs. Electrochemical filtration has been shown to be a cost-effective technique to remove recalcitrant compounds and reduce antibiotic resistance, but limited studies have been done to evaluate the effectiveness of removal of ARGs with electrochemical filtration. Therefore, there is a critical need to evaluate effects of trace levels of antibiotics and CNTs on the development of antibiotic resistance and electrochemical removal of ARGs.

The specific research objectives of this study were to: (1) evaluate selective pressure of sub-inhibitory concentrations of antibiotics on the development of antibiotic resistance and HGT, (2) evaluate development of antibiotic resistance and HGT under exposure to CNTs and antibiotics, and (3) evaluate effectiveness of using an electrochemical MWCNT filter to remove ARGs.

To evaluate the development of antibiotic resistance exposed to sub-MIC of erythromycin, HGT between environmental donor (*E. coli*) and pathogenic bacterial receipt (*B. cereus*) were quantified. The results indicated that extremely low concentration (4×10^{-7} to 4×10^{-3} mg/L) of erythromycin promoted HGT of *erm*80 gene, which is an erythromycin resistance gene. In

addition to traditional culture-based method and quantitative real-time PCR (qPCR), a fluorescence *in situ* hybridization (FISH) approach was used to detect the occurrence and development of ARGs even the bacteria were in the viable but nonculturable (VBNC) state after treatment of sub-lethal level of erythromycin. Multi-walled CNT (MWCNT) was selected as a representative stressor to evaluate on HGT. The results showed that MWCNT HGT above 1 × MIC, which is the lethal level of erythromycin to recipients, and transfer frequency of *erm*80 gene increased up to 101-fold under exposure to 1 × MIC erythromycin and MWCNT as compared to no MWCNT control. However, transfer efficiency of *erm*80 gene under exposure to sub-MIC of erythromycin were inhibited by MWCNTs. Moreover, transfer of antibiotic resistance plasmids was affected by antibiotics and MWCNTs. Although the concentration of individual stressor was not enough to confer antibiotic selection, the effects of antibiotic resistance to both antibiotics and MWCNTs above 1 × MIC could add up and select for antibiotic resistance. The results suggested that CNTs may create additional selective pressure for the spread of ARGs and their effects of HGT should be further investigated. Finally, an electrochemical MWCNT filtration was evaluated to remove genomic DNA and ARGs under the effects of operating conditions, such as pH, phosphate, and NOM, were evaluated. The results showed that electrochemical MWCNT filtration achieved 79% removal efficiency for genomic DNA and 91% removal efficiency for *erm*80 genes. The results suggested that electrochemical MWCNT filtration could be a promising technology for the removal of DNA and ARGs.

Overall, the results improved our understanding of the development of antibiotic resistance and ARGs under various selective pressure. Trace levels of antibiotics promoted the development and spread of ARGs and conjugation transfer of resistance genes exposed to sub-MIC levels of erythromycin and MWCNTs also contributed to the spread and propagation of ARGs. As antibiotic concentrations detected in natural environment are often in trace levels, the results of this study may improve the understanding of health risks of trace levels of antibiotics and help develop effective mitigation strategies to control the spread of antibiotic resistance. Effective removal of ARGs with electrochemical MWCNT filtration may help the development of cost-effective treatment systems to remove ARGs to protect human health and the environment.