

## Changes in the Abundance of Antibiotic Resistance Genes in Biosolids during Wastewater Treatment Processes

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**Background/Objectives.** The prevalence of antibiotic resistant bacteria in wastewater and biosolids poses human health risks although several thorough and well-developed methods are applied to for treatment of the wastewater. The bacteria that possess antibiotic resistance genes (ARG) are often phylogenetically diverse and can therefore be difficult to identify. Bacteria carrying ARG will typically be removed from the aqueous phase during treatment and will thrive in the biosolids fraction throughout the wastewater treatment process. The increased application of antibiotics for treatment of humans as well applied in agriculture have increased the proliferation of ARG in wastewater due to an evolutionary biological response. ARG are rapidly inherited by future generations of bacteria and the possibility of gene survival grows stronger due to the increasing concentration of antibiotics in the environment. ARG, if ingested by humans due to land application of biosolids, have the potential to hinder any treatment of disease through antibiotics. The objective of this study, was to investigate the presence of ARG throughout the wastewater treatment processes in biosolids from an unnamed wastewater treatment plant by use of molecular approaches.

**Approach/Activities.** Biosolids samples were collected throughout the WWTP and DNA was extracted by a MoBio Soil DNA extraction kit. The ARG were detected via PCR and application of three separate ARG primer sets Qint, int1, and int12 that target the class 1 integron int1, which is an integrase gene with a known sequence coding for antibiotic resistance. The presence of the ARG in individual biosolids samples was determined based on the presence/absence of the corresponding PCR product through DNA gel electrophoresis and UV imaging. In addition, a quantitative PCR (Q-PCR) assay was developed that allowed for enumeration of the ARG present throughout the treatment process while being compared to the concentration of solids in the processes.

**Results/Lessons Learned.** The results showed that ARG were observed with the primer sets int1 and int2 throughout the solids phase of the WWTP. Enumeration of the ARG using Q-PCR revealed that  $10E9$ - $10E10$  genes per g biomass were present. The passage of the anaerobic digester showed a reduction of a factor 10-15 compared to locations prior to anaerobic digestion. Based on the results, it was determined that despite a rigorous treatment method in place to eliminate nutrients and pathogenic bacteria from wastewater, ARG are still present in biosolids during all stages of the treatment process. This can potentially cause a spread of ARG in cases, where biosolids are being applied as fertilizer on farmland.