

## 2018 Reciprocal Meat Conference – Consumer Topics

## Meat and Muscle Biology™



## Characterization of Antibiotic Resistance Transmission in the Beef Production System

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### Objectives

Antibiotic resistance (AMR) associated with food animals is a public health concern. While many studies have evaluated AMR through culture-based techniques, shotgun metagenomics for AMR allows for development of an ecological perspective within an entire microbial community, rather than focusing on selected organisms. The objective of this study was to characterize AMR genes throughout a conventional beef production system using targeted shotgun metagenomics approach.

### Materials and Methods

Eighty composite samples from several stages of beef production were collected in such a manner as to follow the same cohorts of cattle: fecal samples ( $n = 21$ ), meat trim samples ( $n = 19$ ), and soil samples, where composted feces were applied as amendments for crops ( $n = 18$ ). In the same timeframe, samples from a nearby human wastewater treatment plant ( $n = 14$ ) and soil where treated waste was applied for crops ( $n = 7$ ) were collected. Collected samples were shipped to Colorado State University and stored at  $-80^{\circ}\text{C}$ . Entire microbial DNA was isolated from each sample (aliquots of samples were used in DNA extraction except for meat which was sampled as a rinsate), and a customized target enrichment system (Agilent, SureSelect XT) was used to build DNA libraries enriched for AMR gene sequences. Libraries were sequenced on a HiSeq 4000 (Illumina;  $2 \times 125$  bp paired-end) at a depth of 4 to 20 samples per lane depending on the expected microbial abundance and amount of off-target background DNA.

### Results

Across all samples, 18 classes, 69 mechanisms, 250 groups, and 1,327 gene accessions associated with AMR were identified. Using this AMR gene target enrichment system, AMR genes were identified in samples from all stages of beef production that were sampled. Human wastewater contained greater numbers of unique gene accessions (richness) compared to other sample matrices, with an average of 621 unique gene accessions per sample. Trim rinsates had the lowest richness (mean = 27 unique gene accessions per sample). Composition of the resistome differed among sample matrices. Fecal resistomes were predominantly associated with tetracycline resistance (74% of hits), while resistome from trim was more diverse; the trim resistome was comprised of 32% elfamycin, 25% betalactam, and 22% rifampin resistance. Additionally, soil samples where composted cattle feces and treated human waste were applied contained similar resistomes: approximately half of sequencing hits related to rifampin resistance and 25% for elfamycin resistance. Finally, treated human waste predominately (36%) contained hits of AMR gene accessions that are resistant to the macrolide, lincosamide, and streptogramin (MLS) class.

### Conclusion

Diversity of AMR genes by location in the beef production system suggested that AMR gene dissemination was not linear throughout production and that there are many factors affecting transmission to humans. Using these data, differing interventions can be designed and implemented based on the specific step in production being targeted to mitigate AMR in and around the beef production system.