BEEF INDUSTRY SAFETY SUMMIT
March 3-5, 2015
Dallas, TX

Project Title: **Shotgun Metagenomic Sequencing Reveals Lack of Antimicrobial Resistance in Beef**

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Category: Pre-harvest research

Published: Unpublished to date

Objective: To describe the genetic antimicrobial resistance profile ("resistome") of pens of cattle as they move through the beef production system, and to track changes in the abundance and diversity of antimicrobial resistance mechanisms.

Experimental Design & Analysis:

Samples (n=48) of feces, water and soil from two pens at four feedlots were aseptically collected at the time of cattle placement and again when the same cattle were shipped for harvest. Subsequently, swab samples (n=8) of the trucks used to transport these same cattle to the packing plant were collected, as well as fecal and water samples (n=16) from the holding pens used to house the cattle. Finally, composite swab samples (n=8) from the trimming belt, round and chuck tables, as well as 400 g of trimmings (n=8) were collected while the same cattle were being processed. Whole community DNA was extracted using Mo-Bio PowerFecal® and PowerSoil® Kits, purified, and sequenced on an Illumina HiSeq. Reads were trimmed and filtered for quality, and *Bos taurus* DNA was removed. Reads were aligned to a database of antimicrobial resistance gene (ARG) sequences using BWA; sequences with >80% gene fraction were considered present. ARGs were grouped by resistance mechanism and relative abundance, richness and Shannon's diversity were calculated and compared across groups of samples using generalized linear models.

Key Results:

406.9 Gb of sequence data were obtained, with an average of 46.3M reads per sample (range 12.0 – 93.4). The mean quality score for all samples was Q35, and fewer than 5% of all reads were removed due to low quality. The proportion of reads classified and removed as *Bos taurus* was highly dependent on sample type. Among bacterial DNA, we identified 318 unique ARGs across all samples, comprising 14 unique classes and 42 unique mechanisms of resistance. In total, reads assigned to ARGs represented 0.044% of all reads (range 0 to 0.11% per sample). No ARGs were identified in the 16 samples collected from the fabrication room. Relative abundance of ARGs decreased from placement through shipping and transport, but this decrease was not statistically significant. Diversity of resistance mechanisms decreased significantly from placement to shipping (P=0.03), increased significantly from shipping to truck (P = 0.01), and decreased again from truck to holding (P = 0.01). Tetracycline resistance was the most commonly identified class of resistance (74% of all ARG-classified reads), and ribosomal protection proteins the most commonly identified type of tetracycline resistance (97% of all tetracycline-classified ARG reads).

Industry Application:

Metagenomics provides a more comprehensive picture of antimicrobial resistance than can be garnered from culture- or PCR-based methods, and thus enables a more comprehensive conclusion about AMR in beef production. This work shows that harvest interventions currently utilized to reduce pathogen load on beef products serve dual-purpose by also reducing the level of ARGs. These results should reduce concerns about transmission of resistance through the beef supply chain. This work also highlights the immense potential of metagenomics as a tool in food safety.

Table 1. Metrics of ARG abundance and diversity, by sampling location. Relative abundance is calculated as the proportion of reads classified as ARGs.

	Arrival	Exit	Truck	Holding	Plant
Average Relative Abundance of ARGs (%)	0.05	0.04	0.03	0.03	0.0
Average Shannon's Diversity Index	0.84^{a}	$0.59^{a,b,c}$	$1.07^{b,c}$	0.60^{c}	0.0
Average Number of Unique Resistance Mechanisms	15.1^{a}	$8.2^{a,b}$	$17.1^{b,c}$	$7.0^{a,c}$	0.0

^{a,b,c} Means with the same superscripts within rows differ significantly ($P < 0.05$).

Figure 1. Composition of ARGs by class of resistance, grouped by sampling location.