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Project Title: **Investigating the Effect of Tulathromycin Exposure on Antimicrobial Resistance Ecology in Feedlot Cattle during the Early Feeding Period Using Shotgun Metagenomics**

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Objective: The goal of this study was to use shotgun sequencing and bioinformatic analysis to understand the impact of metaphylactic tulathromycin (Draxxin) exposure on the profile of antimicrobial resistance genes (resistome) of cattle in the early feeding period. Tulathromycin was chosen because it is a macrolide antibiotic, which is the most commonly used class of antibiotics in livestock production and is typically used to treat pens of cattle metaphylactically to decrease the risk of bovine respiratory disease.

Experimental Design & Analysis:

The advancement of next-generation sequencers now enables a shotgun metagenomic approach to sequence a representative portion of all the DNA in a sample and thus provides a novel approach to investigate how livestock management practices alter microbial communities. In particular, shotgun metagenomics is suited for researching antimicrobial resistance ecology because it does not rely on the aerobic culture of particular organisms and therefore can track the presence of resistance genes regardless of which bacteria actually carries the gene. Two pens of cattle in a Texas feedlot were enrolled for this study. Apart from the administration of 800 mg of tulathromycin for each of the cattle in the treatment group, the cattle in both pens received the standard processing protocol for arrival to the feedlot, were exposed to identical environments, and were fed the same diet throughout the length of the study. Individual fecal samples from the rectal-anal junction were collected at arrival processing and 11 days into the feeding period. Selected fecal samples from treated (n=30) and control (n=30) animals from both sampling times were subjected to total DNA extraction for metagenomic sequencing. Sequencing then creates computer files with millions of nucleotide sequences known as “reads” for each sample and bioinformatic tools were used to trim poor

quality nucleotides and filter out bovine DNA. To evaluate the resistome, sample reads were aligned to a custom database of publically available antimicrobial resistance genes and only genes with >80% gene fraction were considered present for subsequent analysis. Methods for the statistical comparison of bacterial community data from metagenomic samples is under constant progression as the scientific field of metagenomics grows and currently necessitates using multiple techniques such as cumulative sum scaling, Hellinger transformation, and the employment of both zero-inflated multivariate models and non-metric multidimensional scaling of Euclidean distances.

Key Results:

Comparisons of resistome composition revealed that resistome richness and diversity was not significant between groups at either arrival processing or 11 days into the feeding period. Furthermore, when combining both groups, ordination showed a significant separation of the arrival processing and day 11 resistomes, but only the treated group experienced a significant decrease in resistome diversity. These preliminary results suggest that exposure to tulathromycin during arrival processing exerts a relatively small effect on the resistome in treated cattle whereas the transition into the feedlot exerts a greater effect on the resistome of all feedlot cattle.

How can this information be applied in the industry?

The beef industry could leverage the cutting edge technology of next-generation sequencing and shotgun metagenomics to better understand how common management practices can be affecting the critically important issue of antimicrobial resistance.

Figure:

NMDS ordination of arrival processing (pre) and day 11 (post) combined group resistomes, conducted at the gene level. Separation of arrival processing and day 11 resistomes was statistically significant (ANOSIM $P < 0.05$).