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Project Title: **The Beef Fecal Resistome Differs from Other Commodities**

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Objective: To design an integrated sampling-to-analysis pipeline for identifying highly discriminatory SNP profiles in antimicrobial resistance genes (ARGs) within shotgun metagenomic data. Such profiles can potentially be used to track antimicrobial resistance genes through time and space, thus providing crucial information to epidemiological explorations of antimicrobial resistance dynamics, as well as to risk analyses and outbreak investigations.

Experimental Design & Analysis:

AMR genes represent <0.01% of DNA in a metagenomic sample; attaining sufficient sequencing coverage to identify SNPs with high confidence is cost-prohibitive. Bait capture (e.g., Agilent's SureSelect system) can selectively enrich for target genes within metagenomic DNA, but this method has yet to be applied to ARGs. The overall goal of this project was to design an ARG bait capture system and validate its use in metagenomic fecal samples collected from poultry, swine, salmon and beef production and human wastewater treatment plants (WWTP).

In order to validate a comprehensive ARG bait enrichment system, we collected composite fecal samples from large commercial poultry and swine barns, and feedlot cattle pens, as well as treated biosolids from human WWTPs. Total DNA from 5 samples from each site (total N = 20) was extracted. One aliquot of DNA from each site was sequenced on the Illumina HiSeq without pre-enrichment with baits, while a second aliquot from each site was subjected to bait enrichment and then sequenced. Sequence reads were trimmed and filtered, and host DNA was removed. Reads were compared to a database of ARGs in order to characterize the resistome in each sample. Resistome composition between commodities was compared using non-metric multi-dimensional scaling ordination and abundance of ARGs was compared between commodities using zero-inflated Gaussian mixture models. In order to compare the

efficiency of bait enrichment, we compared the resistome composition and sequencing coverage of matched (i.e., enriched and non-enriched) samples.

Key Results:

Sequencing produced ~5.5 billion reads across all 20 non-enriched samples. Fewer than 5% of reads were removed due to low quality, and <5% of trimmed reads were classified as host. The human WWTP samples contained lower abundance of ARGs overall compared to samples from beef, pork and poultry facilities (0.002% of all sequences versus 0.095%, 0.191% and 0.145%, respectively). This is expected due to interventions applied during treatment of municipal wastewater. The resistome of the WWTP samples clustered separately from all livestock samples, indicating a significant difference in ARG composition, driven primarily by macrolide resistance efflux pumps in the WWTP samples (Figure 1). Resistome composition also differed significantly by commodity system. Over 70% of the ARGs identified in the beef and pork samples are known to confer resistance to tetracycline antimicrobials, while the poultry samples contained a majority of ARGs in the macrolide-lincosamide-streptogramin and tetracycline resistance classes (47% and 20%, respectively). Multi-drug resistance mechanisms were also identified in higher relative abundance in poultry samples (13% versus 3% and <1% in pork and beef, respectively), while aminoglycosides were more prevalent in pork (13% versus 4% and 1% in poultry and beef, respectively). Comparative analysis of enriched and non-enriched paired samples indicates that bait enrichment significantly increases sequencing coverage of ARGs in metagenomic data, while also enabling identification of rare ARGs that may not be captured in non-enriched samples. Furthermore, bait enriched sequence data can be used to identify SNP patterns in ARGs from metagenomic data.

How can this information can be applied in the industry?

These results show significant separation between the resistomes of human-associated and livestock-associated samples, which suggests that the bacterial communities in these systems are distinct and do not undergo frequent or large-scale mixing. This should help to reduce concerns about “spill-over” from livestock production facilities into human-associated habitats, but follow-up is needed to confirm the extent of separation of these bacterial ecosystems. Furthermore, this work provides proof-of-concept that a bait enrichment system can be used to target the entire resistome within a metagenomic sample, enabling increased sequencing coverage and thus confident SNP identification. SNPs, in turn, can aid the industry’s food safety efforts in multiple beneficial ways. Beyond their obvious utility as a powerful tool in outbreak investigations, SNP profiles can also help to provide a deeper understanding of how resistance genes may persist and disseminate within integrated livestock production systems. This understanding can help producers implement targeted, practical solutions to mitigating resistance development and persistence within the context of comprehensive food safety and public health systems.

Figure 1. Biplot ordination of samples (dots) from beef (black), poultry (green), pork (red) and human WWTP (blue) samples. Results show significant separation of these samples, indicating that the resistome composition differs between systems. The resistance mechanisms listed were crucial in differentiating resistomes from one another; the closer the label is to a sample (dot), the more closely associated that resistance mechanism was with the sample. Thus, e.g., macrolide efflux pumps were closely associated with WWTP samples.