**BACKGROUND AND MOTIVATION**

Farm-to-fork: is the agricultural setting a key control point for ARG propagation and dissemination?

New mechanisms of resistance can emerge due to selective pressures. For example, the livestock industry is the suspected source of a highly mobilized colistin-resistance gene.1

Each year, at least 2 million people in the U.S. are infected with antimicrobial-resistant bacteria and at least 23,000 people die as a direct result of these infections.2

Up to 28% of E. coli isolates from retail meat carried plasmid-mediated colistin-resistance in a survey conducted in China.3

20% of hospital-acquired infections are from multidrug-resistant phenotypes.1

**QUANTITATIVE METAGENOMIC APPROACH**

**Hypothesis:** Read-level quantification and gene-level annotation will give the most reliable quantification of antimicrobial resistance genes (ARGs).

**DNA Extraction and Sequencing**

- Sampling: 1-hour composite samples were taken from three farms before and after manure treatment (Figure 1).
- Extraction: Duplicate extractions of each composite sample were performed using Power-Fecal Kit then pooled.
- Genomic Spike: A previous study revealed variability in DNA recovery between replicate extractions (Figure 2). DNA extracts were spiked with Marinobacter equilibriae DNA (1% final conc. by mass) to avoid extraction bias and variability.
- Sequencing: PCR-free libraries were prepared then sequenced, one sample per lane, on an Illumina HiSeq 4000.

**Read-Level Quantification**

- Map Reads: Bowtie2
- Calculate \( n_f = \frac{G_{IS}}{V} \times G_{IS} \)

**Gene-Level Annotation**

Using paired reads, Satinsky et al.4 attained only 61.90% protein identity in abundant taxa. Reads in this study will be assembled and binned to increase the robustness of the ARG alignment and annotation.5 The Resfam6 database will be used to identify resistance genes in the metagenome.

**Next Steps:** Evaluate the elimination of ARG in dairy manure treatment.

The DNA forms encoding ARGs: e.g., extracellular DNA (eDNA) or intracellular DNA (iDNA), determine the gene’s fate. Clinically relevant and abundant ARGs identified from the sequencing effort will be quantified using qPCR in iDNA, eDNA and viral DNA fractions throughout manure treatment.

**Extracellular ARG** may be ‘vertically’ transferred to daughter cells. Extracellular plasmid ARG may be horizontally transferred via conjugation.

**Virus-Associated ARG** may spread to bacteria through transduction.

**Metagenomic approach for quantifying a diverse range of antimicrobial resistance genes (ARGs) in environmental reservoirs**

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**References**

1. Satinsky et al. (2015). Quantitative Metagenetic Method

Satinsky et al.1 spliced internal standard (IS) DNA as shown in the schematic below for absolute gene quantification from a metagenome.

The recovered 15 genes were quantified by the number of sequence reads mapped to the protein-encoding genes in the IS genome. A normalization factor was defined to convert the abundance of any gene to absolute copy number per volume as shown in the following equations.

**Quantitative PCR Validation**

ARGs of clinical and/or veterinary relevance will be quantified in the same DNA extracts using qPCR.

**Quantitative Absolute Calculation**

\[ n_f = \frac{G_{IS}}{V} \times G_{IS} \]

\( V \) = Total volume sampled

\( G_s \) = Genome Copies added to sample before lysis

\( G_r \) = Rel. abundance of IS genome recovered

\( G_i \) = Rel. abundance of gene of interest

\( G_a \) = Copies of gene of interest per volume of sample

**Table 1:** Calibration curve with unamplified and assembled reads

<table>
<thead>
<tr>
<th>% IS Genome in DNA extract</th>
<th>% Reads Aligned to IS</th>
<th>% Reads Aligned to IS bin (after assembly)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 %</td>
<td>0.1 %</td>
<td>0.075 %</td>
</tr>
<tr>
<td>1 %</td>
<td>0.83 %</td>
<td>0.73 %</td>
</tr>
<tr>
<td>10 %</td>
<td>9.75 %</td>
<td>9.20 %</td>
</tr>
</tbody>
</table>

**Figure 2:** Extraction recovery of Pseudomonas aeruginosa (PSA) spiked into liquid slurry and compact samples ranged from 50-70%.

**Figure 3:** Coverage of Marinobacter lin observes an IS (%) replication.

**Figure 4:** PI reads aligned to IS bin using Amplification.