Fate of Antibiotic Resistance Genes in Dairy Manure Treated by Anaerobic Digestion and Composting

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Antibiotic resistance genes (ARG) are genes naturally present in bacterial populations and give bacteria the ability to resist the effects of an antibiotic. Antibiotic-resistant bacteria (ARB) can pass their ARG on to their offspring, increasing the abundance of ARB populations over time. ARG are also passed from ARB to unrelated bacteria through ‘horizontal transfer’. Horizontal transfer of ARG from one bacteria type to another can occur in human and in cow digestive tracks, manure, anaerobic digesters, wastewater treatment plants, soil, and water. Refer to the Frequently Asked Questions on Antibiotic Resistance and Dairy Production for more information on antibiotics, ARB, and ARG. A full list of antibiotics labeled for use on dairies can be referenced in Part 1 of Antibiotic Residues in Dairy Manure fact sheet series.

Fate of ARG in Anaerobic Digestion Systems

In general, anaerobic digestion (AD) systems modestly reduce ARG depending on the operating temperature and the specific ARG. Tetracycline resistance genes have not been shown to reduce during AD due to their persistent selective pressure, except possibly at thermophilic (130°F) conditions. Certain other ARG, including sulfonamide resistance genes, have been shown to significantly decrease after AD of cow manure at mesophilic (100°F) conditions. One study found that 11 ARG targets present in the raw manure were lower in the digestate, but only significantly for two of the ARGs.

The digester microbial community changes that occur at higher operating temperatures are presumed to facilitate horizontal transfer of some ARG from manure bacteria to digester bacteria. More research is needed to understand the fate of ARG under varying digester operating conditions.

Fate of ARG in Composting Systems

Many ARG have been shown to persist in finished cattle manure composts, with over 50 detectable in surveys. Cow manure composts at thermophilic conditions have been found to reduce ARG more significantly than composts not exceeding mesophilic conditions. In a study of windrow-composted cattle manure, certain ARG types increased over a 126-day period while most tetracycline and erythromycin ARG levels remained stable.

As observed with AD systems, composts at thermophilic conditions were more capable of reducing ARG than composts remaining at mesophilic conditions. For example, composts at thermophilic conditions were shown to lower normalized levels of tetracycline, sulfonamide, and fluoroquinolone resistance genes by nearly 2 orders of magnitude; however, some ARG remained. Other variables impact the fate of ARG in composted manure, including concentration of residual antibiotics, presence of mobile genetic elements, and content of heavy metals and nutrients.

An on-farm study of a manure treatment system consisting of solid-liquid separation (SLS) and rotary drum (RD) composting of separated solids (SS) measured four ARGs in samples collected over a two-day period at the points indicated (Figure 1). The four ARGs analyzed were:

- **sul1**: associated with sulfonamide resistance
- **blaOXA-1**: associated with β-lactam resistance
- **intI1**: associated with gene transfer
- **tetO**: associated with tetracycline resistance

While 70% or more of the sul1, blaOXA-1, and intI1 gene copies partitioned into the SL, tetO behaved differently with 8 times more copies partitioning into the SS than into the SL. Each of the four ARGs were significantly reduced after the RD composting process of SS with a
98% measured reduction in tetO gene copies. ARG measured results at the sampling locations are shown in Table 1.

<table>
<thead>
<tr>
<th>Sample location</th>
<th>tetO</th>
<th>sul1</th>
<th>blaOXA-1</th>
<th>intI1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>247,000 ± 150,000</td>
<td>49,500 ± 3,680</td>
<td>12,000 ± 2,640</td>
<td>41,000 ± 10,300</td>
</tr>
<tr>
<td>Separated liquids</td>
<td>12,100 ± 14,700</td>
<td>41,000 ± 9,280</td>
<td>12,800 ± 6,240</td>
<td>31,100 ± 7,310</td>
</tr>
<tr>
<td>Separated solids</td>
<td>94,700 ± 28,400</td>
<td>10,700 ± 1,840</td>
<td>2,050 ± 752</td>
<td>13,000 ± 4,770</td>
</tr>
<tr>
<td>RD treated solids</td>
<td>1,620 ± 1,500</td>
<td>2,640 ± 600</td>
<td>256 ± 215</td>
<td>5,180 ± 2,560</td>
</tr>
</tbody>
</table>

Fate of ARG in the Agroecosystem

Background levels of antibiotic resistance in the dairy agroecosystem are not yet well understood. The assessment of baseline levels of ARG in dairy manure from broader sampling, and how those levels change over time in response to farm management, antibiotic usage, and manure treatment, is needed to accurately measure the impact of treated and untreated manures on soils, crops, and the broader environment.

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Figure 1. Schematic of solid-liquid separator and rotary drum composting system (blue), plumbing and conveyors (black arrows), and material flows (grey). Yellow stars indicate sample locations.