Project Title: Horizontal Gene Transfer of Antimicrobial Resistance in Commercial Cattle Production Environments

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Background

3rd-generation cephalosporins (3GCs) are the preferred treatment for serious human *Salmonella enterica* (hereafter *Salmonella*) infections. Currently, 9% of the 1.2 million *Salmonella* infections each year in the U.S. are attributed to beef. Food and Drug Administration (FDA) monitoring of ground beef suggests that beef is not a major source of 3GC-resistant *Salmonella*. However, the Centers for Disease Control and Prevention (CDC) recently concluded that beef is likely a major source of human 3GC-resistant *Salmonella* infections.

Commensal 3GC-resistant *Escherichia coli* are more frequently isolated than 3GC-resistant *Salmonella* in cattle production environments and are theorized "reservoirs" of 3GC resistance. The reservoir designation is primarily based on the isolation of *Salmonella* and *E. coli* harboring incompatibility type A/C2 (Inc A/C2) plasmids with very similar genetic structures including the bla_{CMY-2} gene that confers 3GC-resistance. These Inc A/C2 plasmids contain genes that facilitate the exchange of large fragments of genetic material between different species of bacteria between bacterial species, a process known as horizontal gene transfer. The horizontal gene transfer frequency of clinically important antimicrobial resistance genes into human pathogens in cattle production environments is unknown. Regardless, some scientific journal articles have declared animal feeding operations "horizontal gene transfer hot-spots".

U.S. public health agencies have declared an urgent need to improve the understanding the occurrence of 3GC-resistant *Salmonella* in cattle production environments. Despite its extreme importance the factors contributing to the occurrence of 3GC-resistant *Salmonella* in ground beef and at cattle production sites are poorly understood.

The objective of this research was to determine the relative contribution of horizontal gene transfer from the “3GC-resistant *E. coli* reservoir” to the occurrence of 3GC-resistant *Salmonella* at two beef cattle feedyards. To achieve this goal several stages are required. First, the levels of generic *Salmonella* (defined as *Salmonella* regardless of antimicrobial resistance status), 3GC-resistant *Salmonella,* and 3GC-resistant *E. coli* at two beef cattle feedyards over two-years were determined. Second, 3GC-resistant *Salmonella* and 3GC-resistant were PCR screened to determine the presence of the two prevalent 3GC resistance conferring genes, bla_{CMY-2} and bla_{CTX-M}. Third, whole genome sequence (WGS) will be obtained for representative isolates to identify predominant strains. Finally, horizontal gene transfer rates from representative predominant 3GC-resistant *Salmonella* and 3GC-resistant *E. coli* strains to predominant generic *Salmonella* will be determined in laboratory experiments to compare to the observed populations in commercial cattle feedyards.

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Methodology

Two beef cattle feedyards were enrolled in the study. One feedyard was located in Nebraska (NE) and the other feedyard was located in Texas (TX). At each feedyard five nonadjacent pens were included in the study. Pen surface material (defined as the soil-manure mixture present on the pen surface) was obtained from each of the 10 study pens for consecutive 24 months. During each sample occasion, each pen was divided into 4 quadrants and a pen surface material sample was obtained from each quadrant for a total of 4 samples per pen. A total of 957 samples were cultured to detect and quantify generic *E. coli*, 3GC-resistant *E. coli*, generic *Salmonella*, and 3GC-resistant *Salmonella*. For each positive sample, between 2 and 4 3GC-resistant *Salmonella* and 3GC-resistant *E. coli* colonies were PCR screened to determine the presence of $bla_{\text{CMY-2}}$ and *blaCTX*-M genes. For each month and pen, $bla_{\text{CMY-2}}$ 3GC-resistant *E. coli* and $bla_{\text{CTX-M}}$ 3GC-resistant *E. coli* colony counts were estimated.

At each location the pens with highest and lowest levels of 3GC-resistant *Salmonella* were identified. For these pens (NE pens A & D; TX pens C & E) for each of 24 sampled months three isolates of generic *Salmonella*, 3GC-resistant *Salmonella*, $bla_{\text{CMY-2}}$ 3GC-resistant *E. coli*, and $bla_{\text{CTX-M}}$ 3GC-resistant *E. coli* were obtained from independent samples if available. Additionally, for the remaining pens (NE pens B, C, & E; TX pens A, B, & D) for each of 24 sampled months three isolates of 3GC-resistant *Salmonella* were obtained from independent samples if available. Antimicrobial susceptibility testing of selected *E. coli* and *Salmonella* isolates was performed with the Sensititre broth microdilution system. Genomic DNA was prepared using the QIAamp DNA Mini Kit. Genomic DNA libraries were sequenced on a NextSeq 550 platform (Illumina) Currently, 96 whole genome sequences have been obtained. Genomes were assembled using Shovill version 1.0.4 pipeline. Assembled genome sequences were annotated for serotype, multi-locus sequence type (MLST), plasmid incompatibility (Inc) group, and antimicrobial resistance genes.

Findings

All 684 3GC-resistant *Salmonella* PCR screened in this study harbored $bla_{\text{CMY-2}}$ while none harbored $bla_{\text{CTX-M}}$. However, $bla_{\text{CTX-M}}$ predominated 3GC-resistant *E. coli* both at NE and TX (Figures 1 and 2). This finding was important since it indicates that at both locations the dominant 3GC-resistant *E. coli* population (harboring *blaCTX*-M) was not responsible for horizontal gene transfer of 3GC resistance to *Salmonella*. However, this result in itself did not eliminate the possibility that the minority $bla_{\text{CMY-2}}$ *E. coli* population was maintaining 3GC resistance in *Salmonella* by horizontal gene transfer.

Additional insights into the factors contributing to the persistence of 3GC resistance in *E. coli* and *Salmonella* was and will be obtained by whole genome sequencing (WGS). Presently, high quality WGS sequence has been obtained for a total of 96 isolates, all from NE pen A and equally split (24 each) between generic *Salmonella*, 3GC-resistant *Salmonella*, $bla_{\text{CMY-2}}$ 3GC-resistant *E. coli*, and $bla_{\text{CTX-M}}$ 3GC-resistant *E. coli*. So far, 23 of the 24 generic *Salmonella* isolates are a single subtype, Muenchen/Virginia ST83. The remaining isolate was a pansusceptible Montevideo ST138. For 3GC-resistant *Salmonella*, 23 of 24 isolates were a single subtype, Montevideo ST138. The remaining isolate was a Muenchen/Virginia ST83. All of the 3GC-resistant *Salmonella* Montevideo ST138 harbored an Inc A/C2 plasmid. Since 23 of the 24 3GC-resistant *Salmonella* were the same subtype (Montevideo ST138), with the same plasmid Inc (A/C2), this strongly suggests that 3GC-resistance is maintained in this pen by persistence of this subtype.

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Implications

The preliminary results indicate that persistence of specific *Salmonella* subtypes harboring the *bla*$_{CMY-2}$ gene is the predominant mechanism driving the occurrence of 3GC-resistant *Salmonella* in cattle feedyards, while horizontal gene transfer is a minor contributor. Once whole genome sequencing of the NE feedyard isolates is complete isolates will be selected for laboratory horizontal gene transfer experiments to complement the "real world" results. Then the TX feedyard isolates will be whole genome sequenced followed by laboratory horizontal gene transfer experiments with the dominant strains. This will provide the most detailed examination of naturally occurring 3GC-resistant *E. coli* and *Salmonella* populations at cattle feedyards. This will be a powerful resource for providing empirical answers to conjecture pertaining to the factors contributing to the occurrence of 3GC-resistant *Salmonella* at cattle feedlots.

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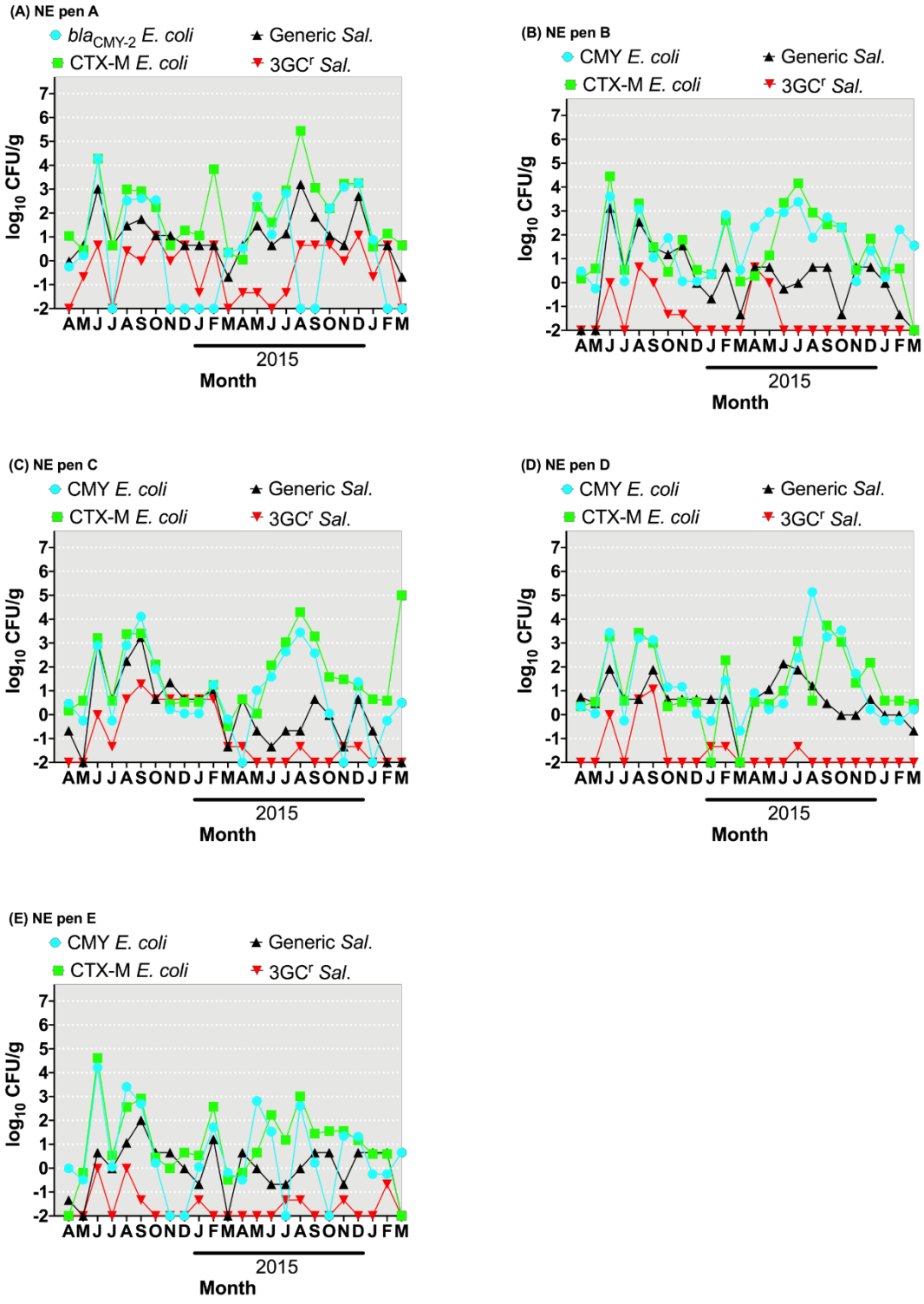

Figure 1. Nebraska (NE) feedyard mean colony counts of *bla*$_{CMY-2}$ third-generation cephalosporin-resistant *Escherichia coli* (cyan circles), *bla*$_{CTX-MY}$ third-generation cephalosporin-resistant *E. coli* (green squares), generic *Salmonella* (black triangles), and 3GCr *Salmonella* (red triangles). (A) pen A, (B) pen B, (C) pen C, (D) pen D, (E) pen E.

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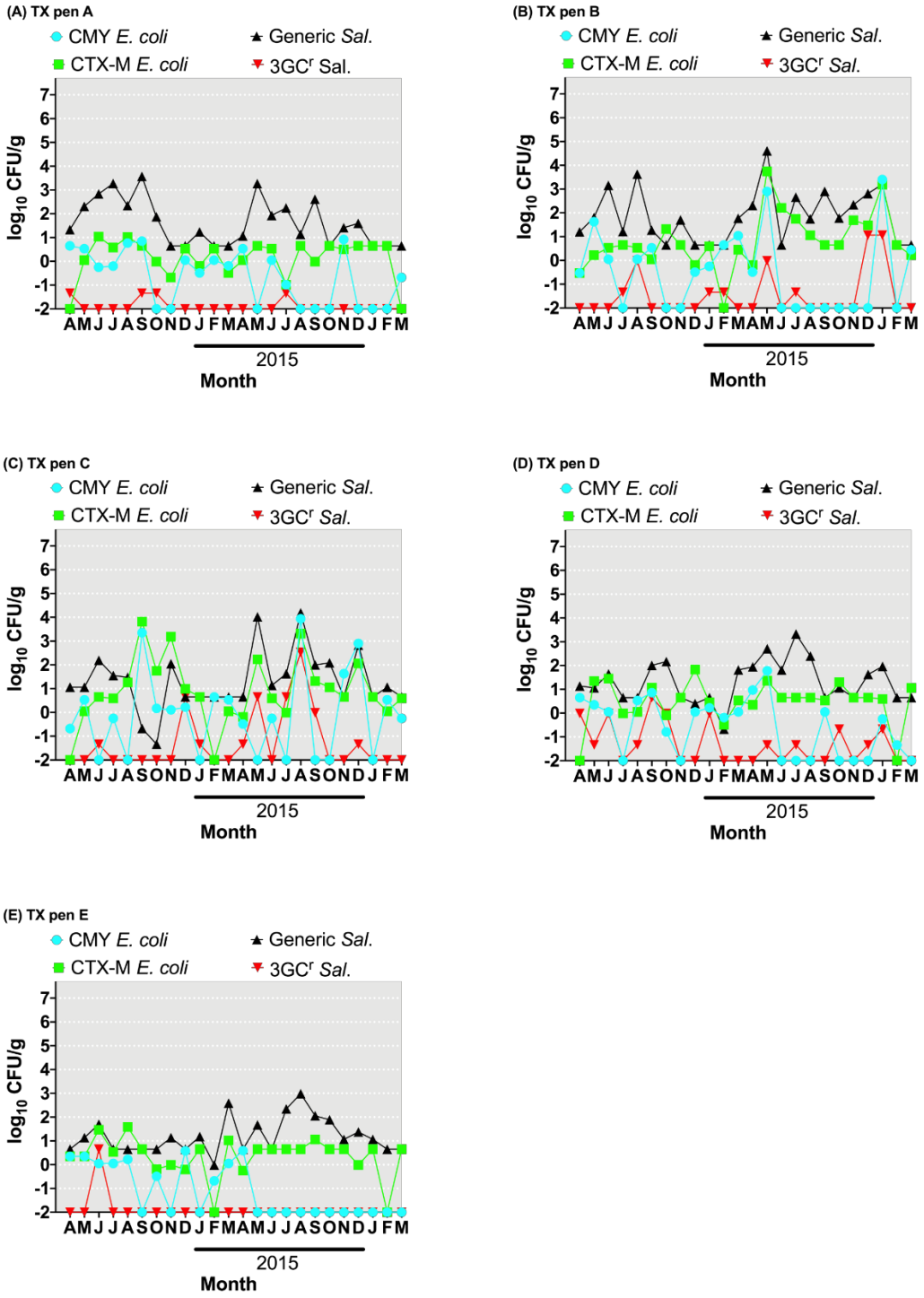

Figure 2. Texas (TX) feedyard mean colony counts of *bla*$_{CMY-2}$ third-generation cephalosporin-resistant *Escherichia coli* (cyan circles), *bla*$_{CTX-M}$ third-generation cephalosporin-resistant *E. coli* (green squares), generic *Salmonella* (black triangles), and 3GCr *Salmonella* (red triangles). (A) pen A, (B) pen B, (C) pen C, (D) pen D, (E) pen E.

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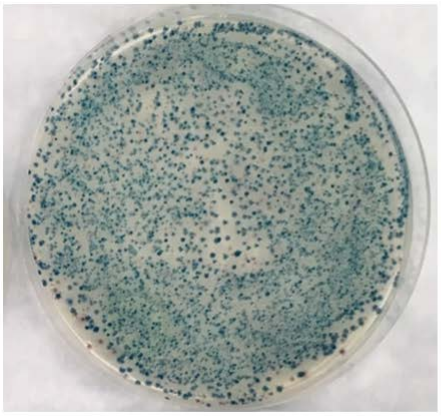

Figure 3. Third-generation cephalosporin-resistant *Escherichia coli*.

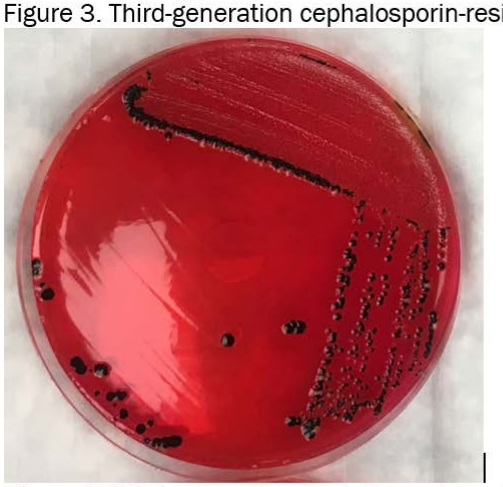

Figure 4. Third-generation cephalosporin-resistant *Salmonella enterica*.

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