**Project Title:** Ecology and Transfer of Antimicrobial Resistant Bacteria and Genes in the Feed-yard and Land-application Environment When Cattle are Fed Different Antibiotics

**Principle Investigator(s):** Ece Bulut[1], Xu Li[2], Galen Erickson[3], Amy Schmidt[3], John Schmidt[4] and Bing Wang[1]

**Institution(s):** [1]University of Nebraska Lincoln, Food Science and Technology
[2]University of Nebraska Lincoln, Civil Engineering
[3]University of Nebraska Lincoln, Animal Science
[4]USDA, Agricultural Research Service, Roman L. Hruska US Meat Animal Research Center (USMARC)

**Completion Date:** June 2018

## Background

Antimicrobial resistance has been recognized as one of the top human health concerns globally, by compromising effective treatments of illnesses in an increasing magnitude. One of the potential critical factors affecting human health is the spread and positive selection of resistance in the environment through manure storage and its application on crop lands, as manure is the major vehicle introducing antibiotic resistance from animals to the environment. However, there is a gap in our knowledge regarding the successful transfer of resistant bacteria from animals to humans via environmental pathways, due to complexity of environmental reservoirs, ecosystem and genetic selection process, as well as uncertainty in survival, acquisition and spread of resistance. Data collection on antimicrobial resistance transferred through beef cattle manure, especially on a quantitative basis, will allow for the assessment of the impact of antibiotic use during beef cattle primary production on the environmental quality and subsequently public health risks imposed by the environmental exposure.

The long-term goal of this project was to enhance antibiotic stewardship by managing the transmission of livestock-originated antibiotic resistance, especially through environment pathways. To achieve the long-term goal, tasks for following specific objectives were conducted:

Objective 1: Evaluate the impact of antibiotic uses on the development of antimicrobial resistance in the beef cattle production environment; and

Objective 2: Investigate the transfer of antimicrobial resistance during primary production to environmental compartments through manure land application on soil.

## Methodology

Three stages were included in this study: beef cattle production (6 months), manure storage as stockpiles (3 months) and manure land application (3 months). During the beef cattle production stage, 96 beef cattle were randomly assigned to three treatment groups: control group for no antibiotic use, Tylan group for tylosin treatment and CTC group for chlortetracycline treatment, with eight steers per pen and four pens per group. Tylan pens were fed ~90mg/day continuously. CTC pens had pulse doses of CTC once in February 1-6, 2017 and



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once in March 1-6, 2017 at 10mg/lb- cattle. Locations of different treatment pens were shown in Figure 1. When animals reached their market weights, manure on each pen surface was scraped and stockpiled by pens on areas with no history of manure application.

Manures from pens in the same treatment group were piled adjacent to each other to form a continuous windrow to represent the size and efficacy of manure stockpiling in reality. After 3-month storage as stockpiles, manure was applied on the land with no history of manure application. Four sites for land application (3X8 m2 each) were chosen, with each divided into 4 smaller plots (2X3 m2 each). Three sites were applied with the manure from different antibiotic treatments (Control, Tylan and CTC). The fourth site represented the baseline soil sampling site without any manure application.

During the cattle production stage, hide sponge samples, fresh rectal feces and aerosol samples at both upwind and downwind directions of feedlot pens were collected at the beginning and end of treatment period, respectively.

During the manure stockpiling stage, manure sampling was done on day 0, 3, 6, 9, 14, week 3, 4, 6, 8, and 12. Aerosol sampling was done on day 0, week 4, 8 and 12. At the manure land application stage, amended soil samples at three treatment sites and the baseline soil site were collected on day 0, 3, 6, 9, 14, week 3, 4, 6, 8, and 12. Aerosol sampling was done on day 0, week 4, 8 and 12. Example aerosol sampling setting is shown in the photo.

All samples were subject to microbiological tests for the presence/absence and enumeration in the positive samples of generic *E.coli*, tetracycline resistant *E.coli*, macrolide resistant *E.coli*, *Enterococcus spp*., tetracycline resistant *Enterococcus*, macrolide resistant *Enterococcus*, *Salmonella enterica*, tetracycline resistant *Salmonella*, and macrolide resistant *Salmonella.* Presumptive positive isolates were confirmed by MALDI-TOF mass spectrometry at the Nebraska Veterinary Diagnostic Center.

## Findings

Generic, tetracycline or macrolide resistant *Salmonella* were not identified in any samples during this project, including cattle production, manure stockpiling and manure land application. Hence, microbiological test results in this study include data only for *E. coli* and *Enterococcus*.

Primary production stage – fecal and hide samples

In general, generic, tetracycline and macrolide resistant *E.coli* and *Enterococcus* can be found in the majority of fecal and hide samples at both the beginning (prevalence equal to 100% for all sample types and treatment groups) and the end of treatment samplings (prevalence ranging from 75-100%). Among samples that are enumerable, rectal fecal samples harbor higher level of *E.coli* and *Enterococcus* and their resistant counterparts than the hide samples. Within each sample type, the contamination load in various sample types in general follow an order of generic bacteria > tetracycline resistant ones ≥ macrolide resistant ones. However, there was not statistically significant difference in the microbiological contamination levels between antibiotic treatment groups.

Manure stockpiling stage – manure samples

During the 3-month period of manure storage as stockpiles, the prevalence of generic *E.coli* and *Enterococcus* dropped from 100% to around 25-50%, and the concentration in



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enumerable samples dropped from ~5log$_{10}$CFU/g to 2- 3log$_{10}$CFU/g. Resistant bacteria started from a level lower than the generic bacteria and by the end of the storage period there was no resistant *E.coli* or *Enterococcus* detected or enumerated in almost all samples. In addition, macrolide resistance was reduced to a non-detectable level sooner than tetracycline resistance. There was not significant difference in the decreasing trend between manure stockpiles from different antibiotic treatment groups. Results are detailed in the full project report.

Manure application stage – amended soil samples

At the beginning of application, generic *E.coli* and *Enterococcus* were detected from 75%-100% of the amended soil samples at a level of 2-3log$_{10}$CFU/g. After the first week, no generic *E.coli* was detected and after the third week, no *Enterococcus* was detected in the samples. Tetracycline resistant *E.coli* or *Enterococcus* can be detected in 25-50% of samples from different treatments, but none can be enumerated at the beginning of land application. No macrolide resistant *E.coli* or *Enterococcus* was detected in any samples throughout this stage. There was not significant difference in the trend between soil samples amended by manure from different antibiotic treatment groups. Results are detailed in the full project report.

All stages – aerosol samples

In general, no *E.coli* or *Enterococcus* was detected in the upwind samples throughout all study stages, but some samples collected in the downwind direction contained the target bacteria at a relatively low level (1-800CFU/1000L air volume). Note that adults breathe around an average of 500ml of air in one normal breathing. A higher level of bacteria in the aerosol was observed in the downwind of manure stockpiles (~400CFU/1000L) than feedlot pens (<250CFU/1000L) or land application sites (<30CFU/1000L). *Enterococcus* were more likely to be observed in the aerosol samples. The only detected resistance was tetracycline and macrolide resistant *Enterococcus* at the downwind direction of stockpiles (<75 CFU/1000L). No resistant bacteria of interest was detected at the feedlot or land application sites.

Similar to other sample types, there was not significant difference in the bacteria detection between treatment groups. Results are shown in Table 2, 8, 15 and 20 in the full project report.

## Implications

The use of Tylan or chlortetracycline at the cattle feedlot in this study did not significantly increase the within-herd prevalence or concentration of macrolide or tetracycline-resistant *E. coli, Salmonella* or *Enterococcus* in feces or on hides. Effective manure management is helpful in reducing both overall bacteria and the antibiotic resistant ones, which can subsequently help eliminate the transmission to croplands. Based on this study, airborne transmission of macrolide and tetracycline resistant *E. coli*, *Salmonella* and *Enterococcus* from the beef cattle feedlot, manure storage and land application sites is limited.



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Figure 1. Rectangular areas shown in red are the pens of treatment animals. Remaining pens in UNL ENREC (previously ARDC) were filled with Tylan fed cattle.

Image 1. Impinger and impactor systems placed approximately 50m downwind from the manure stockpile from cattle fed with chlortetracycline for bioaerosol collection.



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