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Project Title: **Characterization of Enterococci, *Salmonella* spp., and Generic *Escherichia coli* Isolated from the Feces of Cattle Fed Rations With and Without Tylosin Phosphate**

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Objective: The use of antimicrobials drugs (AMD) in animal production has been criticized because of their suspected contribution to antimicrobial resistance. Specific to beef cattle, the use of tylosin phosphate, a macrolide, in the rations of finishing cattle to control liver abscesses has been the subject of extensive scrutiny. As macrolides are considered a critically important AMD, efforts to understand and mitigate macrolide resistance are imperative. Thus, the objective was to evaluate differences in the prevalence and antimicrobial susceptibility of *Salmonella* spp., generic *Escherichia coli,* and Enterococci isolated from the feces of feedlot cattle fed finishing rations with and without tylosin.

Experimental Design & Analysis:

A feedyard in Colorado cooperated in sourcing and procuring cattle for sampling. Pens of crossbred cattle representing conventional and "natural" production systems were identified after feedlot arrival (n = 8 pens/ system; n = 2,210 conventional and 1,656 "natural"). Cattle were processed and managed identically with the exception of tylosin utilization—only cattle in the conventional pens were supplemented with tylosin. Approximately 12 weeks after arrival, fecal samples were collected from the floors of each pen. Approximately 25 g of composited fecal sample from each pen was diluted with 225 ml of tryptic soy broth (TSB) for enumeration and enrichment. The remaining fecal sample was frozen and stored for later metagenomic analyses. Samples for enumeration were diluted and plated onto Enterococcosel (EC) or MacConkey (MC) agars and incubated for 24 (MC) or 48 (EC) h at 43°C before enumeration of Enterococci or *E. coli* colonies. Samples for enrichment were incubated at 37°C for 24 h before

plating onto EC or MC agars and incubation as described above for isolation of Enterococci and *E. coli*. Additionally, samples for *Salmonella* isolation were further enriched in tetrathionate (TT) or Rappaport-Vassiliadis (RV) broths at 43°C for 24 h. Following secondary enrichment, RV and TT samples were plated onto xylose-lysine-tergitol-4 (XLT-4) and brilliant green sulfa (BGS) agars and incubated at 43°C for 24 h. Representative colonies from EC, MC, XLT-4, and BGS agars were streaked, twice, onto selective agars and incubated as described above. Confirmation of isolate etiology was performed using standardized procedures. The susceptibility of isolates to AMDs, including macrolides, will be assessed in early 2016.

Key Results:

Preliminary data (Table 1) indicate similarity in the populations of generic *E. coli* in cattle feces ($P = 0.89$); however, the data suggest higher populations of Enterococci in fecal samples collected from cattle belonging to a "natural" production system. Conversely, the prevalence of *Salmonella* was higher in the pen fecal samples of cattle in conventional pens (25%) versus those in "natural" pens (0%). Although these data are preliminary, assessment of the antimicrobial susceptibility of isolates will be performed in early 2016.

How can this information can be applied in the industry?

These data were collected as part of a larger study intended to assess the influence of tylosin and cattle finishing location on AMR on isolated microorganisms, but also metagenomic evaluations of the resistome of fecal, liver, and environmental samples. Although the data presented in the current abstract are preliminary in nature, they do suggest gross differences in Enterococci isolated from the feces of cattle in conventional and "natural" production systems. As macrolide-resistant Enterococci from cattle are suspected in facilitating the co-selection of enterococci that are resistant to other macrolides (including erythromycin), these differences yield key insights into the influence production system (i.e. tylosin inclusion) on resistance acquisition. These data, when paired with the metagenomic evaluations of the resistome, will aid in determining the impact of macrolide use in beef production on the acquisition and expression of AMR determinants.

Table 1. Populations (log cfu/g) of Enterococci and generic *Escherichia coli* in the feces of feedlot cattle fed rations supplemented with and without tylosin phosphate.

Production System	Enterococci	Generic *Escherichia coli*
Conventional- with Tylosin	3.11	5.68
"Natural"- without Tylosin	3.79	5.79
P-value	0.05	0.89
SEM	0.33	0.45