

Title: Antibiotic resistance gene diversity and mobility in bacterial communities from post-weaning swine fed antibiotics or alternatives (NPB #18-184)

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Scientific Abstract: Antibiotic-resistant bacterial infections are a global health crisis, which has resulted in calls for improved antibiotic prudence both in human medicine and animal agriculture. One way to improve antibiotic prudence in agriculture is through the use of alternatives to antibiotics to improve animal health and decrease the need for antibiotic use pigs. In addition to testing the efficacy of non-antibiotic feed additives for improving animal health, evaluating the impacts of these additives on antibiotic resistance gene diversity in the gut bacteria is essential to defining whether the non-antibiotic additive is effectively mitigating the antibiotic resistance problem. Heavy metals, including therapeutic levels of zinc and copper, have emerged in the US as one alternative to antibiotics, though the impact on antibiotic resistance is not well defined. To examine the impact of therapeutic heavy metals on gut bacteria and antibiotic resistance genes, samples collected from two different animal trials experiments were assessed. For the first experiment, pigs fed an unamended diet, amended diet with chlortetracycline, or amended diet with zinc oxide and copper chloride. For the second experiment, pigs were fed an unamended diet, amended diet with chlortetracycline, or amended diet with zinc oxide. On day 14 (experiment 1) and day 23 (experiment 2), rectal swabs were performed for feces collection. Swabs were cultured in MacConkey media to enrich for *Enterobacteriaceae* and DNA extracted from bacteria. The DNA was sequenced using both short (n=54) and long (n=21) read technology to assess antimicrobial resistance and mobility gene profiles. No treatment effect was observed in the sequenced material, but the IncFIB, IncI1, and Col(MG828) plasmid replicons were present in a majority of samples (83%, 64%, and 60%, respectively). Antibiotic resistance genes present in a majority of samples included *tetB* (85%), *aph(3')-Ib* (74%), *mdfA* (74%), *aph(6)-Id* (72%), *blaCMY-2* (70%), *tetA* (64%), and *blaTEM-1B* (53%). The *blaCMY-2* gene co-occurred with the IncI1 plasmid replicon, which suggests this plasmid type may be of special interest when assessing the potential for resistance to beta-lactams. Additionally, DNA was extracted directly from feces to evaluate gene abundance by qPCR, and no differences were detected in gene abundance among treatment groups for the genes examined. However, these genes represent a small selection of genes responsible for antibiotic resistance and antimicrobial resistance gene transfer. Further work should examine different culturing techniques and *Escherichia coli* retrieval, as zinc may reduce *Escherichia coli* strain diversity.

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