PROGRAM & PROCEEDINGS

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disseminated primarily in the US, while blaNDM-1 has primarily disseminated in SE Asia. Because we have previously recovered enteric bacteria producing carbapenemase from wastewater treatment plant influent, and because wastewater is not sterilized during treatment, we hypothesized that enteric bacteria harboring plasmid-borne carbapenemase genes were discharged into the environment in effluent. During the summer of 2015, we collected samples weekly at the Jackson Pike Wastewater Treatment Plant in Columbus Ohio. Each week, one liter samples were collected pre-effluent, effluent, post-effluent, as well as upstream and downstream of discharge. Samples were filtered and enriched in nutrient broth with meropenem, incubated overnight then inoculated to MacConkey agar supplemented with meropenem to identify isolates expressing the carbapenemase phenotype. Carbapenemase-producing coliform bacteria we recovered from each sampling site, with prevalence rates ranging from 20% to 100%. The most common genotype was blaKPC although other genotypes were recovered. Our results indicate that carbapenemase-producing enteric bacteria are commonly disseminated into the environment from wastewater treatment plants. Humans, wildlife, companion animals, and livestock downstream from the discharge may be exposed and become colonized by these CREs in the environment, posing a risk to public and animal health.

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Effect of copper, zinc, and essential oil supplementation on antimicrobial resistance of fecal Escherichia coli in nursery piglets

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Copper, zinc, and essential oils are alternatives to antibiotics (ATA) that have been suggested to improve growth performance. The effects of these ATA on the resistance features of fecal Escherichia coli in nursery piglets have not been fully explored. Therefore, we compared the effects of in-feed copper, zinc, and oregano oil (alone, or in combinations) with those of in-feed low or high doses of chlortetracycline on antimicrobial resistance of fecal E. coli. Study consisted of 350 weaned piglets 21 days of age were randomly assigned to 70 pens (5 pigs per pen). On day 5, pens were randomly allotted to one of 10 treatment groups in a 2×2×2 (+2) factorial design with main effects of Cu (0 vs. 125 ppm Cu), Zn (0 vs. 3,000 ppm Zn from d 5 to 12 and 2,000 ppm Zn from d 12 to 33), and oregano oil (0 vs. 0.1%). Two additional treatment groups were fed sub-therapeutic or therapeutic levels of chlortetracycline (CTC; 55 or 441 mg/kg of feed). Fresh fecal samples were collected weekly over 42 days by gentle rectal massage from three pigs in each pen. Isolation and enumeration of E. coli was done by plating fecal samples from days 0 and 28, on MacConkey agar (MAC), MAC+ Tetracycline (16 µg/ml) , MAC+Ceftriaxone (4 µg/ml), and MAC+Cu (1 to 8 ppm) plates. A triplex PCR was done on E. coli isolates to detect tetA, tetB and blacmy-2 genes. Minimum inhibitory concentrations were determined by sensititer procedure. Whole genome sequencing was performed on a subset of isolates to compare genotypic data with phenotypic resistance. The data were analyzed using generalized linear mixed models (STATA MP v. 12.1). Copper, zinc, and oregano oil did not show any effects on expanding antibiotic resistance; in fact, copper fed alone had a sparing effect on multi-drug resistance and ceftiofur resistance. A majority of the isolates were resistant to tetracycline (99%) while 5.7% of the isolates were positive for qnrB gene and resistant to ciprofloxacin.

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Antimicrobial susceptibility of enteric Gram-negative facultative anaerobe bacilli in aerobic versus anaerobic conditions

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Purpose: Antimicrobial drug use in farm animals results in antimicrobial exposure of the host's enteric bacteria, some of which are potential foodborne pathogens. Assessing how this exposure impacts the enteric bacteria necessitates development of pharmacodynamic models of the drug action against the bacteria in the anaerobic conditions of intestine. The models require measurements of bacterial antimicrobial susceptibility, such as the minimum inhibitory concentrations (MIC) of the drugs. Currently, the measurements obtained in aerobic conditions in vitro are utilized. However, the Gram-negative bacilli among foodborne pathogens, Escherichia coli and Salmonella, are facultative anaerobes which experience physiological changes in anaerobic conditions. The objective of this study was to investigate differences in antimicrobial susceptibility under aerobic and anaerobic conditions of generic E. coli and Salmonella isolates from cattle feces. We focused on bactericidal antimicrobials and included all such drug classes used in cattle: cephalosporins, older β-lactams (aminopenicillins), aminoglycosides, and fluoroquinolones.

Methods: The susceptibility of each bacterial isolate to ceftriaxone, ampicillin, kanamycin, gentamycin, and enrofloxacin was measured using E-test method under aerobic conditions and anaerobic conditions following a 24-hour period of adaptation of the bacterial culture. A total of over 80 isolates of E. coli and Salmonella were tested.

Results: The susceptibility of E. coli and Salmonella isolates to aminopenicillins, aminoglycosides, and fluoroquinolones in anaerobic conditions differed from that in aerobic conditions. This was observed for the isolates susceptible (based on the clinical breakpoint interpretation) to the studied antimicrobials when tested in aerobic conditions.

Conclusion: The results demonstrated that for modeling and assessing the impact of antimicrobial use in cattle on their enteric bacteria that are Gram-negative facultative anaerobe bacilli, the measurements of bacterial susceptibility to the antimicrobials reaching the intestine may need to be obtained in anaerobic conditions.

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Comparing the resistome of poultry, swine, cattle and salmon production and nearby human waste water treatment plants

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Purpose:

Metagenomics holds immense potential for understanding how antimicrobial resistance (AMR) genes move within and between livestock

production systems and human habitats. The ability to interrogate the entire resistance potential of a given sample also enables comprehensive investigation of how different livestock production processes - including different antimicrobial use protocols - impact the risk of AMR transmission to humans.
Antimicrobial use patterns differ widely between poultry, swine, cattle and salmon production in North America. We hypothesized that the resistome of poultry, swine, cattle and salmon feces would differ and that these differences could inform design of waste management processes tailored to each production system. Furthermore, we hypothesized that the resistome of biosolids from human wastewater treatment plants (WWTPs) located near these facilities would differ from the feces of each commodity.
Methods:
To test these hypotheses, we collected composite fecal samples from large commercial poultry and swine barns, farmed salmon sea cages, and feedlot cattle pens; in addition, we collected treated biosolids from nearby human WWTPs. Total DNA from 5 samples from each site (total N = 25) was extracted and shotgun sequenced on the Illumina HiSeq. Reads were compared to a database of AMR gene sequences to characterize the resistome in each sample. Resistome composition was compared using non-metric multi-dimensional scaling ordination. Abundance of different AMR mechanisms and classes were compared between samples using zero-inflated Gaussian mixture models.
Results:
Shotgun sequencing produced ~5.5 billion reads across all 25 samples. Fewer than 5% of reads were removed due to low quality, and &gt 200 AMR genes across all samples. Resistome composition differed by commodity species, and the resistome of human WWTP samples clustered separately from all livestock samples.
Conclusions:
These results demonstrate that the fecal resistomes of chicken, swine, beef cattle and salmon differ significantly, suggesting that manure management systems may need to be tailored for each commodity system.

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Use of shotgun metagenomic to evaluate the microbiome in cattle feces following tulathromycin metaphylaxis

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Shotgun metagenomics, facilitated by next-generation sequencing, represents a novel approach to investigate microbial communities. The goal of this study was to use a metagenomic approach to understand the impact of metaphylactic tulathromycin exposure on the microbiome of cattle in the early feeding period. Two pens of cattle in a Texas feedlot were selected for this study. One pen was chosen to receive 800 mg of tulathromycin while the other was chosen for the control. Individual fecal samples from the rectum were collected at arrival processing and 11 days into the feeding period. Fecal samples from treated (n=30) and control (n=30) animals from both sampling times were subjected to DNA extraction for metagenomic sequencing. After sequencing, low quality sequences and bovine DNA were removed using Trimmomatic and Burrows-Wheeler Aligner softwares, respectively. Then, a taxonomic sequence classifier (Kraken) was used to assign taxonomic labels to non-bovine DNA sequences. Kraken aligns sequenced reads to the Reference Sequence database (National Center for Biotechnology Information, NCBI) and determines the bacterial composition of samples based on nucleotides matches. Next, the number of reads that map to different NCBI taxonomic labels were normalized to account for differences in sequencing depth across samples and get an estimate of the relative abundance of each taxonomy group. A non-metric multidimensional scaling ordination was used to determine whether the overall microbiome differed between groups of cattle. In order to identify specific taxonomic labels that were significantly different between groups, multivariate models were built using zero-inflated Gaussian mixture distributions. The proposed pipeline successfully characterized the microbiome in the feces of cattle with or without exposure to metaphylactic tulathromycin. However, more comprehensive genome databases are required to strengthen the classification of metagenomic reads. Scientists and industry partners need to join efforts to understand the use of metagenomics in food production environments and to develop a better understanding of the biological relevance of the metagenomic results.

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Characterizing variation in the microbial resistome between natural and conventional beef operations

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Routine antibiotic use has drawn criticism and led to the creation of alternative practices in food animal production, such as the “natural” label, which establishes guidelines for decreased hormone and antibiotic use. It is thought that less antimicrobial use will lead to less selective pressure for Antimicrobial Resistance (AMR) and result in a more secure food supply. However, direct characterizations of microbial populations in feedlots are rare, and more evidence is needed to support this claim. This study aims to characterize the microbial communities and resistomes in natural and conventional beef feedlots using metagenomics.
Composite fecal (N=12) and wastewater samples (N=13) were taken from four feedlots in Western Canada. One feedlot contained both conventional and natural cattle. Engineered wetland (N=1) and human wastewater treatment plant (N=6) samples were taken from the surrounding area. Total DNA was extracted and sequenced on the Illumina HiSeq platform. Low quality bases were removed using Trimmomatic. Host DNA was removed by filtering reads that aligned against the Bos taurus genome using BWA. The remaining reads were profiled by alignment against our AMR gene database using BWA. AMR gene composition was validated using Hidden Markov Models trained on our AMR database and applied to the raw sequencing reads. Microbiomes were profiled using Kraken and MetaPhlAn. The coverage of functional pathways was determined using HUMAnN2.