COMPARING FECAL AND MEAT RESISTOMES IN U. S. BEEF, PORK, AND BROILER PRODUCTION

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I. INTRODUCTION

Antibiotic resistance (AMR) is a global public health concern [1]. Use of antibiotics in food producing animals is a growing concern over the fear that administration of antibiotics could increase resistance in meat-borne pathogens, leading to untreatable human cases of infection [1]. To manage AMR risk, first there needs to be an understanding of the current state of AMR in food producing animals. While previous work has described different stages of food animal production, there has not been a study to understand the similarity between feces and meat from the same group of animals. The objective of this study was to understand if fecal and meat resistomes from the same groups of animals are correlated.

II. MATERIALS AND METHODS

Sample Collection. Sixty-one (N = 61) composite fecal samples were collected from the pen/barn/house floor of one geographical site of beef cattle (n =21), pigs (n = 20) and broiler chickens (n = 20) 24 hours before harvest. Following harvest at a commercial abattoir, meat trimmings from the same cohorts of animals were collected (n = 19 for beef and n = 20 for pork and chicken). All samples were shipped to Colorado State University (Fort Collins, CO) for storage at -80°C until further processing.

DNA Extraction to Sequencing. Ten grams of each fecal composite sample was thawed and DNA was extracted using the Mo-Bio PowerMax Soil DNA isolation kit (Mo Bio Laboratories, Inc.) Four-hundred grams of meat trimmings from each sample was rinsed with 90ml of buffered peptone water and the liquid was centrifuged (4°C for 10 mins at 4300 x g) to form a pellet to use in the Mo-Bio PowerMax Soil DNA isolation kit. Libraries were created using the SureSelect^{XT} Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library (Agilent Technologies) with the custom-designed MEGaRICH bait set described in Noyes *et al.* [2]. All libraries were sequenced on a HiSeq 2500 (Illumina) with 2 × 125 bp paired-end reads using HiSeq SBS Kit v4 reagents (Illumina) at a depth of 8 samples per lane.

Bioinformatics and Statistics. Raw fastq files were analyzed using AMR++ pipeline and MEGARes database [3] for AMR gene classification and quantification. Procrustes included in vegan R package (version 2.4-4) was used to compare congruence of the fecal resistome and the meat resistome ordinations based on $\alpha = 0.05$, correlation coefficient (r) and measure of fit (m^2) at the class and gene level.

III. RESULTS AND DISCUSSION

Sequencing. A total of 5.4 billion reads were sequenced with an average Phred score of 37.8. Filtering of host DNA resulted in an average removal of 0.10% of reads in feces and 95.87% in trim samples. Eighteen classes, 60 mechanisms, 236 groups, and 1025 genes associated with antibiotic resistance were identified across all samples. Overall, tetracycline was the predominant class of resistance across all feces, while predominant classes of resistance across meat samples were more varied (Figure 1). The alignment used in the AMRplusplus pipeline is not sensitive enough to detect single nucleotide polymorphisms (SNP). However, some of the genes that confer resistance do so via a point mutation. As a result, we have chosen to leave all presumptive AMR genes in the samples. While this inflates counts across samples in a uniform matter, Elfamycin and Rifampin resistance comprised 34% and 55%, respectively, of SNP confirmed genes, meaning these were likely inflated.

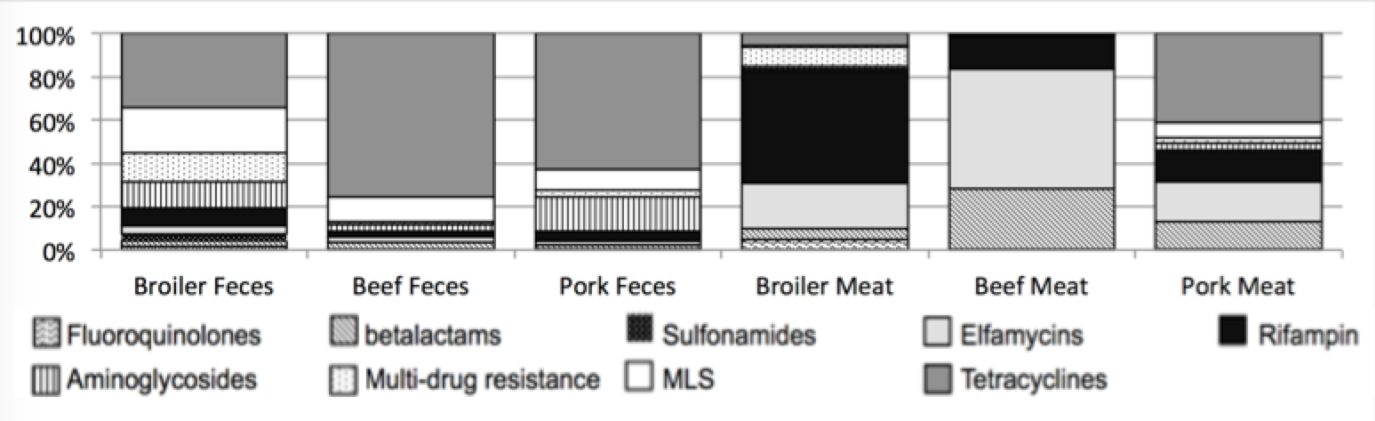

Figure 1. Proportions of class of antibiotic resistance by each sample group

Procrustes. When procrustes analysis was performed on each feces/meat pair within species, no significant correlations were found (Table 1). Hence, there was not a significant correlation between the fecal and meat resistome (i.e., they are not related). This dissimilarly indicates that a meat resistome is not related to the fecal resistome. Because of these differences, it is likely that fecal contamination is not a key component in the meat resistome; if it were, microbial communities would be more similar to each other.

Table 1. Procrustes statistics to determine whether antibiotic resistance genes in feces and antibiotic resistance genes in meat were correlated by superimposing the fecal resistome and meat resistome ordination plots at the class and gene level.

	Species	Parameters		
		r[1]	m2[2]	*P*[3]
Class Level	Beef	0.07	0.99	0.99
	Broilers	0.14	0.98	0.73
	Pork	0.29	0.91	0.33
Gene Level	Beef	0.24	0.94	0.58
	Broilers	0.09	0.99	0.88
	Pork	0.42	0.82	0.17

[1] r = coefficient of correlation between fecal resistome and meat resistome ordinations;
[2]m[2]: residual sum of squares after contrasting fecal resistome and meat resistome ordinations
[3]*P* = probability values for each procrustes comparison, significance at $P < 0.05$

IV. CONCLUSION

Fecal resistomes are more similar to each other between species than meat resistomes. Fecal and meat resistomes are not similar to each other, so fecal contamination on carcasses is likely not a major driver in meat resistome composition.

ACKNOWLEDGEMENTS

This work was funded by USDA NIFA grant 2015-68003-23048.

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