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A Comparison of the Resistome between Natural and Conventional Retail Ground Beef Products

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Objectives

The threat of antimicrobial resistance (AMR) in beef products is an emerging health issue and a driving force in consumer purchasing decisions. Analyzing retail ground beef products using culture-independent techniques allows for the investigation of AMR prior to consumption. The objective of this study was to characterize the resistome of retail ground beef products processed from conventionally and naturally raised cattle using whole-genome sequencing.

Materials and Methods

Samples of natural (n = 50, raised without antibiotics) and conventional ground beef products (n = 50) were purchased from various retailers throughout Fort Collins, CO. All samples were of different brands, packaging types, and lean points. Samples were processed 48 h following collection. 30-g aliquots of ground beef were washed with 100 mL phosphate-buffered saline (PBS) and the supernatant was centrifuged for 10 min at 10,000 × g. DNA was isolated using QIAGEN PowerFecal kit. A 515F/806R primer pair was used to amplify the V4 region of the 16S rRNA gene region of the isolated DNA. A subset of 16 samples (n = 8) from the original 100 were selected for resistome analysis. Libraries were built for the 16 samples using a customized bait-pulldown system (Agilent, SureSelect XT HS) targeting specific AMR genes within the MegaRes database. All libraries and 16S amplicons were sequenced on a HiSeq 4000 (Illumina) platform with 2 × 125 bp paired-end reads and sequenced across 8 lanes.

Results

Of the 100 samples subjected to 16S rRNA gene sequencing, 96 passed quality checks and were analyzed. Shannon's Diversity Index was used to compare α diversity between treatment groups and found no difference in microbiome α diversity (PERMANOVA, P = 0.13). Weighted UniFrac was used to compare β-diversity between treatment groups and samples of varying packaging types. Differences in β-diversity between conventional and natural products were found to be significant (PERMANOVA, P = 0.001). Furthermore, pairwise PERMANOVA comparisons suggest the microbial community of chub packaging differs from tray-overwrapped, vacuum sealed, and store ground product (P = 0.002, 0.001, 0.001, respectively). Similarly, tray-overwrapped product also differed from vacuum sealed samples (PERMANOVA, P = 0.003).

The 16 samples of ground beef collectively had hits to 12 classes, 23 mechanisms, 23 groups, and 105 gene accessions associated with antimicrobial resistance. An ANOVA test comparing resistance gene counts in natural and conventional products suggested no differences in AMR gene counts (P = 0.361). Furthermore, an Analysis of Similarity, which considers the ratio of α and β diversity of the samples, suggested that the resistome in natural and conventional retail products were similar to each other (ANOSIM, R = –0.0079). Among the gene accessions detected, the majority was associated with tetracycline resistance (84%). Other resistance gene accessions detected included macrolide-lincosamide-streptogramin (8%), elfamycin (2%), β-lactam (1.5%), rifampin (1%), and multi-drug (2%).

Conclusion

Among the 2 treatments, it appears that packaging type is the driving force behind the differences seen in the microbiome. The resistome of conventional and natural products were similar, suggesting that differences in production practices have little influence on contribution to antimicrobial resistance in retail ground beef products.