Colorado State University
Microbial Ecology Group
Colorado State University
Fort Collins, Colorado 80523
http://MicrobialEcology.colostate.edu / http://meg.colostate.edu

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Take-Home Summary: Discussion of research from the CSU Microbial Ecology Group

By Dr. Xiang Yang

Title: **Use of metagenomic shotgun sequencing technology to detect foodborne pathogens within microbiome in beef production.**
Authors: Yang X, Noyes NR, Doster E, Martin JN, Linke LM, Magnuson RJ, Yang H, Geornaras I, Woerner D, Jones KL, Boucher C, Morley PS, Belk KE.
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Lead author is Dr. Xiang Yang, postdoctoral fellow in the Department of Animal Sciences.

Principal investigators are:

- Dr. Keith Belk, MS, PhD, Professor of Meat Safety and Quality, Monfort Chair in Meat Science, Department of Animal Sciences, Colorado State University.
- Dr. Paul Morley, DVM, PhD, DACVIM, Professor of Epidemiology and Infection Control, Department of Clinical Sciences, Director of Infection Control, James L. Voss Veterinary Teaching Hospital, Colorado State University. Professor of Epidemiology, Colorado School of Public Health.

This is the first study showing quantitative changes in foodborne pathogens/indicators within their microbiome using a shotgun metagenomics approach.

- This study addressed a knowledge gap relating to use of metagenomics to detect foodborne pathogens in a longitudinal study of the beef production chain. We elaborate on the distribution and persistence of bacterial pathogens as beef moves through the supply chain using cattle traced and sampled as they moved through the system. To our knowledge, no published studies have quantified changes in pathogen populations in the context of the larger microbiome.

- Our study was the first to longitudinally follow the same cattle using shotgun metagenomics as they moved across time and space of the production supply chain. Sample points included cattle entry into the feedlot, as they exited the feedlot, the transport trucks, abattoir holding pens, and both product and equipment at the end of fabrication system as the beef moved towards ultimate consumers.
  - DNA read counts classified as pathogens per million reads for *Salmonella enterica*, *Listeria monocytogenes*, generic *Escherichia coli*, *Staphylococcus aureus*, *Clostridium* (*C. botulinum*, *C. perfringens*), and *Campylobacter* (*C. jejuni*, *C. coli*, *C. fetus*) decreased as cattle and beef moved through the production and processing system.
    - This result showed that total bacterial loads (based on genomic reads), including pathogens, were reduced as cattle were processed, which supported the effectiveness of using multiple sequential antimicrobial interventions in beef abattoirs.
  - Normalized read counts for *Salmonella enterica*, *Escherichia coli*, and *Clostridium botulinum* were greater in the final product (Market-Ready samples).

        - The sequences generated of Market-Ready beef samples yielded a majority (99%) that were classified as *Bos taurus*. As such, there is a large difference among the different sample matrices in the proportion of reads belonging to the bacterial microbiome relative to the total reads obtained from samples. To address this issue, we employed quantile normalization to investigate the change in proportion of these pathogens within the total microbiome, and also adjusted sequence depth based on the distribution of counts that were assigned to all bacteria within each sample.
        - Our results indicated that the proportion of these bacteria increased (the effect on absolute numbers was unknown) within the remaining microbiome. The unequal efficacy of antimicrobial interventions against pathogenic bacteria—either due to internalization or cross protection—provides a scenario in which the diversity of the microbiome, though shrinking, may result in higher relative abundance of *Clostridium botulinum* and *Salmonella enterica*.
        - These results should draw industry's attention to the need for concerted control of these specific pathogens/bacteria regarding to food safety.

- The assessment of using a metagenomics approach on pathogen detection in our study also identified several challenges in this analytic method.
    - One of the main challenges was identifying specific pathogens from which the sequence reads originated.
    - Moreover, misclassification was inherent to short read lengths, an inability to get deep coverage of DNA sequencing from the pathogenic organisms in the sample due to the existence of other prokaryote and eukaryote DNA within the sample, and the impossibility of obtaining a comprehensive database containing genetic sequences for all possible pathogens and bacteria.
        - Many bacterial whole genome sequences have been submitted to NCBI, but their accuracy is variable and any contamination in sequence data in the database can cause sequence misclassification. In long term, as we add more sequences to create a comprehensive database, it is necessary to evaluate the accuracy of these published genome sequences.
    - Government agencies, such as the Food Safety and Inspection Service (FSIS), have started to investigate use of sequence-based methods, including metagenomics, for pathogen detection. Our results strongly indicate that the shotgun metagenomic approach is not yet practically ready for pathogen identification for regulatory purposes.

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Ongoing research by the CSU Microbial Ecology Group is described here:

- http://meg.colostate.edu
- http://source.colostate.edu/csu-researchers-trace-superbug-genes-better-understand-antibiotic-resistant-germs