Characterization of the microbial ecology of two fresh pork cuts throughout a production schedule at a pork fabrication facility.

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Background

Recent outbreaks and regulatory initiatives have put the elimination of *Salmonella* at the forefront of food safety initiatives at meat and poultry processors. Common interventions used for reducing the overall load of *Salmonella* also have the potential to alter the overall microbial ecology of fresh pork. However, a baseline understanding of the inherent microbiome of fresh pork in the absence of chemical intervention and how it is conserved throughout a production day, week, or month is not well defined. The objective of this study is to assess the microbial ecology of two common fresh pork cuts across multiple timepoints within a production day, week, and month. Additionally, this study aims to determine if potential relationships exist between the microbial ecology of fresh pork to both the overall microbial load and presence of *Salmonella* in the meat.

Materials and Methods

Two common pork cuts representing trim used for comminuted pork (Bootjack Trim, “BJ”) and whole muscle pork tenderloins (“TL”) were sampled at the beginning, middle, and end of first shift at a major pork fabrication facility. Sampling occurred daily on Monday through Thursday and was conducted for two consecutive weeks during each of three consecutive months (June, July, and August). A 500 g sample of TL and BJ was collected at each sampling time point and subjected to culture-based analysis for aerobic plate count (APC) and presence and load of *Salmonella*; as well as rinsate collection for DNA extraction and subsequent 16S sequencing of the V3-V4 hypervariable regions. Sequence data were analyzed using the DADA2 pipeline. Primary analysis outcomes were APC counts, *Salmonella* presence/absence, and microbiome diversity and composition at the genus level. Differences in these outcomes by sample type (BJ versus TL) and time period (shift time, day, and month) were evaluated using multivariable models.

Results

Aerobic plate counts for the TL differed significantly between periods within a production shift, with the beginning of the shift having significantly higher APCs than samples taken at the middle or end of the production shift. Crude prevalence of *Salmonella* was 8.3% in BJ and 4.1% in the TL. *Salmonella*-positive samples tended to cluster around two processing windows of less than 3 days each, during the months of June and July. All positive samples from both the BJ and TL had a Salmonella load of less than 1 cfu/g. qPCR-generated copy numbers of the 16S gene were significantly higher in the BJ compared to the TL ($P = 0.02$), but both meat types had a low microbial load as evidenced by very low 16S qPCR copy numbers overall. Genus-level alpha diversity (richness and Simpson’s) was significantly different between BJ and TL samples, but not by shift time period or Salmonella status of the sample. Overall microbiome composition was significantly different between the two different meat cuts as measured by PERMANOVA ($P = 0.001$, $R2 = 0.054$) and also between shift time period ($P = 0.003$, $R2 = 0.021$), day of the week ($P = 0.001$, $R2 = 0.064$), and month ($P = 0.001$, $R2 = 0.029$). However, the total R2 for all statistically significant variables only explained 16.8% of the total variation observed in beta diversity.

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SafePork 2023: The 14th International Symposium on the Epidemiology and Control Biological, Chemical and Physical Hazards in Pigs and Pork, New Orleans, LA, United States, May 15–17, 2023
https://doi.org/10.31274/safepork.16344

Conclusions

The microbial ecology of both the BJ and TL was relatively consistent at the phylum level across all timepoints; however, sample-to-sample variability was relatively high, especially at higher taxonomic resolution such as the species and genus levels. Additionally, this variation was not well explained by the factors measured in this study, i.e., APCs, sample type, *Salmonella* status or time point. Furthermore, both the prevalence and load of *Salmonella* was low, which limited the ability to determine if relationships exist between the presence of *Salmonella* and the microbiome observed on each individual fresh pork cut. These results suggest that sampling time point might not be an important study design factor for future microbiome studies of fresh pork, however the temporal clustering of *Salmonella*-positive samples suggests that studies investigating microbiome-*Salmonella* associations may need to account for temporal variability in *Salmonella* prevalence. This is especially pertinent given the relatively low crude prevalence of *Salmonella* we observed in this study. Additionally, further work is needed to understand the drivers of the stochasticity that we observed in the microbiome.