The microbiome of *Escherichia coli* and culture-negative nonsevere clinical mastitis: Characterization and associations with linear score and milk production

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Introduction

Culture-negative and *Escherichia coli* cases are uncommonly treated in pathogen-based protocols for nonsevere clinical mastitis (CM). High-throughput sequencing might reveal missed treatment opportunities in regards to the presence of other pathogens and can provide information on microbial diversity. We wished to explore the milk microbiome and its association with milk production and postevent linear score (LS) for cows with CM characterized as negative or *E. coli* by culture. Our first objective was to explore the microbiome of cows with *E. coli* CM to determine if microbial profiles differ between cows that had positive (no drop in milk production or a next-test LS of <4.0) or negative (a drop in milk production or a next test LS of ≥4.0) outcomes when not treated. Our second objective was to explore the microbiome of cows with culture-negative CM to determine if microbial profiles differ between cows that had positive or negative outcomes. Characterizing these associations will improve our understanding of CM, allowing for more effective treatment strategies.

Materials and Methods

Fifty *E. coli*-positive and 35 culture-negative milk samples from CM cases were enrolled. No cases were treated. Follow-up samples were taken 14 d post-mastitis for the *E. coli*-positive quarters to determine chronicity. DNA extraction, isolation, and purification were performed on all samples followed by amplification of the V4 hypervariable region of the bacterial 16S rRNA gene using the Illumina MiSeq platform. Sequences were processed and taxonomy assigned through the QIIME2 software pipeline. Within-sample diversity or α-diversity was assessed using the Shannon index. Between-sample diversity or β-diversity was assessed through phylogenetic-based Unifrac distances; the matrix produced was used for comparisons between outcome groups. Spearman's rank correlation and Kruskal-Wallis tests were used to determine associations between α-diversity and outcomes, or between α-diversity and relative abundances of taxonomic families. For β-diversity, Permutational Analysis of Variance was used on Unifrac matrices to determine relationships to the outcomes. Analysis of Composition of Microbiomes (ANCOM) in QIIME2 was used to identify the operational taxonomic units driving the differences.

Results

On culture of the follow-up samples, no cases were positive for *E. coli*. A difference in α-diversity was present between enrollment and follow-up samples (3.8 vs 5.1). When α-diversity was explored for enrollment *E. coli* samples, there was no relationship between Shannon index and subsequent LS. There was a decrease in α-diversity as losses in milk production increased for *E. coli*-positive cows. This difference was contributed by a greater relative abundance of the family Enterobacteriaceae (67.8% vs 38.4%) for cows that dropped in production versus those that did not. ANCOM identified 1 phylum, *Proteobacteria*, that differed between *E. coli*-positive cows that dropped in production and those that did not. Evaluation of β-diversity found no statistical relationship between post-mastitis LS groups and microbiome. Although 9 culture-negative quarters had profiles dominated by taxonomic families containing common mastitis pathogens, no associations were found for production changes and post-mastitis LS when evaluating α- and β-diversities and composition of the microbiomes. The findings suggest that a contributing factor to negative outcomes in *E. coli*-positive cows is relative abundance of this pathogen, and that no single or collective group of bacterial families contributes to milk production changes or post-mastitis LS in culture-negative quarters.

Significance

The absence of associations between outcomes and pathogen profiles in this study indicates that we are not missing opportunities by not treating *E. coli*-positive and culture-negative quarters.