Materials and Methods

Cows diagnosed with clinical mastitis with a gram-negative pathogen or no visible growth on aerobic culture (n=103) were randomly allocated to either treatment group, which received 5 intramammary infusions of ceftiofur hydrochloride at 24-h intervals only on the affected quarter, or an untreated control group. Serial samples were collected from the affected quarter and an ipsilateral quarter for microbiome analysis. The 16S rRNA gene was amplified from genomic DNA and sequenced using the Illumina MiSeq platform. Sequences were processed via the MiSeq Reporter version 2.5 and QIIME version 1.7.0-dev, for classification of reads, quality filtering, and calculation of number of OTUs, Chao1, and Shannon diversity indexes. Microbiome changes occurring over time and in response to intramammary antibiotic therapy were analyzed at the phylum and family levels using JMP Pro 11. The effect of clinical mastitis on the microbiome was assessed through response screening analysis. P-values were adjusted for false discovery rate. The GLIMMIX procedure of SAS was used to assess the effects of clinical mastitis and intramammary treatment in OTU numbers, Shannon, and Chao1 indexes.

Results

In cows diagnosed with *E. coli*-associated mastitis, the relative abundance of *Enterobacteriaceae* was greater in the milk from mastitic quarters when compared to healthy quarters, which presented a more diverse microbiome. No differences in the rate of change of *Enterobacteriaceae* were observed between treatment groups. Milk of mastitic quarters classified as negative culture by standard laboratory methods did not exhibit major shifts in the bacterial population, nor did treatment with ceftiofur hydrochloride result in major shifts in milk microbiome. Intramammary treatment with ceftiofur hydrochloride did not improve clinical and bacteriological cures of mastitis compared with untreated controls in either Gram-negative or culture-negative mastitis cases.

Significance

Next generation sequencing revealed significant differences in the metagenome of healthy and mastitic quarters from cows diagnosed with Gram-negative pathogens. However, the bacterial profile of healthy and mastitic quarters from cows diagnosed with no bacterial growth under aerobic conditions did not differ. There were no differences in clinical cure or relative abundance of the most abundant bacterial family from mastitic samples between treatment and control groups in any of the bacterial groups evaluated.

Longitudinal characterization of mastitis causing pathogens previously identified as "other Streptococcal species", including *Lactococcus*

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Introduction

Identification of *Streptococcus agalactiae* and *Streptococcus dysgalactiae* isolated from milk using standard microbiological methods is very accurate when compared to 16S sequencing (Wyder, 2011). The remaining Gram-positive, catalase-negative cocci (GPCN) found in milk, including species in the genera *Streptococcus, Lactococcus, Enterococcus,* and *Aerococcus*, cannot be easily or economically differentiated using biochemical tests (Fortin et al, 2003). As a result, these pathogens are frequently reported as "*Streptococcus* species," making it difficult to assess the clinical significance of the differing organisms (Fortin et al, 2003). The objective of this study was to compare bacteriological cure, risk of recurrent mastitis, and longevity within the herd among different GPCN organisms on 5 farms in northern New York.

Materials and Methods

Five farms having >20 cows with GPCN intramammary (IMM) pathogens in the summer of 2014, including *Lactococcus lactis*, were enrolled in this study in April of 2015. All milk samples from those 5 farms submitted to Quality Milk Production Services (QMPS) were cultured using standard microbiological methods (NMC Handbook, 1999). All GPCN samples were then speciated using MALDI-TOF (Bruker Daltonics, The Woodlands, TX) technology to confirm the bacteria present. All cows enrolled were resampled 14 to 28 days after the initial sample in order to assess bacteriological cure, if they remained in the herd. Cows identified with GPCN infections were tracked with Dairy Comp 305 records after test day for days-in-milk (DIM) at time of sampling, parity, milk production, and previous and future incidents of mas-

titis. Data was collected from the test day before sampling through 4 test days after sampling. Continuous outcome variables were analyzed by ANOVA and binary responses for pathogen effect using 2x2 tables and Pearson's Chi-squared tests. Impact on milk production was assessed with multivariable analysis in SAS controlling for parity, lactation and DIM.

Results

Two hundred and twenty-nine animals were identified with GPCN infections accurately identified to the genus and species level. Of the organisms identified, 67 were identified as *Streptococcus dysglactiae* (26.5%), 28 as *Streptococcus uberis* (11.1%), 118 as *Lactococcus lactis* (46.6%), 2 as *Lactococcus garviae* (0.8%), 6 as *Enterococcus saccharolyticus* (2.4%), 3 as *Enterococcus facecaum* (1.2%), 2 as *Enterococcus faecium* (0.8%), and 1 each of *Enterococcus thailandicus, Streptococcus equinus* and *Streptococcus mitis* (0.4% each). There was a statistically significant difference between the bacteriological cure rate for *L. lactis* (59% cures, n=66) when compared to both *S. dysgalactiae* (92% cures, n=49; $P < 0.001$) and *S. uberis* (89% cures, n=18; $P = 0.02$). At least 1 second clinical mastitis event was recorded for 26% of cows with *S. dysgalactiae*, 31% of *L. lactis*, 14% of *S. uberis*, and 0% of *E. saccharolyticus*. The difference between *S. uberis* and *L. lactis* indicated a potential trend for an increased risk of recurrent mastitis for those cows with *L. lactis* infections ($P = 0.10$; risk ratio=2.2). There was a trend toward a difference in number of cows leaving the herd after a *S. dysgalactiae* infection (19%) compared to those with *S. uberis* (36%; $P = 0.09$) or *L. lactis* (31%; $P = 0.10$). Among the species of bacteria causing the intramammary infections investigated here, impact on milk production did not appear to differ (P=0.83).

Significance

Lactococcus lactis appears to be an important intramammary pathogen on some dairy farms in northern New York, resulting in a mastitis case that is less likely to result in a bacteriological cure when compared to common *Streptococcus* species, irrespective of treatment. This IMM pathogen is also potentially associated with an increasing risk for recurrent mastitis and may increase the risk of cows leaving the herd.

Randomized non-inferiority trial comparing two commercial intramammary antibiotics for the treatment of non-severe clinical mastitis in dairy cows

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Introduction

No new antimicrobials have been approved for the treatment of mastitis since 2006; it would be beneficial to perform controlled field trials comparing 2 existing treatments. Non-inferiority trials are valuable in this regard, as they do not require a negative control as in a randomized, controlled FDA drug trial. If a therapy with similar efficacy is available, decision making criteria can include convenience, lower costs, less side effects, improved delivery systems, and better integration into current protocols. The purpose was to perform a non-inferiority comparison of 2 intramammary treatments for clinical mastitis (CM). We intended to show that hetacillin (HP; Hetacin K, Boehringer Ingelheim, St. Joseph, MO) had comparable efficacy to the reference treatment, ceftiofur (CH; Spectramast, Zoetis, Kalamazoo, MI) when considering cure and survival indices.

Materials and Methods

Cows from 6 New York dairies were considered. Cows with non-severe CM were randomly assigned to 5 d or 3 d treatment with CH or HP, respectively. Milk samples were collected sterilely on d 1, 14, and 21. The cow was given a clinical score (CS) on this day and d 2 to 5, 14 and 21. Cultures were performed on samples according to NMC guidelines. Primary outcomes were bacteriological, clinical, and pathogen cures. A cow was defined as a bacteriological cure when the initial pathogen was absent from both post-treatment samples. A clinical cure was defined when the CS became "0." If both