Uterine microbiome, antibiotic resistance genes and virulence factors of metritic treated cows that cure or failed to cure from metritis

Z. Zhou, M.S. Gomes, I.F. Canisso, E.F. Garrett, J.S. Stewart, F.S. Lima
University of Illinois Urbana-Champaign, Urbana, IL

Metritis is major postpartum disease in dairy cows causing reduced milk production, impaired fertility, and substantial economic losses. Antibiotics are the main therapeutic option, however, ~35% of the cows fail to recover from the disease after treatment. Furthermore, ceftiofur, the major drug used to treat a multitude of dairy cows diseases, has been linked to emergence and dissemination of antibiotic resistance for β-lactamase CMY-2. Ampicillin is an efficacious alternative to treat metritis with similar, but faster cure rates when compared with ceftiofur. However, ampicillin is also a β-lactam antibiotic that binds to specific penicillin-binding proteins belonging to the same antibiotic cluster as ceftiofur. Although from a practical standpoint ampicillin is an effective alternative to treat metritis, it is unclear if ampicillin can mitigate the selective pressure of ceftiofur, influence antimicrobial resistance genes dissemination or it is related differently to pathogens virulence factors. Herein, we used whole genome shotgun sequencing to shed light on uterine microbiome, antimicrobial resistance genes (ARGs), and virulence factors genes (VFGs) of cows that cured or failed to cure from metritis after treatment with ceftiofur or ampicillin. A cohort of 24 metritic primiparous cows healthy and not exposed to dry cow therapy and were randomly allocated to receive either ampicillin trihydrate (n = 12) or ceftiofur hydrochloride (n =12) for 5 days. Uterine swab samples for each cow were collected at metritis diagnosis (d1) and five days later (d6) one day after treatments finished. Half of the cows (12/24) recovered after treatment (ampicillin = 7 and ceftiofur = 5). Our analysis revealed that over time (from d1 to d6) the mean relative abundance (MRA) of the genera Bacteroides, Prevotella, Alistipes, Fusobacterium, and Tannerella were reduced (P < 0.01), whereas Porphyromonas was increased (P < 0.01) independent of treatment (P > 0.05). For cows that recovered from metritis, only Streptococcus MRA was increased when compared with counterparts that did not recover from metritis. We found the beta-diversity of microbiome communities follow a similar pattern, with microbiome diversity decreasing (P < 0.01) after treatment independent of treatment type (P > 0.05) and cure status (P > 0.05). Antibiotic treatment independent of type decreased VFGs abundance, but increased ARGs abundance. The resistome of metritic cows was dominated by tetracycline resistance genes, but beta-lactam ARGs such as CMY-2 were not affected by treatment or time (P > 0.05). The ARGs TetT and TetW increased over time (P < 0.01) independent of treatment (P > 0.05) or recovery status (P > 0.05). A higher MRA of VFs such as translation elongation factor Tu for Streptococcus spp. and heat shock protein 70 from Vibrio cholerae suggests that further investigation of the potential role of these bacteria and VFs on metritis pathogenesis is warranted. In conclusion, antibiotic treatment over time independent of type altered uterine microbiome, reduced VFGs abundance, and increased ARGs abundance, thus ampicillin is an alternative to ceftiofur for metritis treatment that has not direct impacts on uterine microbiome, AGRs, and VFGs.

Keywords: Metritis, microbiome, antibiotic resistance gene, virulence factors, cure.