TITLE: GENOMIC, TRANSCRIPTOMIC AND *IN VITRO* ANALYSIS OF ACQUIRED ANTIBIOTIC RESISTANCE GENES IN THE RUMEN MICROBIOME

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ABSTRACT:

Horizontal gene transfer is one of the main mechanisms involved in the evolution of pathogens, being frequently associated with the spread of antibiotic resistance among commensal and pathogenic bacteria. Exchange of genetic material is facilitated in densely populated ecosystems where microbial species live in close physical contact, such as the gastrointestinal tract of livestock animals. In this work, the objectives were: 1) to identify the presence of acquired antibiotic resistance genes (ARGs) in 435 ruminal microbial genomes; 2) to confirm the resistance phenotypes *in vitro*, and 3) to evaluate ARG expression in ruminal metatranscriptomes. Resfinder was used to identify acquired ARGs *in silico* and some ruminal cultures corresponding to the analyzed genomes were evaluated *in vitro* to confirm the presence of the antibiotic resistance phenotype. Moreover, fifteen datasets of ruminal microbial metatranscriptomes from sheep, dairy and beef cattle were downloaded from Sequence Read Arquive (SRA/NCBI) and used to evaluate ARG expression, which was represented as "reads per kilobase million" (RPKM). Resfinder predicted 141 genes distributed in 72 ruminal bacterial genomes. The *tet*(W) gene was the most abundant, with hits being found in 29 genomes belonging to two distinct phyla (*Firmicutes* and *Actinobacteria*), which reveals the widespread distribution of this gene. Nonetheless, some ARGs, such as vancomycin resistance genes were only detected in the phylum *Firmicutes*. In the 12 bacteria analyzed *in vitro*, 11 predicted resistance phenotypes were confirmed out of 15 evaluated, which included eight resistances to tetracycline and one resistance to clindamycin, vancomycin and macrolide. These *in vitro* results confirmed the resistance phenotype for the majority of the ARGs predicted *in silico*. The evaluation of the expression levels in metatranscriptome datasets showed that the beta-lactam resistance gene *blaACl*-1, the macrolide resistance gene *mef*(A), the vancomycin resistance genes *vanR*-C and *vanR*-D and several tetracycline resistance genes, including *tet*(32), *tet*(40), *tet*(J) and mainly *tet*(O), *tet*(Q), *tet*(W) and *tet*(37), were expressed in the rumen datasets. Our results demonstrate the presence and active role of acquired ARGs in the rumen microbiome, with potential implications to animal and human health. Future experiments will investigate mechanisms that could mediate the spreading of these ARGs among rumen bacteria.

Keywords: ruminal bacteria, antibiotic resistance, Resfinder, metatranscriptome