

CHARACTERIZATION OF ANTIBIOTIC RESISTANCE GENES AND IDENTIFICATION OF MOBILE ELEMENTS IN THE GASTROINTESTINAL MICROBIOME OF NELORE BULLS

Leticia Maria de Aquino^{1,2}; Liliane Costa Conteville²; Bruno Gabriel Andrade³; Adhemar Zerlotini Neto⁴; Gerson Barreto Mourão⁵; Luiz Lehmann Coutinho⁵; Luciana Correia de Almeida Regitano²

¹. São Carlos, SP, Brazil. Center of Biological Sciences and Health, Federal University of São Carlos; ². São Carlos, SP, Brazil. Embrapa Southeast Livestock; ³. Cork, Ireland. Computer Science Department, Munster technological University; ⁴. Campinas, SP, Brazil. Embrapa Agricultural Informatics; ⁵. Piracicaba, SP, Brazil. Center for Functional Genomics, Department of Animal Science, University of São Paulo

Abstract:

The presence of antibiotic resistance genes (ARGs) is a global public health concern, and multidrug resistance has been increasingly incident in clinical pathogens. The emergence of resistance to "last-resort" antibiotics spurs the search for mechanisms to control bacteria that harbor and may propagate ARGs. The predominant use of antibiotics for the treatment of livestock infection as well as human infections not only compromises therapeutic efficacy, but also contributes to the emergence of reservoirs of antibiotic multidrug resistance (AMR) that can be transmitted through the food chain and environmental waste. However, few studies use metagenomic data to examine ARGs, especially in commensal bacteria present in food-producing animals, such as ruminants. Furthermore, mobile elements such as plasmids and bacteriophages can transfer their genetic material horizontally through their insertion into bacterial cells during the infection process, which is a propagation pathway for ARGs. The purpose of this study was to explore 916 metagenome-assembled genomes (MAGs) from the rumen and feces of 52 Nelore bulls (*Bos indicus*), in order to characterize ARGs and identify mobile elements. These microbial genomes were searched against the MEGARes database using ABRicate. Based on contigs' sequences, we used CheckV for virus identification. Only contigs classified as "Complete" (with length of ≥ 5 kilobases), with viral genes identified, and contamination $< 1\%$ were considered for further analysis. For mobile elements classification, we analyzed these filtered data in GeNomad. In total, 36 bacterial MAGs harbor resistance genes, being 30 from feces and six from rumen. Some fecal MAGs harbor more than one ARG, including one MAG that harbored 42 ARGs. Several identified ARGs are associated with the resistance to antibiotics used in veterinary and human medicine to combat important infectious agents, like beta-lactams, penicillins and tetracyclines. In terms of taxonomic abundance, these genes are mainly in fecal MAGs classified as *Firmicutes* (72.4%), *Bacteroides* (24.1%) and *Actinobacteria* (3.4%). In rumen, ARGs are identified in MAGs classified as *Bacteroidetes* (80.0%) and *Firmicutes* (20.0%). We classified 1418 contigs as potential viral genomes using CheckV, 977 from feces, and 441 from rumen. Using GeNomad, 967 viruses found in fecal contigs were classified, being mostly as bacteriophages of the orders *Caudoviricetes* (96.2%) and *Crassvirales* (2.2%) and as family *Microviridae* (0.9%). Other viruses belong to families such as *Schitoviridae* (0.4%) and *Autographiviridae* (0.1%); and orders *Imitervirales* (0.1%) and *Cirlivirales* (0.1%). In the ruminal contigs, 425 viruses were classified, being mostly as *Caudoviricetes* (96.5%), *Crassvirales* (1.2%), and *Microviridae* (1.6%), and others in the orders *Petitvirales* (0.2%) and *Geplafuvirales* (0.5%). Furthermore, three contigs from feces and 6 from rumen were classified as plasmids, but none of them had AMR genes. Further transcriptomics and proteomics analyses may provide more assertive information on the impact of these genes, whether to describe the activity of these mobile elements in the microbiome or to offer insights to combat their resistance to antibiotics used in human and veterinary medicine.

Palavras-chave: Antibiotic Resistance Genes; resistome; virulome; mobile elements; metagenomics

Support / Acknowledgment

Funded by National Council for Scientific and Technological Development, CNPq (grant number: 456191/2014-3) and São Paulo Research Foundation, FAPESP (grant number: 2019/04089-2). L.M. Aquino is funded with a fellowship from the CNPq (process number 128568/2022-4). L.L.Coutinho and L.C.A. Regitano are CNPq research fellows.