

**TITLE:** ANALYSIS OF THE KEFIR MILK AND WATER RESYSTOM OF BRAZIL AND ARGENTINA AND ITS CLINICAL RELEVANCE

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### **ABSTRACT**

Antimicrobial resistance represents one of the most worrisome threats to public health in the 21st century. Bacteria harboring antibiotic resistance genes (ARGs) can reach the digestive tract through food ingestion and ARGs can be exchanged through the horizontal transfer mechanism. The aim of this work was to evaluate the expression of antibiotic resistance genes from water and milk kefir, focusing on clinically relevant resistance genes in mobile genetic elements. The metatranscriptome was made in Myseq (Illumina) of six kefirs: AA1 and AA2 (milk kefir from Argentina), KLSA and KLCU (milk kefir from Bahia and Curitiba - Brazil, respectively) and KASA and KACU (water kefir from Argentina Bahia and Curitiba – Brazil, respectively). To determine the resistance genes, the transcripts were aligned with the CARD database (The Comprehensive Antibiotic Resistance Database) with parameters of 60% identity and e-value of  $10^{-5}$ . The location of the transcripts was determined by aligning with the NCBI Plasmid RefSeq database and the ARGs were classified for risk according to literature criteria. A total of 203 ARGs were identified, with the KASA drink being the most diverse (130 genes). Most resistance genes were located on the chromosome (mean 84.91%). KASA and KACU had a higher frequency of ARGs located in plasmids (7.13 and 6.51%, respectively), the other drinks had frequencies ranging from 5.52 to 1.62%. The expressed genes located in plasmids exclusive to water drinks and present in KACU and KASA were AAC(6')-Ie-APH(2'')-Ia and otr(A) which encode resistance to aminoglycosides and tetracycline, respectively. In milk beverages, the AAC(6')-Ib7, CTX-M-50, kdpE, and tet(C) genes were exclusive to KLCU, the aadA15 gene was exclusive to AA1. BRP(MBL) was common to KLSA and KLCU. All genes mentioned belong to Level I, which represents a high risk for contributing to resistance and dissemination of ARGs through mobile genetic elements. Our findings suggest that foods such as kefir play an important role in the dissemination of antimicrobial resistance genes used in clinical medicine, which may pose risks to human health.

**Keywords:** Antimicrobial resistance, metatranscriptome, resistome, kefir.

**Development Agencies:** CAPES, CNPq and FAPEMIG.