TITLE: CHARACTERIZATION OF THE CRISPR-*cas* SYSTEM IN MULTIDRUG-RESISTANT *Enterococcus faecalis* ISOLATES FROM SWINE

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ABSTRACT:

In bacteria and archaea, clustered, regularly interspaced short palindromic repeat (CRISPR) loci have been associated with acquired defense against plasmids and phages. Recently, a highly significant inverse relationship between CRISPR-*cas* and antibiotic resistance was established in enterococci. While hospital-adapted strains generally lack CRISPR-*cas*, commensal strains have conserved CRISPR-*cas*. *Enterococcus* spp. are gut commensal bacteria from human and other animal species. Therefore, investigating how CRISPR-cas regulates the flux of mobile elements under the selective pressure of antimicrobial agents in veterinary settings will broaden the understanding of antimicrobial resistance. We aimed to characterize CRISPR-*cas* loci of epidemiologically unrelated multidrug-resistant *E. faecalis* (ST29, ST330, ST591, ST710, ST711) isolated from healthy nursery pigs in different Brazillian States (DF, PR, MG, SP, MT and SC). Genomic DNA of 12 *E. faecalis* isolates from pig faeces were sequenced using an Illumina Miseq platform. Libraries were prepared using the Illumina Nextera XT DNA sample preparation kit (Ilumina Inc., USA) with changes for 2x250pb paired-end reads. CLC Genomics Workbench 8.0.3 was used for *de novo* assemblies. Rapid Annotation Server (RAST) and Prokaryotic Genomes Annotation Pipeline (NCBI_PGAP) were accessed for genome annotations. CRISPRfinder was used to identify putative CRISPR loci in the 12 *E. faecalis* genomes. BLAST functions were used for comparisons and data analysis. CAS-Type IIA was detected in ST591 *E. faecalis* (5 isolates), ST711 *E. faecalis* (1 isolate), and ST330 *E. faecalis* (2 isolates). However, 4 CRISPR-*cas*-deficient *E. faecalis* strains (ST29, ST710, ST711, ST330) were also detected. A CRISPR array (431 bp; 6 CRISPR spacer sequences) was common to ST591, ST710 and ST711 *E. faecalis* strains, but we found that CRISPR loci were variable among them. A CRISPR array of 631 bp/ 9 CRISPR spacer sequences was also detected in ST591 and ST710, and a CRISPR array of 499 bp/ 7 CRISPR spacer sequences, in ST711 *E. faecalis* strains. In Brazil, little is known about how the spread inter- species/genera of resistance genes has been driven in bacteria of animal origin. Further studies are required to expose the role of the CRISPR-*cas* system in genome evolution of commensal enterococci exposed to antimicrobial use in veterinary settings.

Keywords: CRISPR-*cas*, transferable resistance, *Enterococcus faecalis*.

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