TITLE: IS CRISPR-CAS ASSOCIATED WITH *ENTEROCOCCUS FAECALIS* ADAPTATION TO DIFFERENT ENVIRONMENTS AND THE EMERGENCE OF HIGH-RISK LINEAGES?

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ABSTRACT:

Enterococcus faecalis is responsible for healthcare-associated infections that are often difficult to treat due to acquisition of antimicrobial resistance and virulence-associated genes, a process that may be subject to CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and Associated Proteins) interference. The aim of this study was to investigate the CRISPR content in 90 *E. faecalis* isolates from coastal waters, wild birds and hospitalized human patients, and the possible associations of these elements with the acquisition of accessory traits and how they might impact adaptation. CRISPR loci (CRISPR1-Cas and CRISPR3-Cas) and accessory genes (antimicrobial resistance and virulence associated) were screened by PCR, genetic diversity was determined by MLST, and whole genome sequences of 1446 *E. faecalis* genomes from GenBank were assessed for complementary analysis. CRISPR-Cas were detected in 47.8% (43/90) of the isolates, with CRISPR1-Cas being the most frequent (36/43). Most of the CRISPR-positive isolates were recovered from coastal waters (48.8%), followed by those from wild birds (30.2%), and humans (21.0%). Accessory genes more frequently detected in human isolates, such as *aac(6')-Iaph(2'')-Ia, cylA, asa1* and *agg*, were associated with CRISPR1-Cas absence. In contrast, frequencies of other genes (such as *ant(6')-Ia, ermA, tetM* and *esp*) were significantly higher when CRISPR1-Cas was present. Genome analysis confirmed additional negative correlations between *van*A/*van*B genes and CRISPR-Cas. The data suggest that the absence or disruption of CRISPR-Cas may be associated with *E. faecalis* adaptation to the hospital environment, as uniformly observed in genomes from high-risk lineages, such as STs 2, 6, 9 and 28. On the other hand, intra-ST variation of CRISPR1-Cas content was observed in lineages that were not restricted to the hospital setting, such as STs 16, 21, 23 and 40. The occurrence of missense mutations in *cas9* lead us to speculate their possible contribution to the emergence of vancomycin resistance in ST116. Finally, we detected a *cas9* displacement from CRISPR3-*cas* operon in genetically related STs 72, 443 and 712. We conclude that CRISPR-Cas screening may be a useful strategy in the identification of *E. faecalis* lineages that are more likely to cause hospital outbreaks due to increasing acquisition of traits involved in persistence, being a key factor for adaptation, genetic diversity and evolutionary trajectory of the species.

Keywords: *Enterococcus faecalis*, CRISPR, antimicrobial resistance, virulence, adaptation

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