

**TITLE:** CRISPR LOCI DISTRIBUTION AMONG *ENTEROCOCCUS FAECALIS* ISOLATES FROM DIFFERENT SOURCES AND ITS CORRELATION WITH THE OCCURRENCE OF ANTIMICROBIAL RESISTANCE AND VIRULENCE DETERMINANTS

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**ABSTRACT:**

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) loci have been recognized as an acquired defense system against acquisition of foreign DNA, implying that the loss of this mechanism might have allowed the acquisition of features associated with adaptation of pathogens to the hospital environment. The aim of this study was to perform a comparative analysis of CRISPR loci frequency and diversity among 93 *Enterococcus faecalis* isolates recovered from different sources. The occurrence of associations between the presence or absence of CRISPR with antimicrobial resistance and virulence profiles was also evaluated. The isolates were characterized by MALDI-TOF MS. CRISPR loci (CRISPR1-cas; CRISPR2; CRISPR3-cas) were identified by PCR. Antimicrobial susceptibility profiles were determined by disk-diffusion tests and resistance- and virulence-associated genes were detected by PCR. CRISPR1-cas and CRISPR3-cas were detected in 38 (40.9%) and 8 (8.6%) isolates, respectively, while the orphan locus CRISPR2 was present in all the isolates. Among the 38 isolates harboring CRISPR1-cas, 23 (60.5%) were recovered from sea water, 9 (23.7%) from human patients and 6 (15.8%) from wild birds, while the 8 isolates harboring CRISPR3-cas were recovered from wild birds. Statistically significant correlations were shown between the absence of CRISPR1-cas system and high frequency of the following antimicrobial resistance and virulence genes: *aac(6')-laph(2'')-la* ( $p=0.0052$ ), *cylA* ( $p=0.0001$ ), *asa1* ( $p=0.0009$ ) e *agg* ( $p=0.0045$ ). However, depending on the isolation source of the isolate, the occurrence of the following other genes was correlated with the presence of CRISPR1-cas instead of its absence: *ant(6')-la* ( $p=0.0001$ ), *erm(A)* ( $p=0.0001$ ), *tet(M)* ( $p=0.0012$ ), *esp* ( $p=0.0001$ ). No significant correlations were identified between the frequency of accessory genes and CRISPR3-cas locus, except for *cylA*, which was more frequently detected among isolates carrying this locus ( $p=0.0299$ ). Therefore, our results demonstrate that, at least for some genes, the presence of CRISPR loci might be negatively or positively associated with the acquisition of foreign DNA in *E. faecalis* genome. Analysis of these correlations among isolates from different sources may contribute to a wider comprehension of the dynamics of CRISPR system function and its impact on bacterial accessory genome.

**Keywords:** antimicrobial resistance, CRISPR, *Enterococcus*, virulence factors

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