TITLE: CRISPR3-CAS9 MUTATIONS POTENTIALLY INVOLVED IN *ENTEROCOCCUS FAECALIS* ST116 TRANSITION FROM A LOW-RISK UBIQUITOUS LINEAGE TO A VANCOMYCIN-RESISTANT HUMAN PATHOGEN: AN EMERGING THREAT TO GLOBAL ONE-HEALTH

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ABSTRACT:

Enterococcus faecalis is an opportunistic pathogen responsible for a wide range of nosocomial infections and represents a global public health threat due to its ability to acquire multidrug resistance. Endogenous CRISPR-Cas systems provide defense against invading DNA, being directly linked to the adaptation of multidrug-resistant lineages to the hospital environment. This study aimed to investigate the occurrence of microevolutionary events involving CRISPR3-*cas* potentially associated with the emergence of vancomycin-resistant enterococci (VRE) of sequence-type (ST) 116 in human patients. ST116 isolates have been recovered from humans, animals, and environmental sources worldwide. Three ST116 wild bird isolates from a bacterial collection were tested by PCR for CRISPR *loci* and *van* genes. In addition, we screened 1,441 *E. faecalis* genomes from GenBank for in-depth analysis of CRISPR-*cas* and *van* gene distribution in this species using CRISPRCasFinder, ResFinder 4.1, and *in silico* PCR, followed by statistical analysis of the correlation between these genetic traits. Our results show that CRISPR3-*cas* is ubiquitous in ST116 isolates and was detected in all 12 genomes of this ST deposited in GenBank. However, 15 missense mutations were identified in the ST116-Cas9 protein sequence, which could explain the failure to detect its gene by both *in vitro* and *in silico* PCR, but only by CRISPRCasFinder. Statistically significant negative correlations were found between CRISPR-*cas* and *van* gene frequencies. Only a few *E. faecalis* exhibited the co-occurrence of CRISPR-*cas* and *van*A, including four ST116 genomes (three from human sources, including blood, urine, and stool, and one from turkey meat). The nucleotide content of the ST116 CRISPR3 array also showed different patterns between *van*A-positive and *van*A-negative genomes. Finally, SNP-based whole genome phylogenetic analysis (performed by CSI Phylogeny 1.4) confirmed that *van*A-positive ST116 genomes and two other ST116 genomes with fragmented CRISPR3-*cas* were more closely related than others from this same lineage. We conclude that CRISPR3 spacer deletion, *cas* cluster and array fragmentation, and mutations in the conserved internal region of the Cas9 sequence may be associated with the acquisition and maintenance of the *van*A gene in ST116. Thus, our data suggest that CRISPR3-*cas* inactivation may be involved in the transition of ST116 from a lineage with low antibiotic resistance to a VRE human pathogen.

Keywords: *Enterococcus faecalis*, CRISPR-Cas9, antibiotic resistance, VRE, One-health

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