TITLE: A MULTIPLEX PCR FOR SCREENING AND TYPING CRISPR-Cas SYSTEMS IN COAGULASE-NEGATIVE STAPHYLOCOCCI

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ABSTRACT:

Coagulase-negative staphylococci (CoNS), long considered as harmless habitants of the human microbiota, have emerged as one of the most common causes of healthcare-associated infections. It has been proposed that CoNS may act as reservoirs of genes that, after horizontal gene transfer, enhance the potential of *S. aureus* to resist antimicrobial treatment. However, this role could be contradicted by the fact that CRISPR/Cas systems, which are estimated to exist in the genomes of about 50% of bacteria, interfere with plasmid uptake in staphylococci. CRISPR is an array of short direct repeats interspaced by small segments of DNA derived from previous exposure to foreign DNA, either viruses or plasmids. These elements are accompanied by CRISPR-associated proteins (Cas), which recognize an invader genetic material, cleave it and incorporate a fragment of it within the bacterial genome. To investigate the abundance of CRISPR-Cas systems in CoNS, we aimed to develop a PCR reaction based on the CRISPR ubiquitous gene *cas1* and analyze its presence in staphylococci clinical strains. For that purpose, the *cas1* gene sequence of 15 strains belonging to seven different CoNS species were obtained from Genbank and aligned with Clustal Omega. A Baeysian inference of the phylogeny of this gene was then performed with Mr. Bayes 3.2. From the phylogenetic tree we observed that the strains were grouped in those with CRISPR systems of types II or III, which are differentiated by their number and types of associated genes. Then, two pairs of primers were designed to target the conserved region of *cas1* from each CRISPR type. These primers were tested together in a multiplex PCR, using the detection of the 16S rRNA as an endogenous control and the *S. epidermidis* strains 10L and RP62A as controls for the amplification of types II and III *cas1*, respectively. The reaction was tested in 55 CoNS clinical strains of eight species: *S. saprophyticus* (n=24), *S. haemolyticus* (n=10), *S. epidermidis* (n=6), *S. capitis* (6), *S. cohni* (n=5), *S. warneri* (n=2), *S. lugdunensis* (n=1) and *S. Simulans* (n=1). Among them, only 4% were positive for the *cas1* gene, both belonging to type III CRISPR systems. Our results validate a multiplex PCR for screening and typing CRISPR/Cas in CoNS and show that these systems are more rare in these bacteria than in others, which is consistent with their role as antimicrobial resistance genes reservoirs.

Keywords: CRISPR, *cas1*, staphylococci, multiplex-PCR, CoNS

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