

**P2844 CRISPR-Cas system molecular organization and functionality in clinical MDR *Staphylococcus epidermidis***

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**Background:** Antibiotic resistance is a worldwide threat for public health. According to the WHO, deaths attributable to infections by Multi-Drug Resistant (MDR) bacteria will increase year-by-year whether new "fighting strategies" will not be found soon. In this scenario CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeat and CRISPR-associated genes), a heritable adaptive immune-system targeting foreign DNA or RNA to protect bacteria from phage infection (PI) and mobile genetic element acquisition, could provide new ground-breaking approaches against plasmid horizontal gene transfer or for the therapeutic use of bacteriophages.

**Materials/methods:** In 10 clinical MDR *Staphylococcus epidermidis*, the CRISPR-Cas molecular organization was in depth investigated analyzing CRISPR-Cas genetic characterization and Single Nucleotide Polymorphisms (SNPs) by colony-PCR and Sanger-sequencing. Furthermore, CRISPR-Cas functionality was tested by phage infection sensitiveness by plaque assays and plasmid acquisition by transformation with pCRISPR-Nickase and subsequent conjugation with pGO400 (harboring also nickase-gene) in chloramphenicol/mupirocin selectable strain harboring a complete CRISPR-Cas locus.

**Results:** In 3 out of 10 *S.epidermidis*, CRISPR-Cas genetic characterization was in synteny with the prototype RP62A reference *S.epidermidis* CRISPR-Cas type III-A organization even though containing more upstream spacers, and only one strain showed 56 non-synonymous SNPs in all CRISPR-Cas genes with the exception of *cas2*. Nevertheless conjugation assays showed pGO400 positive transconjugants indicating the CRISPR-Cas system no-functionality despite its perfect match with the prototype. The remaining strains harbored at least one of the CRISPR-Cas genes. Among the three strains having the RP62A-like CRISPR-Cas system, two strains were resistant and 1 was sensitive to the PI with tested phages. Finally, a new phage able to infect one of our phage resistant MDR *S.epidermidis* was isolated from Tuscaloosa wastewater plant.

**Conclusions:** Our data revealed that a complete CRISPR-Cas system, with or without non-synonymous SNPs, is not frequently found in MDR *S.epidermidis*, whilst defective CRISPR-Cas loci are more commonly spread. Interestingly, RP62A *S.epidermidis* CRISPR-Cas type III-A organization containing more spacers was associated to a permissive plasmid acquisition and phage infection resistance suggesting that different CRISPR-Cas modulation activity can occur *versus* phages infection or plasmid acquisition.