# *IN SILICO* DESIGN OF sgRNAs TARGETING THE *bla*$_{KPC}$ VARIANTS AS A CRISPR-Cas9 STRATEGY AGAINST ANTIMICROBIAL RESISTANCE

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Antimicrobial resistance (AMR) represents a major global health threat, leading to increasing treatment failures in recent years[1]. In 2019, multidrug-resistant (MDR) bacterial infections were associated with 4.95 million deaths, of which 1.27 million were directly attributed to AMR. Moreover, projections estimate that by 2050, AMR could cause 10 million deaths annually and economic losses of up to $100 trillion[2]. β-lactam family (penicillins, cephalosporins, carbapenems, and monobactams) has been particularly impacted. The widespread and indiscriminate use of these antibiotics has contributed to the emergence of antibiotic-resistant bacteria. Resistance in gram-negative bacteria is mainly driven by extended-spectrum β-lactamases (ESBLs). Although β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam are available, their efficacy is limited to class A, with poor effectiveness against classes B, C, and D. Therefore, the objective of this study was to design single-guide RNAs (sgRNAs) targeting the *bla*$_{KPC}$ (class A beta-lactamase) gene for subsequent *in vitro* evaluation. Firstly, the nucleotide sequences were retrieved from the Beta-lactamase Database, and single guide RNAs (sgRNAs) were generated using the CHOPCHOP online server based on the *bla*$_{KPC-3}$ sequence. Multiple alignment of all *bla*$_{KPC}$ variants was performed with MEGA X (ClustalW) to identify highly conserved regions. A total of 142 sgRNAs were predicted. Among these, three (sgRNA1, sgRNA8, and sgRNA9) were selected according to the following criteria: absence of self-complementarity, cutting efficiency > 70%, and a GC content of 40- 60%. These sgRNAs were mapped within the initial 600 bp of the target sequence and were conserved in over 80% of the aligned *bla*$_{KPC}$ variants. Finally, the *in-silico* prediction of sgRNAs validates their potential for *in vitro* evaluation and confirms their efficacy. This approach has the potential to serve as a therapeutic alternative capable of directly eliminating the genetic determinants of resistance, thereby overcoming the limitations of traditional β-lactamase inhibitors.

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