Engineered and Construction of PDS132:ΔvirG as Suicide Vector for Targeted Gene Deletion of VirG From Shigella Flexneri 2a in Order to Generation a Live Attenuated Shigella Vaccine

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Background & Objectives: Shigella are gram negative bacteria capable of inducing their entry into non-phagocytic cells via secretion of various effector proteins called invasion plasmid antigens (Ipas). The most important of Ipas is VirG protein. Live attenuated Shigella vaccines have indicated promise in inducing protective immune responses in human clinical trials. In current situation, constructions of Shigella vaccine candidate strains based on classical allelic exchange systems are considered. The aim of this research was to engineered and construction of pDS132::ΔvirG as a suicide plasmid fortargeted deletion regions of virG gene by using allele exchange methods in Shigella flexneri 2a.

Methods: In this applied study, species and serotype of shigella was confirmed by using serological and Polymerase Chain Reaction (PCR) tests. Detection primers of virG gene were designed and cloned to pGEM-5zf vectorand finally, sequencing was done. According to virG restriction enzyme map, 1751 bp of virG gene was removed by using of HincΠ restriction enzyme and the ΔvirG was successfully constructed. The pGEMΔvirG vector was digested by use of SphI and SalI enzymes and then cloned to pSD132 as suicide vector. Precision of process were verified through phenotypeand genotype experiment.

Results: The Shigella flexneri type 2a strain was verified by serological and PCR tests. Sequence of the virG gene in native strain was sequentially identical with the strains submitted in the Gene-Bank database. Since the pDS132::ΔvirG contains 1484 bp which derived from virG gene, therefore, it can be utilized for the interference in virG gene as specific suicidevector in shigella flexneri 2a.

Conclusion: Application of suicide systems facilitated mutant construction in more specific and effective methods in comparison with the other early techniques such as serial passage.

Keywords: Allelic Exchange; VirG, Shigella Flexneri 2a; Shigellosis; Suicide Vector