TITLE: *In silico* development of a multi-epitope vaccine against *P. aeruginosa* infection

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ABSTRACT:

Pseudomonas aeruginosa is an opportunistic human pathogen that causes nosocomial infection in diverse healthcare settings, such as catheter-associated infections and neonatal meningitis. This bacterium is resistant to most antibiotics used in therapy, and despite the high morbidity and mortality rate associated with infection, there are no effective preventive measures available. In this study, we used an immunoinformatics approach to design a multi-epitope peptide with potential use as vaccine against *P. aeruginosa* infection. Initially, a total of 174 complete genomes of *P. aeruginosa* available at the NCBI database was explored to obtain a set of shared genes comprising the core genome. Implementation of a reverse vaccinology workflow provided 7 candidate antigenic proteins that were submitted, along with 5 quorum sensing-related virulence proteins screened from the literature, to prediction of T-cell (MHC-I and MHC-II) and linear B-cell epitopes. Immunoinformatics analyses allowed identification of 24 highly antigenic and non-toxic T-cell epitopes with high levels of population coverage. These epitopes were used to design eight different chimeric peptide constructs that were evaluated for potential use as immunogens. Two of these constructs were found to be highly immunogenic, non-allergenic, non-toxic, and non-homologous to natural human proteins, therefore presenting the best features for vaccine development. Their structural models were predicted and exhibited well-defined and strong hydrogen-bonding interactions with the Toll-like receptor 4 (TLR4) and myeloid differentiation factor 2 (MD2). Immune simulation analyses using the two constructs showed high levels of T-cell and B-cell activities and long-lasting antibody production, which indicates the elicitation of the adaptive immune response. Lastly, the coding sequences of the two constructs were in silico cloned into the pET28 vector with all codons optimized for Escherichia coli K12. This work provided substantial results that will drive efforts to produce effective recombinant immunogens against *P. aeruginosa* infection. However, experimental validation is required to ensure the effectiveness and safety of these proposed vaccines.

Keywords: *Pseudomonas aeruginosa*; multi-epitope vaccine; immunoinformatics.

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