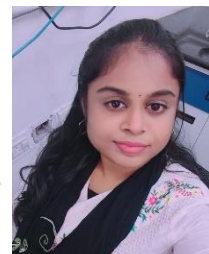


Reverse vaccinology–based prioritization of hypothetical proteins for multi-epitope vaccine development against MDR *Pseudomonas aeruginosa* JJPA01**Bhuvaneswari Narthanareeswaran^a and Jeyakanthan Jeyaraman^{a*}**

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**Abstract**

Antimicrobial resistance is a growing global health threat as multidrug-resistant (MDR) pathogens rapidly spread and diminish the effectiveness of current antibiotic therapies. *Pseudomonas aeruginosa*, a critical ESKAPE pathogen, represents this challenge due to its complex resistance mechanisms and the absence of any licensed vaccine. Developing vaccines that target MDR clinical strains rather than standard laboratory isolates is essential to ensure real-world applicability and effectiveness. This study investigated hypothetical proteins from the MDR clinical isolate *P. aeruginosa* JJPA01 to identify novel antigenic candidates for multi-epitope vaccine (MEV) development—an area often neglected in traditional vaccine design pipelines. Functional annotation of 430 hypothetical proteins resulted in 26 high-confidence proteins, among which three outer membrane proteins with strong antigenic and non-allergenic characteristics were selected for further analysis. Reverse vaccinology approaches identified 8 B-cell, 19 cytotoxic T-cell, and 8 helper T-cell epitopes. These epitopes were rationally assembled into an MEV construct incorporating human β -defensin and a PADRE sequence to improve immunogenicity and broaden HLA representation. Four MEV models were designed, and model V4 demonstrated the most favourable binding interactions with TLR2, TLR4, MHC I, and MHC II receptors, achieving 99.73% global population coverage. Molecular dynamics simulations confirmed the stability of the receptor–vaccine complexes, while in-silico cloning into the pET-28a(+) vector validated its expression feasibility. Immune simulations further revealed strong B-cell and T-cell responses with effective memory generation. Overall, the proposed MEV candidate offers a promising foundation for experimental validation and represents a step forward in combating *P. aeruginosa* infections amid rising antimicrobial resistance.

Keywords: Antimicrobial Resistance; Multidrug resistance; ESKAPE pathogens; Reverse Vaccinology; Multi-epitope vaccine; Immunoinformatics;