Structural and Mechanistic Analysis of *WaaQ* in Coordinating LPS Core Assembly in Multidrug-Resistant *Klebsiella pneumonia* through Computational Approaches

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Abstract

Lipopolysaccharide (LPS) biosynthesis is an essential process in Gram-negative bacteria, playing a significant role in maintaining outer membrane integrity and contributing to virulence. The *WaaQ* protein, a heptosyltransferase, is integral to the assembly of the LPS core by facilitating the transfer of heptose residues. However, the exact mechanism of its function is not fully understood, with varying reports regarding its enzymatic activity. In this research, *Klebsiella pneumoniae*, a prominent multidrug-resistant pathogen, was chosen as a model organism to study *WaaQ*, guided by phylogenetic and statistical analyses of its protein sequences. A three-dimensional structural model of *WaaQ* was created, and molecular docking studies were conducted to examine its interaction with *WaaC* and *WaaF* it co enzyme. Our molecular dynamics simulations clarify the structural dynamics that underpin *WaaQ*'s pivotal role in the inner core biosynthesis of lipopolysaccharides (LPS). The *WaaQ–WaaC* complex demonstrates significant conformational stability, indicating a tightly regulated and resilient interface that is optimized for precise substrate reception from the upstream heptosyltransferase. Conversely, the *WaaQ–WaaF* interaction is marked by enhanced flexibility, especially in surface loops, suggesting a more transient association that facilitates efficient product release and transfer to the downstream enzyme. This variation in interaction strength—stable upstream and dynamic downstream—reflects the directional flow of

enzymatic activity during LPS core assembly and reinforces the concept of *WaaQ* acting as a central molecular hub. By integrating structural stability with strategic flexibility, *WaaQ* promotes both accuracy and efficiency in the sequential heptosylation processes of LPS biosynthesis. These findings enhance our understanding of the dynamic coordination among glycosyltransferases in bacterial envelope assembly and may guide future initiatives aimed at disrupting LPS biosynthesis as a therapeutic approach.

Keywords: *Klebsiella pneumoniae*, Carbapenemases, Heptosyltransferase, β-lactam antibiotics, Gram-negative