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Mutations in conserved N-terminal amino acid residues of *Neisseria gonorrhoeae* FtsI (PBP2) modify interactions with the cell division protein FtsW and penicillin-binding

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Background: Penicillin-binding protein 2 (PBP2, $FtsI_{Ng}$) is an important molecular target for β-lactam antibiotics used to treat *N. gonorrhoeae* (Ng) infections. $FtsI_{Ng}$ is also a cell division protein comprising an N-terminal domain, with uncharacterized function, and a C-terminal transpeptidase domain which also functions as the penicillin-binding site. Mutation(s) at or near the active site have a pronounced effect on the MIC of Ng to β-lactam drugs. We identified four conserved amino acids (R75, R167, G180, and E193) in the N-terminus and have investigated their role in the ability of $FtsI_{Ng}$ to interact with the cell division protein $FtsW_{Ng}$ and on penicillin-binding.

Aim/Methods: Site directed mutagenesis was used to introduce arginine to alanine/glycine (R75A/G, R167A/G), glycine to glutamic acid/arginine mutations (G180E/R), and glutamic acid to alanine/glycine (E193A/G) amino acid substitutions into $FtsI_{Ng}$. Wild-type and mutated histidine-tagged $FtsI_{Ng}$ proteins were then purified by nickel affinity chromatography. Isothermal titration calorimetry (ITC) experiments were performed to determine the effect of R167G and G180R mutations on penicillin-binding. Bacterial two-hybrid (B2H) assays were used to test the effect of these mutations on interactions between $FtsW_{Ng}$ with wild-type and mutant $FtsI_{Ng}$.

Results: Penicillin binding, as measured by ITC, was significantly increased for $FtsI_{Ng}$-R167G (2.32 times) and $FtsI_{Ng}$-G180R (2.14 times) compared to wild-type $FtsI_{Ng}$ (p-value < 0.05). The $FtsI_{Ng}$-G180E mutation did not have any effect on penicillin-binding. The residual β-galactosidase activity testing the interaction of $FtsI_{Ng}$ mutants R75A/G, R167A/G and E193A/G with $FtsW_{Ng}$ ranged from 56% to 95%, while the wildtype $FtsI_{Ng}$-$FtsW_{Ng}$ interaction had 35% residual β-galactosidase activity. This shows that these mutations in the N-terminus of $FtsI_{Ng}$ are implicated in the interaction between $FtsI_{Ng}$ with $FtsW_{Ng}$.

Conclusions: This research shows that conserved amino acid residues (R75, R167, G180, E193) in the N-terminus of $FtsI_{Ng}$ play an important role in its interaction with the cell division protein $FtsW_{Ng}$, and that R167G and G180R mutations of $FtsI_{Ng}$ influence penicillin-binding.