Inactivation of the glycoside hydrolase NagZ attenuates antipseudomonal beta-lactam resistance in Pseudomonas aeruginosa.

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ABSTRAC:

The overproduction of chromosomal AmpC β-lactamase poses a serious challenge to the successful treatment of *Pseudomonas aeruginosa* infection with β-lactam antibiotics. Induction of *ampC* expression by β-lactams is mediated by disruption of peptidoglycan (PG) recycling and the accumulation of cytosolic 1,6-anhydro-*N*-acetylmuramyl peptides, catabolites of PG recycling that are generated by an *N*-acetyl-β-D-glucosaminidase encoded by *nagZ* (PA3005). In the absence of β-lactams, *ampC* expression is repressed by three AmpD amidases encoded by *ampD*, *ampDh2* and *ampDh3*, which act to degrade these 1,6-anhydro-*N*-acetylmuramyl peptide inducer molecules. Inactivation of *ampD* genes results in a stepwise upregulation of *ampC* expression and clinical resistance to antipseudomonal β-lactams due to accumulation of the *ampC* inducer anhydromuropeptides. To examine the role of NagZ on AmpC mediated β-lactam resistance in *P. aeruginosa*, we inactivated *nagZ* in *P. aeruginosa* PAO1 and in an isogenic triple *ampD* null mutant. We show that inactivation of *nagZ* represses both intrinsic β-lactam resistance (up to 4-fold) and the high antipseudomonal β-lactam resistance (up to 16-fold) that is associated with the loss of AmpD activity. We also demonstrate that AmpC mediated resistance to antipseudomonal β-lactams can be attenuated in PAO1 and in a series of *ampD* null mutants using a selective small molecule inhibitor of NagZ. Our results suggest that blocking NagZ activity could provide a strategy to enhance the efficacy of β-lactams against *P. aeruginosa* and other Gram-negatives that encode inducible chromosomal *ampC* and to counteract the hyperinduction of *ampC* that occurs from the selection of *ampD* null mutations during β-lactam therapy.

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