Understanding a bacterial fitness landscape through substrate specificity and inhibition of a novel beta-lactam antibiotic inhibiting enzyme in Klebsiella pneumoniae.

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Background: Klebsiella pneumoniae carbapenem-hydrolyzing beta-lactamase-2 (KPC-2) is a broad spectrum, class A carbapenemase that confers resistance to imipenem, ceftazidime and piperacillin/tazobactam. In patients infected or colonized with K. pneumoniae harboring mutations coding for the creation of the KPC-2 enzyme, mortality is high (30-70%) due to our inability to treat them effectively with extant anti-microbial agents. Based upon the crystal structure of KPC-2, we constructed a variety of mutants that explore the binding of beta-lactams to the active site of the enzyme. Our goal was to understand the genetic and amino acid sequence requirements of KPC-2's ability to hydrolyze carbapenems and cephalosporins and confer resistance to inhibitors (e.g. clavulanic acid, sulbactam and tazobactam).

Methods: We utilized site-directed and site-saturation mutagenesis, standard enzyme kinetic studies, and antimicrobial susceptibility testing to test a range of anti-microbial agents and inhibitors on K. pneumoniae strains harboring the KPC-2 mutation.

Results and conclusions: A series of >100 variants engineered by mutagenesis studies show that the resistance to clavulanic acid is a more dominant phenotype than resistance to carbapenems or cephems. These observations suggest that the genesis of this carbapenemase occured as a result of a substrate preference for olivanic aicds, the precursor of clavulanic acid and thienamycin. The overall results of this study have allowed for the creation of a robust fitness landscape for K. pneumoniae harboring this new mutation and yield a new tool for strategic therapeutic theory and practice. Further, these notions have implications for the evolution of other resistance genes occurring in other nature.

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