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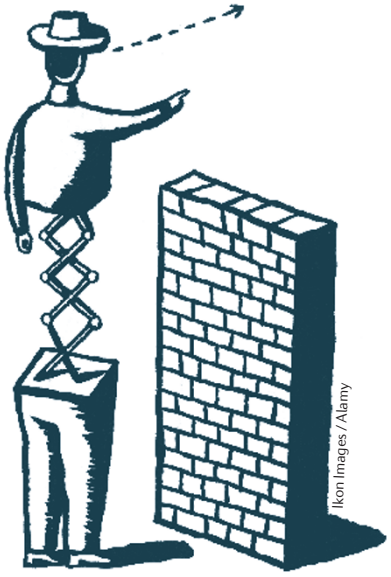
ANTIMICROBIALS

β-lactam sensor discovered

One of the most common mechanisms of antibiotic resistance is the expression of β-lactamases. When resistant bacteria are exposed to β-lactam antibiotics, production of these enzymes is induced and they degrade the antibiotics; however, the triggers of this response are incompletely understood. Li, Wang *et al.* have now identified a sensor histidine kinase in *Vibrio parahaemolyticus* that serves as a receptor for β-lactams and upregulates β-lactamase expression through its cognate response regulator.

In Gram-negative bacteria, the induction of β-lactamases was thought to be mainly an indirect process in response to antibiotic-mediated cell-wall damage and, unlike in Gram-positive bacteria, a direct role of β-lactams has not so far been shown. When Li, Wang *et al.* deleted histidine kinase genes in *V. parahaemolyticus*, they noticed that the *Δvpa0920* mutant was unable to grow on plates containing β-lactams, whereas wild-type cells and mutant cells with *vpa0920* expressed in *trans* showed no growth defect. *vpa0920* encodes a transmembrane protein with domains predicted to be a histidine kinase and an ATPase, and the downstream gene *vpa0919* is predicted to encode a response regulator; both genes are present in all *Vibrio* species. RNA-seq analysis revealed that several genes were downregulated in *Δvpa0920* cells compared with wild-type cells, including genes involved in peptidoglycan synthesis and antibiotic resistance, such as penicillin-binding proteins. Of all genes, the biggest fold-change was observed for *blaA*, which encodes a class A β-lactamase. Thus, the authors termed this two-component system VbrK and VbrR, for *Vibrio* β-lactam resistance sensor kinase and regulator, respectively. Deletion of the genes encoding either VbrK, VbrR or BlaA abolished β-lactam resistance and a biochemical assay confirmed that all three components were required for β-lactamase activity. Furthermore, both β-lactamase activity and autophosphorylation of VbrK could be induced by β-lactams, but not other lactams, suggesting that VbrK specifically recognizes β-lactams.



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To test whether VbrK is a β-lactam receptor, the authors mutated amino acid residues in the extracellular region of VbrK, where the putative ligand binding pocket is located. One of the mutations, L82A, abolished VbrK autophosphorylation and β-lactamase production in response to β-lactams and penicillin binding of the purified sensor domain. Interestingly, two other mutations, L95A and P125A, reduced the ligand specificity — VbrK carrying either of these mutations responded not only to the β-lactam carbapenicillin but also to the lactams δ-valerolactam, ε-caprolactam and 2-azacyclononanone. These lactams do not induce cell-wall damage, which further supports the role of VbrK in direct sensing of β-lactam antibiotics. In addition, phosphorylation of VbrK could be triggered *in vitro* when membrane extracts of *Escherichia coli* expressing VbrK were incubated with carbapenicillin and ATP, confirming that VbrK is a direct sensor of β-lactams. Finally, point mutations of the predicted phosphorylation sites in VbrK and VbrR abolished *blaA* expression, β-lactamase activity and β-lactam resistance.

Taken together, these results show that the histidine kinase VbrK binds to β-lactam antibiotics and, through the response regulator VbrR, triggers the expression of a β-lactamase that mediates antibiotic resistance. Direct sensing of β-lactams allows bacteria to respond rapidly to the presence of antibiotics, before cell-wall damage occurs; this study provides the first example of such a direct response in Gram-negative bacteria.

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> “the histidine kinase VbrK binds β-lactam antibiotics and, through the response regulator VbrR, triggers the expression of a β-lactamase, mediating antibiotic resistance”

ORIGINAL ARTICLE Li, L. *et al.* Sensor histidine kinase is a β-lactam receptor and induces resistance to β-lactam antibiotics. *Proc. Natl Acad. Sci. USA* **113**, 1648–1653 (2016)
FURTHER READING Blair, J. M. A. *et al.* Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* **13**, 42–51 (2015)