ANTIMICROBIALS

Promoting tolerance

Tolerance to antibiotics in genetically susceptible bacteria poses major problems for the treatment of infectious diseases and provides a source of resistant strains. Two papers published in *Science* now provide insight into the diverse mechanisms underlying this process.

When bacteria are starved of nutrients, as often occurs in the setting of an infection, they cease to grow, and tolerance to virtually all classes of antibiotics can arise. It had been assumed that this was due to the inactivity of the antibiotic targets in the non-growing cells. However, growth arrest resulting from nutrient starvation is associated with a range of physiological changes. Nguyen *et al.* sought to investigate the potential role of one of these starvation-induced changes, the stringent response, in antibiotic tolerance. The stringent response is activated on production of the alarmones guanosine pentaphosphate and guanosine tetraphosphate ((p)ppGpp), which are synthesized by the *relA* and *spoT* gene products and regulate the expression of a range of genes. The authors found that disruption of both *relA* and *spoT* in a strain of *Pseudomonas aeruginosa* eliminated production of (p)ppGpp in response to starvation and that this correlated with a dramatic reduction in the levels of ofloxacin tolerance compared with levels in wild-type cells, despite the fact that both strains were growth arrested. Similar reductions in tolerance were observed for four other antibiotics with diverse mechanisms of action, suggesting that it is the stringent response and not growth arrest that has a role in antibiotic tolerance. Furthermore, inactivation of the stringent response improved outcomes in experimental infections and eliminated the emergence of antibiotic-resistant clones.

> “bacteria tolerate antibiotics through managing the resulting oxidative effects.”

Recent work identified oxidative stress caused by the production of hydroxyl radicals ($OH^{\bullet}$) as a common mechanism by which antibiotic action results in bacterial cell death. Accordingly, Nguyen *et al.* observed a high level of $OH^{\bullet}$ in the *relA spoT* mutant strain, owing to increased production of pro-oxidant 4-hydroxy-2-alkylquinolones (HAQs). Interestingly, increasing HAQ levels in wild-type cells had little effect on antibiotic susceptibility, leading the authors to investigate whether the *relA spoT* strain also has impaired antioxidant defences. They found that, in addition to increased HAQ levels, the *relA spoT* strain had decreased levels of both catalase and superoxide dismutase, indicating that disruption of the stringent response leads to reduced antibiotic tolerance owing to both greater $OH^{\bullet}$ levels and impaired antioxidant defences.

Bacteria can also protect themselves against antibiotic-mediated oxidative stress by the production of nitric oxide (NO), which acts as a signalling molecule or gasotransmitter. H_2S is also known to act as a gasotransmitter in mammalian systems, although in bacteria the production of H_2S was considered to be only a by-product of sulphur metabolism. Interestingly, orthologues of mammalian enzymes that synthesize H_2S can be found in most bacterial genomes, leading Shatalin *et al.* to investigate possible physiological functions. They inactivated the H_2S synthesis genes in four clinically relevant and evolutionarily distant bacterial pathogens and subjected the resulting strains to analysis using phenotypic microarrays. The strains exhibited little or no defects in growth but were more susceptible to antibiotic-induced oxidative stress. Furthermore, increased levels of NO were observed in these mutants, whereas increased levels of H_2S were observed in NO biosynthesis mutants, suggesting that endogenously produced H_2S and NO act synergistically to confer multidrug resistance by acting as antioxidants.

Taken together, these papers provide important insights into the mechanisms by which bacteria tolerate antibiotics through managing the resulting oxidative effects. This work also provides new potential therapeutic avenues through which it might be possible to increase the efficacy and prolong the shelf life of our dwindling antibiotic arsenal.

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PHOTODISC

ORIGINAL RESEARCH PAPERS Nguyen, D. *et al.* Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. *Science* **334**, 982–986 (2011) | Shatalin, K. *et al.* H_2S: a universal defense against antibiotics in bacteria. *Science* **334**, 986–990 (2011)

FURTHER READING Kohanski, M. A. *et al.* How antibiotics kill bacteria: from targets to networks. *Nature Rev. Microbiol.* **8**, 423–435 (2010)