Antibiotics and antibiotic resistance made news on several fronts in the past year. Many public health organizations, including the CDC, used terms such as “crisis”, “catastrophic consequences”, and “nightmare scenario” to highlight the rapid emergence and spread of antibiotic resistance. A report from the Pew Commission on Industrial Farm Animal Production, on the fifth anniversary of the publication of its landmark 2008 report, noted that state and federal legislative efforts to limit non-therapeutic use of antibiotics in animal production were thwarted by drug and food animal industries. In its lobbying disclosures, the Farm Bureau stated that such efforts to limit use of animal antibiotics were “based on emotion and no credible peer reviewed science.” Meanwhile, there have been inexorable advances in our understanding of the molecular mechanisms by which antibiotics induce diversity and resistance in bacteria. This article reviews one study that probed the role of the bacterial general stress response in sub-inhibitory antibiotic-induced mutagenesis and antibiotic resistance.

Alterations induced by sub-inhibitory antibiotic doses include changes in gene expression, horizontal gene transfer, and mutagenesis. Antibiotic-induced gene expression can impact virulence, while increased mutagenesis and horizontal gene transfer can promote antibiotic resistance and spread. In bacterial and eukaryotic cells alike, low levels of antibiotics stimulate the generation of reactive oxygen species. Off-target effects of antibiotics on eukaryotic cells may explain their growth-promoting properties, as well as the specific side effects observed during therapeutic use.

In a recent study, Guttierez et al. explored the mechanism of antibiotic-induced mutagenesis in Escherichia coli, Vibrio cholerae, and Pseudomonas aeruginosa. Their results implicate a role for the general stress response, mediated by the alternate sigma factor RpoS (σS). Increased mutations likely resulted from the stabilization of the error-prone DNA polymerase PolIV and were facilitated by a decrease in MutS-dependent DNA mismatch repair.

Based on a detailed dissection of the phenomenon in E. coli, as well as other published work, the authors propose the following model: Sub-inhibitory antibiotic concentrations induce the general stress response, manifested by an increase in rpoS mRNA. Regulatory small RNAs (sRNA), and their chaperone Hfq, play a role in this antibiotic-mediated increase in rpoS mRNA. There is also an increase in misfolded and unfolded proteins in the stressed cells, and these are refolded or degraded by the ClpP-ClpX protease-chaperone complex. It is presumed, but not shown, that the...
ClpP-ClpX levels are not altered in the treated cells. Titration of ClpP-
ClpX spares several proteins normally downregulated by this complex, such as
RpoS and the error-prone polymerase PolIV. A role for both these proteins was
confirmed by examining ΔrpoS and ΔdnaH (ΔdinB encodes PolIV) strains: sub-
inhibitory antibiotics did not increase mutagenesis in these strains. Does RpoS
inhibit sub-inhibitory concentrations of antibiotics? Does RpoS and the error-
prone polymerase PolIV interact? ClpX spares several proteins normally
downregulated by this complex, such as RpoS and the error-prone polymerase
polymorphisms.

Antibiotics induce ROS production, and an earlier study implicated these
molecules in mutagenesis via perturbation of the TCA cycle. In their study,
Gutierrez et al. showed that the antibiotic treatment of both WT and ΔrpoS strains
resulted in similar increases in ROS, but antibiotic-induced mutagenesis was decreased in antibiotic-treated cells, and MutS overproduction abolished antibiotic-induced mutagenesis. Probing the role of RpoS in reducing MutS levels, the investigators showed that the RpoS-
induced small RNA SdsR bound to
mutS mRNA and inhibited translation. Consistent with this, antibiotic-dependent mutagenesis was significantly reduced in strains deleted for sdsR in and those
overexpressing MutS.

Several studies in the past year have continued to debate the role of reactive oxygen species (ROS) in antibiotic-mediated cell death. While a key 2007 paper made the case for ROS, specifically hydrogen radicals, as a common determinant of death induced by diverse antibiotics, three recent papers presented data inconsistent with this model. Even at sub-inhibitory levels, antibiotics induce ROS production, and an earlier study implicated these molecules in mutagenesis via perturbation of the TCA cycle. In their study, Gutierrez et al. showed that the antibiotic treatment of both WT and ΔrpoS strains resulted in similar increases in ROS, but antibiotic-induced mutagenesis was decreased in antibiotic-treated cells, and MutS overproduction abolished antibiotic-induced mutagenesis. Probing the role of RpoS in reducing MutS levels, the investigators showed that the RpoS-
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