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Off-label abuse of antibiotics by bacteria

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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.

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Sub-inhibitory and sub-therapeutic antibiotic concentrations influence many cellular responses and alter bacterial and eukaryotic cell physiology.[1] Bacteria may be exposed to low levels of antibiotics in the environment or in livestock animals where the compounds are used for growth promotion. Even during therapeutic use, bacteria encounter a range of antibiotic concentrations depending on the body site they occupy or their location within a biofilm.

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.[2]

In a recent study, Guttierez et al. explored the mechanism of antibiotic-induced mutagenesis in *Escherichia coli*, *Vibrio cholerae*, and *Pseudomonas aeruginosa*.[3] Their results implicate a role for the general stress response, mediated by the alternate sigma factor RpoS (σ^S). 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:[4] 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 Δ*rpoS* and Δ*dinB* (*dinB* encodes PolIV) strains: sub-inhibitory antibiotics did not increase mutagenesis in these strains. Does RpoS induce *dinB* transcription, as has been observed in carbon starvation-induced stress?[5] Curiously, no, since *dinB* mRNA was not significantly altered in antibiotic-treated cells.

What, then, is the role of RpoS in this process? The general stress response mediated by RpoS results in the altered expression of hundreds of genes, any of which could play a role in sub-inhibitory antibiotic-mediated mutagenesis. The clue to the relevant molecule(s) was in the nature of mutations induced by ampicillin: overall, the frequency of frame-shift mutations, base substitutions, and insertion sequence mobility were increased. Notably, there were >9-fold increases in GC→AT and AT→GC transitions, characteristic of a deficiency in mismatch repair. Indeed, MutS, which detects and binds to mismatches, 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* and in those overexpressing MutS.

Several studies in the past year re-ignited the debate about the role of reactive oxygen species (ROS) in antibiotic-mediated cell death.[6] While a key 2007 paper made the case for ROS, specifically hydroxyl radicals, as a common determinant of death induced by diverse antibiotics, three recent papers presented data inconsistent with this model.[6] Even at sub-inhibitory levels, antibiotics induce ROS production, and an earlier study implicated these molecules in mutagenesis via perturbation of the TCA cycle.[7] In their study, Guttierez et al. showed that the antibiotic treatment of both WT and Δ*rpoS* strains resulted in similar increases in ROS, but increased mutagenesis was observed only in the former.[3] The implication is that even if ROS played a role in increased mutagenesis (for instance, by generating the misfolded proteins that titrate ClpP-ClpX levels), these effects are mediated via RpoS.

The present study, as well as others in this vein, has implications well beyond the induction of antibiotic resistance. Antibiotic-induced mutagenesis likely accelerates bacterial adaptive evolution. Low levels of antibiotics contribute to strain diversification in organisms such as *Pseudomonas aeruginosa*, though the precise mechanisms are still in debate.[8] Is it possible that antibiotics also trigger similar signaling and mutagenic alterations in commensal bacterial populations? Sub-inhibitory concentrations of piperacillin and/or tazobactam, for instance, were recently shown to induce broad proteomic alterations in *Bacteroides fragilis*.[9] The persistent mutagenesis and selection environment distinct to each individual may thus constantly engineer the microbiome for optimal adaption to unique environments—the differences being observable not so much at the species level, but rather at the level of nucleotide polymorphisms.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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