

Alternative explanation for indole-induced antibiotic tolerance in *Salmonella*

We were interested to read the recent publication by Vega et al. (1), which suggests that indole is an interspecies signal that causes *Salmonella* to become less susceptible to antibiotics due to activation of the oxidative stress response. However, we believe that there is an alternative explanation for their data: the drug tolerance phenotype is due to increased production of one or more multidrug efflux pumps. The drugs tested by Vega et al., ciprofloxacin and carbenicillin, are known substrates of the AcrB transporter, and indole has been shown to induce the production of efflux pumps in both *Escherichia coli* and *Salmonella* (2). In *Salmonella*, induction is mediated by increased expression of the transcriptional activator RamA, which regulates expression of *acrAB* (2). Therefore, the indole-induced drug tolerance seen by Vega et al. could result from induction of the multidrug resistance (MDR) AcrAB-TolC efflux system, leading to increased tolerance to these two drugs. This hypothesis is noted by the authors but dismissed after their RT-PCR experiments showed that expression of the *ramA* gene was reduced in the presence of indole. We are perplexed by this observation because it conflicts with all previous studies including measurements using gene reporter constructs, RT-PCR, microarray, and Western

blotting (2), which reveal that *ramA*/RamA is induced by indole in numerous different *Salmonella* strains including strain LT2 used by Vega et al. in their experiments. Furthermore, the authors used these RT-PCR data as evidence that efflux is not involved in the phenotype. We believe that this is a mistaken assumption for two reasons. First, efflux was not measured. This is surprising because efflux of ciprofloxacin and other compounds can be measured easily using one of several published methods to quantify accumulation or efflux of fluorescent substrates (3, 4). Second, regulation of MDR efflux pumps in Gram-negative bacteria is complex and multifactorial. For instance, other transcription factors, including MarA, SoxS, and Rob, can also regulate expression of MDR efflux pumps, and indole induces expression of *soxS* in *E. coli* (5).

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