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Thiol oxidation and CoA depletion cause antibiotic tolerance

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The widespread use of antibiotics promotes the development and dissemination of resistance mechanisms. While resistant bacteria are able to grow in the presence of antibiotics, tolerance limits killing by bactericidal drugs, often yielding subpopulations of persisters that can seed regrowth and serve as a reservoir for resistance development. Despite of its role in treatment failures, the molecular understanding of antibiotic tolerance is still limited, hampering effective diagnostic and therapeutic approaches. Here, we apply a non-biased approach based on global proteome and metabolome analyses of hyper-tolerant *Pseudomonas aeruginosa* isolates in combination with an iterative machine learning approach that identified thiol groups as accurate predictors of antimicrobial tolerance. Single cell analyses of thiol oxidation directly linked tolerance to cysteine hyper-oxidation, arguing that unbalanced redox physiology represents a general driver of antibiotic tolerance. In line with this, we identify coenzyme A depletion as a key parameter mediating drug tolerance in *P. aeruginosa*. Boosting coenzyme A availability with engineered catalysts from *Staphylococcus aureus* largely restored drug susceptibility of hyper-tolerant *P. aeruginosa* strains, establishing a causal link between redox physiology and drug susceptibility. We show that these physiological aberrations can robustly predict tolerance in clinical isolates of *P. aeruginosa* opening up new ways for rapid tolerance diagnostics. By defining redox imbalances as a major driver of drug tolerance in an important human pathogen, this study provides an entry into deciphering the molecular and cellular underpinnings of antibiotic resilience and paves the way to novel diagnostic and therapeutic lines of attack against infection relapses.