Suppression of reactive-oxygen-species accumulation accounts for paradoxical bacterial survival at high quinolone concentration

Gan Luan[1], Yuzhi Hong[1], Karl Drlica[1], Xilin Zhao[1,2#]

[1]Public Health Research Institute and Department of Microbiology, Biochemistry, and Molecular Genetics, New Jersey Medical School, Rutgers Biomedical and Health Sciences, Rutgers University, 225 Warren St., Newark NJ 07103

[2]State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics, School of Public Health, Xiamen University, South Xiang-An Road, Xiang-An District, Xiamen, Fujian Province, 361102, China

Two supplementary figures:

Fig. S1. Effect of chloramphenicol or bipyridyl plus thiourea on bacterial growth rate, survival, and ROS accumulation. At sub-MIC concentrations, the selected agents slow bacterial growth rate to the same extent, but the bipyridyl-thiourea combination is far more effective at blocking killing and the accumulation of ROS.

Fig. S2. Exogenous ROS can eliminate inhibition of nalidixic acid-mediated killing by chloramphenicol. Nalidixic acid, presumably due to DNA lesions introduced, causes sublethal concentrations of hydrogen peroxide to become lethal. Inhibition of protein synthesis by chloramphenicol has no effect on peroxide-mediated killing and is therefore unlikely to affect the introduction of peroxide-sensitive lesions by nalidixic acid.

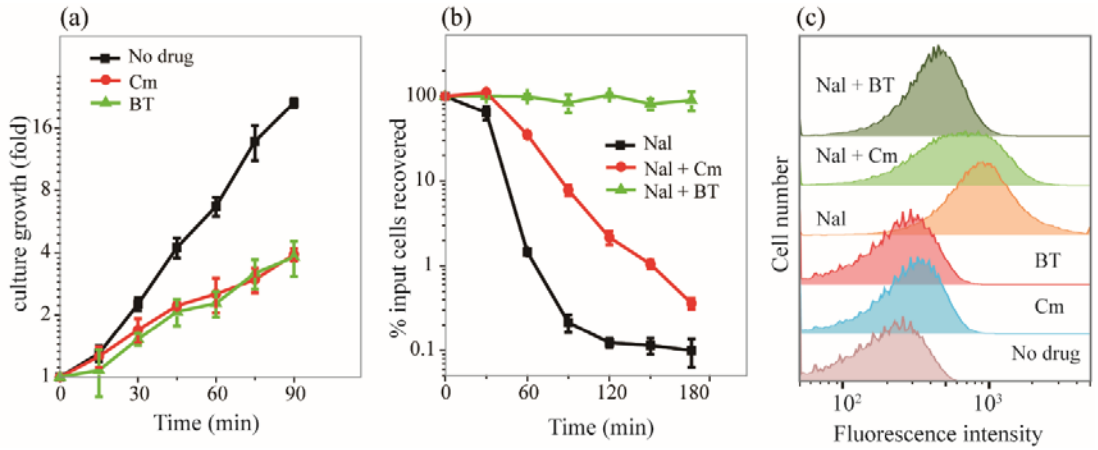

Fig. S1. Effect of chloramphenicol or bipyridyl plus thiourea on bacterial growth rate, nalidixic acid-mediated killing, and ROS accumulation. (a) Reduction of growth rate. Exponentially growing cultures of wild-type *E. coli* (strain 3505) were treated with the indicated compounds, and CFU were measured at various times. (b) Bacterial survival. Exponentially growing cultures of wild-type *E. coli* were treated with the indicated inhibitors, and then survival was measured relative to an untreated control assayed at the time of inhibitor addition. For panels (a) and (b), data represent means from three independent experiments; error bars represent standard error of mean. (c) ROS accumulation. Exponentially growing cultures of wild-type *E. coli* were treated with the indicated compounds plus carboxy-H2DCFDA (10 μM) for 3 h. Cells were observed by flow cytometry as described in Methods and Materials in the main text. Three independent experiments gave similar results. Abbreviations: Nal, nalidixic acid at 100 μg/ml; BT, bipyridyl plus thiourea, both at half MIC; Cm, chloramphenicol at 2 μg/ml; Na + BT, combination of nalidixic acid at 100 μg/ml with BT at half MIC; Nal + Cm, combination of nalidixic acid at 100 μg/ml with chloramphenicol at 2 μg/ml.

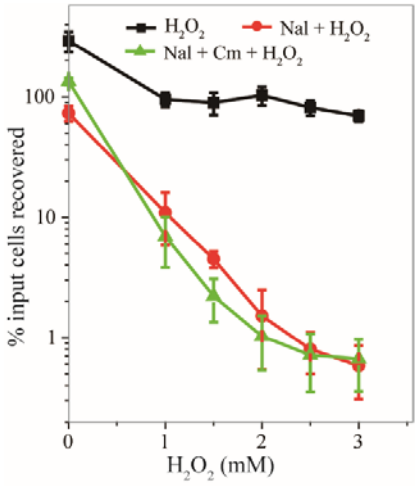

Fig. S2. Exogenous ROS can eliminate inhibition of nalidixic acid-mediated killing by chloramphenicol. Exponentially growing cultures of wild-type *E. coli* (strain 3505) were treated with indicated concentrations of hydrogen peroxide (H_2O_2) alone or either plus nalidixic acid (Nal + H_2O_2) or plus both nalidixic acid and chloramphenicol (Nal + Cm + H_2O_2) for 30 min. Survival was measured as described in Methods in the main text. Nalidixic acid concentration was 100 μg/ml, while chloramphenicol concentration was 20 μg/ml.