Supplemental Information: Pervasive and diverse collateral sensitivity profiles inform optimal strategies to limit antibiotic resistance

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The Supplemental Information (SI) contains six additional figures (S1-S6) and one table (S1).

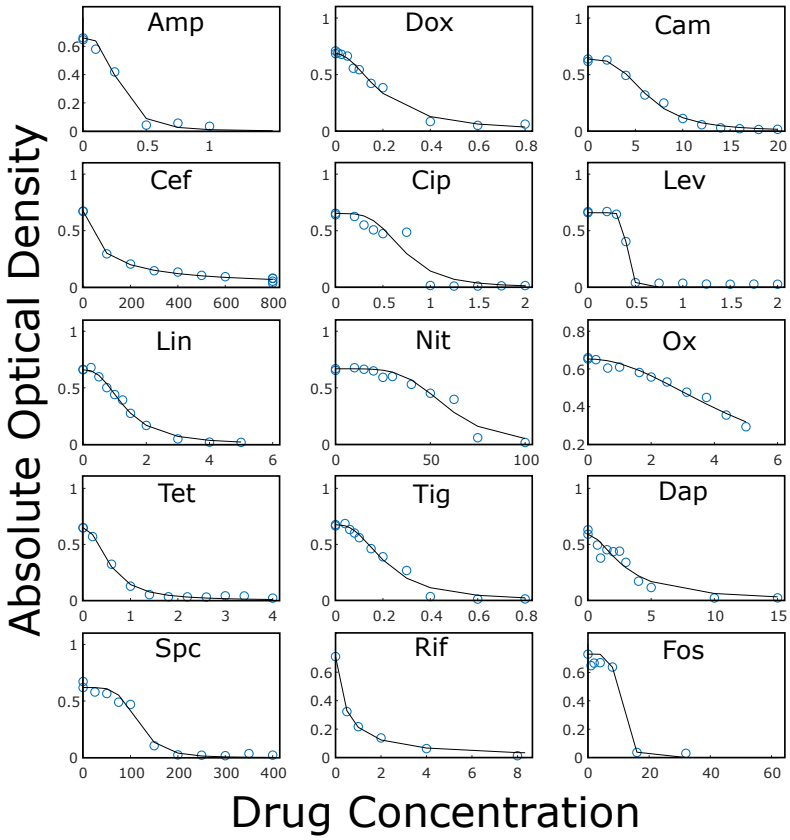

FIG. S1. **Example dose response curves for each drug** Optical density (OD) of V583 cultures after 12 hours of incubation at various drug concentrations (blue circles). All drug concentrations are measured in μg/mL. Lines: fit of normalized dose response curve to Hill-like function $f(x) = (1 + (x/K)^h)^{-1}$, with K the IC_{50} and h a Hill coefficient.

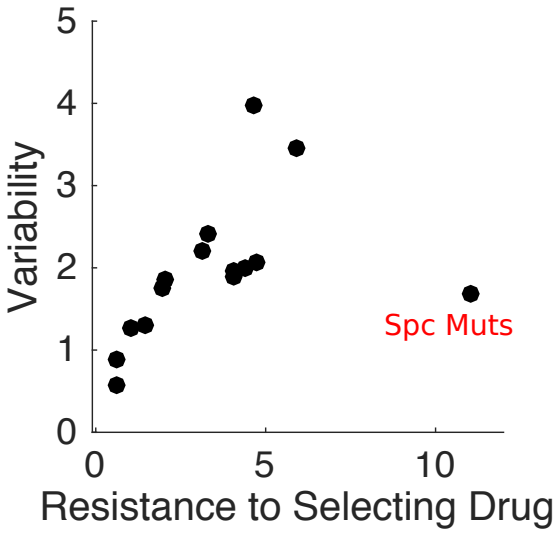

FIG. S2. **Variation in collateral profiles is correlated with resistance to selecting drug.** Variability in collateral profiles between mutants selected by the same drug is defined by first representing each mutant's collateral profile as a vector $\bar{C}$ in 15-dimensional drug space. Dimension i represents the $\log_2$-scaled fold increase in IC_{50} (relative to wild-type) for drug i. The variability for a set of mutants evolved to the same drug is then given by the average Euclidean distance d_i for a mutant from the centroid. Scatter plot between the variability (with effects of selecting drug included) and the ($\log_2$-scaled) fold increase in IC_{50} to the selecting drug (Spearman correlation of 0.70, $p = 0.005$ including the spc mutants; 0.87, $p < 10^{-4}$ without the spc mutants.).

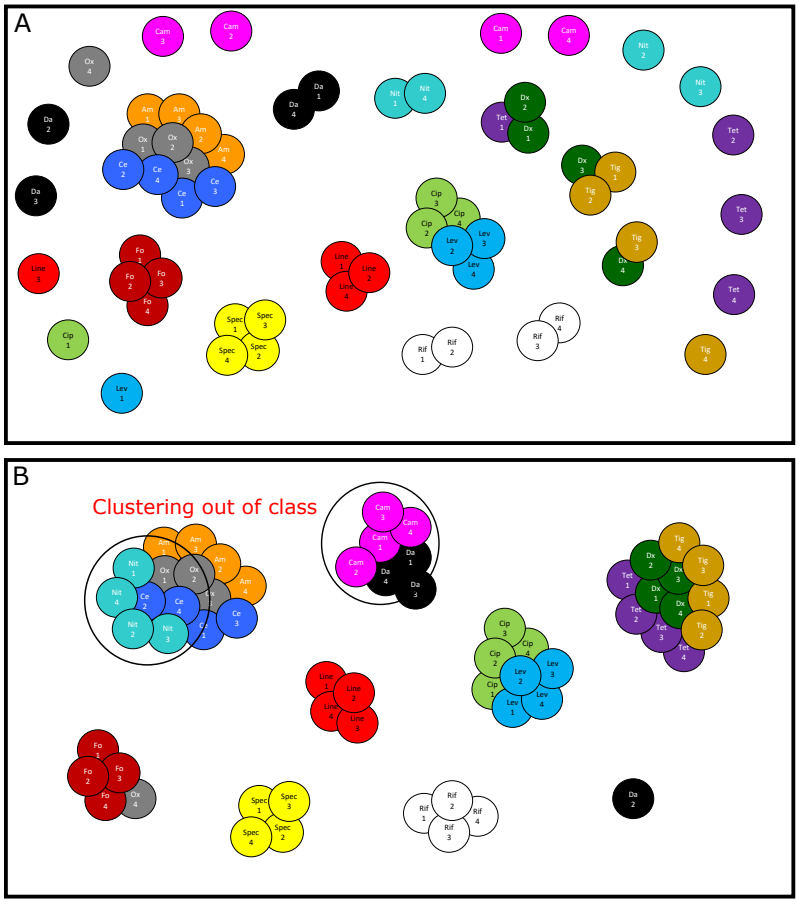

FIG. S3. **Hierarchical clustering of collateral sensitivity profiles partitions mutants into groups selected by known drug classes** A. Each circle represents a single mutant. Color depicts drug used for selection. Low-level clustering is largely characterized by grouping of mutants evolved to the same drug (i.e. replicate evolution experiments). However, in several cases mutants selected by one drug (e.g. Cip) cluster with mutants selected by a different drug (Lev) of the same class. B. At later stages of clustering, mutants evolved to drugs from a similar class–or with similar mechanisms of action– tend to cluster together. However, the two drugs for which high-level resistance was not achieved (Nit and Cam) cluster with drugs from different classes (black circles).

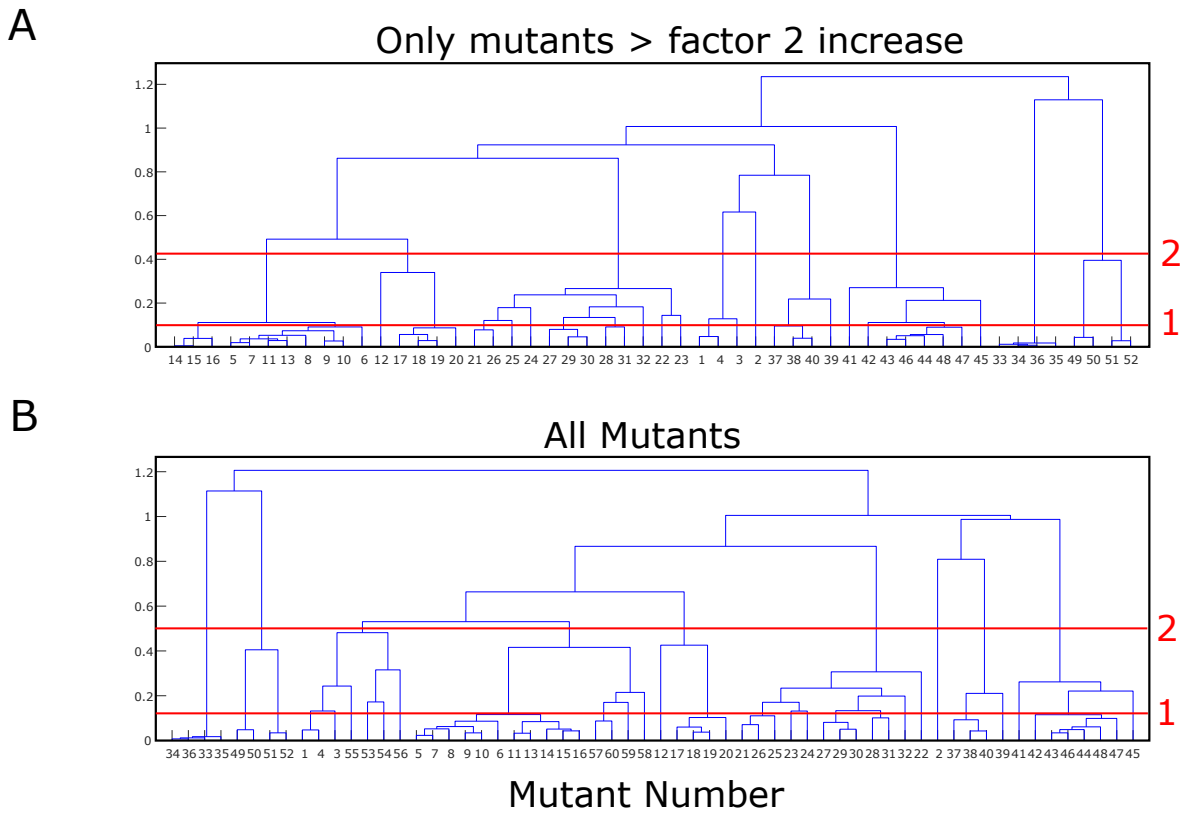

FIG. S4. **Dendrograms generated by hierarchical clustering** Dendograms generated by hierarchical clustering for mutants that exhibit high-level (more than 2x increase in IC_{50}) resistance (A) and for all mutants (B). Red horziontal lines indicate clustering levels depicted in Figure 4 and Figure S3. See Table S1 for mutant number key.

TABLE I. Mutant Number Table For Dendrograms

Mutant Number	**Drug Name**
1-4	Daptomycin
5-8	Ampicillin
9-12	Oxacillin
13-16	Ceftriaxone
17-20	Fosfomycin
21-24	Tetracycline
25-28	Doxycycline
29-32	Tigecycline
33-36	Spectinomycin
37-40	Linezolid
41-44	Ciprofloxacin
45-48	Levofloxacin
49-52	Rifampicin
53-56	Chloramphenicol
57-60	Nitrofurantoin

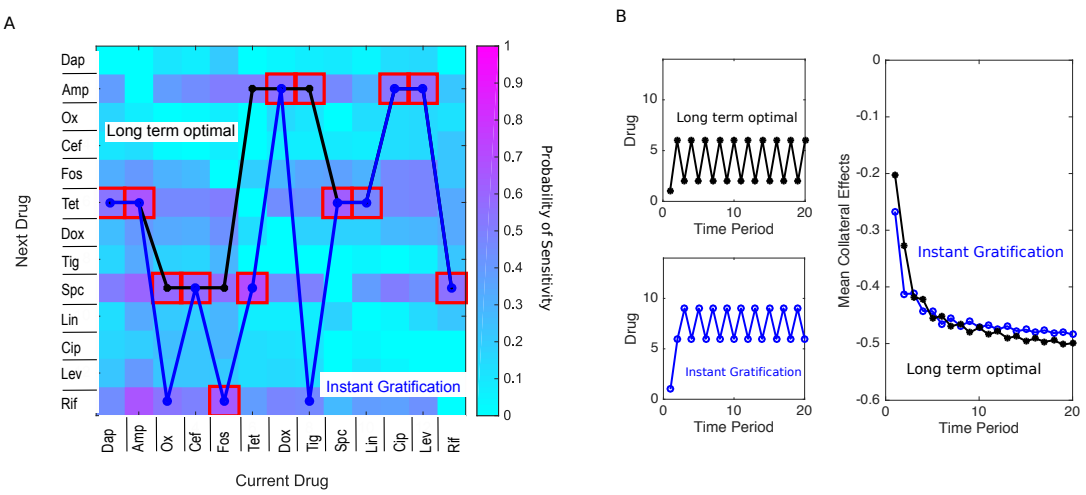

FIG. S5. **MDP model with integer cost function and allowed transitions to any sensitivity profile with resistance to the current drug** A. Heat map indicates the probability (over available states) of collateral sensitivity to the next drug (rows) given a particular selecting drug (columns). At each time step, a transition can occur to any sensitivity profile exhibiting resistance to the current drug. Black stars: optimal short term policy (instant gratification; $\gamma = 0$)); blue circles: optimal long-term policy ($\gamma = 0.95$). Red squares indicate maximum of each column. Note that because the MDP minimizes cost rather than maximizing probability of sensitivity, even the short-term solution does not always maximize the probability of sensitivity at the next step (because resistance is punished). B. Left panels: optimal drug cycles, starting from drug 1 (Dap), for long term (upper panel) and instant gratification (lower panel) strategies. Long-term strategy asymptotically approaches a cycle between drugs 6 (Tet) and 2 (Amp); the instant gratification strategy approaches a cycle between drugs 6 (Tet) and 9 (Amp). Right panel: mean collateral effects (cumulative) for the long-term strategy (black), instant gratification strategy (blue), and random drug cycles (red, dashed). The mean (cumulative) collateral effect at time step t_i is given by $\langle \sum_{t=0}^{t_i} \frac{r_t}{t+1} \rangle$, where brackets indicate an average over 1000 independent simulations of the MDP. Here r_t is -1, 0, or 1 if the profile at the current time step is sensitive to, not affected by, or resistant to the current drug, respectively.

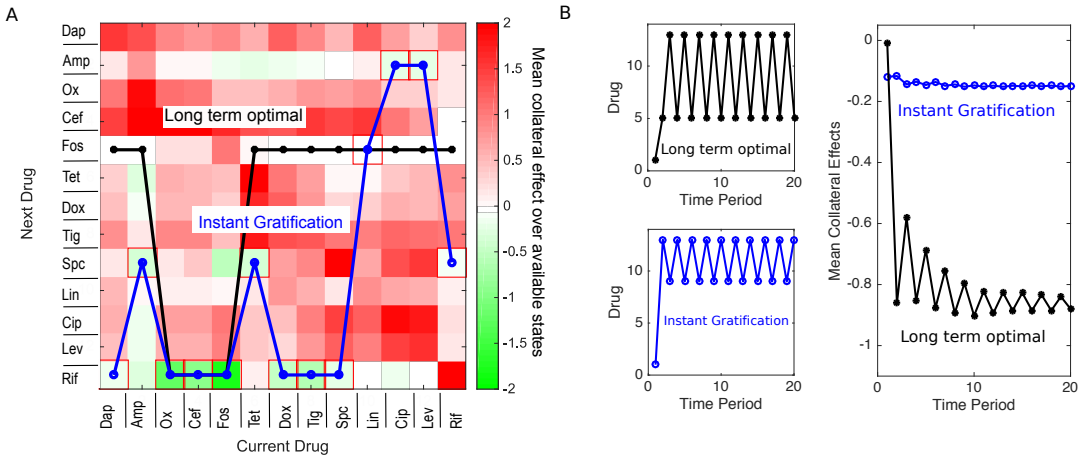

FIG. S6. **MDP model with non-integer cost function and allowed transitions to any sensitivity profile with resistance to the current drug** A. Heat map indicates the mean collateral sensitivity (over available states) to the next drug (rows) given a particular selecting drug (columns). At each time step, a transition can occur to any sensitivity profile exhibiting resistance to the current drug. Black stars: optimal short term policy (instant gratification; $\gamma = 0$)); blue circles: optimal long-term policy ($\gamma = 0.95$). Red squares indicate minimum of each column. B. Left panels: optimal drug cycles, starting from drug 1 (Dap), for long term (upper panel) and instant gratification (lower panel) strategies. Long-term strategy asymptotically approaches a cycle between drugs 5 (Fos) and 13 (Rif); the instant gratification strategy approaches a cycle between drugs 9 (Spc) and 13 (Rif). Right panel: mean collateral effects (cumulative) for the long-term strategy (black), instant gratification strategy (blue), and random drug cycles (red, dashed). The mean (cumulative) collateral effect at time step t_i is given by $\langle \sum_{t=0}^{t_i} \frac{r_t}{t+1} \rangle$, where brackets indicate an average over 1000 independent simulations of the MDP. Here r_t is the value C of collateral sensitivity or resistance to the current drug.