SUPPLEMENTARY INFORMATION for

Evaluating the potential efficacy and limitations of a phage for joint antibiotic and phage therapy of *Staphylococcus aureus* infections

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I. Host range

In our report, we summarized the evidence that PYO^{Sa}'s host range included all 83 clinical isolates of *Staphylococcus aureus* tested. Much of those data are from LabCorp. The methods used are summarized in the following and the results obtained are summarized in Supplemental Table S1.

Methods

The methods LabCorp employed, as detailed below, to test the sensitivity of these strains to PYO^{Sa} were somewhat different than those we employed and presented in our report (See Results Section 1).

(i) The *S. aureus* strains tested were streaked for single colonies on TSB agar.

(ii) These single colonies were inoculated in 3 ml LB broth and grown overnight with shaking at 37°C.

(iii) The overnight cultures were diluted by 1:30 in 3ml LB broth and grown with shaking at 37°C to an OD 600nm of between 0.3-1.0.

(iv) 300ul of these cultures were added to 3ml LB soft (0.3% or 0.5%) agar supplemented with 3ul 1M $CaCl_2$ and 3ul 1M $MgCl_2$ which were spread onto LB agar plates to form a lawn.

(v) 5µl of the PYO^{Sa} lysates were spotted onto the lawns and incubated overnight.

(vi) Lysates were prepared from original spot plates and then re-spotted on the original spot plate host bacteria following the above conditions.

The Key to scoring the results of this assay follows:

Key	
-	No lysis
-/+	Weak lysis
+	Partial lysis (cloudy)
++	Complete lysis

Table S1 Assay for the susceptibility of *S. aureus* to phage PYO^{Sa}. Data provided by LabCorp.

Species	Strain	Spot	Re-spot
Staphylococcus aureus	27660	++	++
Staphylococcus aureus	RN6390	+	-/+
Staphylococcus aureus	502A	++	++
Staphylococcus aureus	6538	++	++
Staphylococcus aureus	25923	++	++
Staphylococcus aureus subsp. aureus	12600	++	++
Staphylococcus aureus	RN4220	++	++
Staphylococcus aureus subsp. aureus	BAA-1721	++	++
Staphylococcus aureus subsp. aureus	14775	++	++
Staphylococcus aureus	AR0461	++	++
Methicillin-resistant *S. aureus*	017-243	++	++
Methicillin-resistant *S. aureus*	041-332	+	-/+
Methicillin-resistant *S. aureus*	045-188	++	++
Methicillin-resistant *S. aureus*	038-401	+	++
Methicillin-resistant *S. aureus*	049-841	++	++
Methicillin-resistant *S. aureus*	USA300	+	-
Methicillin-resistant *S. aureus*	002-639	+	+
Methicillin-resistant *S. aureus*	049-60	++	++
Methicillin-resistant *S. aureus*	026-485	++	++
Methicillin-resistant *S. aureus*	011-719	++	++
Methicillin-resistant *S. aureus*	045-696	++	++
Methicillin-resistant *S. aureus*	BAA-1707	+	-/+
Methicillin-resistant *S. aureus*	BAA-1717	+	+
Methicillin-resistant *S. aureus*	BAA-1720	++	++
Methicillin-resistant *S. aureus*	BAA-1747	+	-
Methicillin-resistant *S. aureus*	BAA-1754	++	+
Methicillin-resistant *S. aureus*	BAA-1761	++	++
Methicillin-resistant *S. aureus*	BAA-1763	++	++
Species	**Strain**	**Spot**	**Re-spot**
Methicillin-resistant *S. aureus*	BAA-1764	++	-/+
Methicillin-resistant *S. aureus*	BAA-1766	++	+

Methicillin-resistant *S. aureus*	BAA-1768	++	++
Methicillin-resistant *S. aureus*	BAA-41	++	++
Methicillin-resistant *S. aureus*	BAA-42	++	-/+
Multidrug-resistant resistant *S. aureus*	BAA-44	++	+
Methicillin-resistant *S. aureus*	BAA-1683	++	+
Methicillin-resistant *S. aureus*	BAA-2094	++	++
Methicillin-resistant *S. aureus*	BAA-2313	++	++
Methicillin-resistant *S. aureus*	33592	++	+
Methicillin-resistant *S. epidermis*	700583	-/+	-
Methicillin-resistant *S. haemolyticus*	700564	-/+	-
Staphylococcus aureus subsp. aureus	BAA-1718	++	++
Methicillin-resistant *S. aureus*	AR0462	++	-
Methicillin-resistant *S. aureus*	AR0463	++	++
Methicillin-resistant *S. aureus*	AR0464	-/+	-/+
Methicillin-resistant *S. aureus*	AR0465	++	+
Methicillin-resistant *S. aureus*	AR0466	+	-
Methicillin-resistant *S. aureus*	AR0467	++	+
Methicillin-resistant *S. aureus*	AR0468	++	+
Methicillin-resistant *S. aureus*	AR0469	++	+
Methicillin-resistant *S. aureus*	AR0470	++	+
Methicillin-resistant *S. aureus*	AR0471	++	-
Methicillin-resistant *S. aureus*	AR0472	++	++
Methicillin-resistant *S. aureus*	AR0473	++	++
Methicillin-resistant *S. aureus*	AR0474	++	+
Methicillin-resistant *S. aureus*	AR0475	+	-
Methicillin-resistant *S. aureus*	AR0476	++	++
Methicillin-resistant *S. aureus*	AR0477	++	++
Methicillin-resistant *S. aureus*	AR0478	++	++
Methicillin-resistant *S. aureus*	AR0479	++	++
Species	**Strain**	**Spot**	**Re-spot**
Methicillin-resistant *S. aureus*	AR0480	+	+
Methicillin-resistant *S. aureus*	AR0481	++	++

Methicillin-resistant *S. aureus*	AR0482	++	++
Methicillin-resistant *S. aureus*	AR0483	++	++
Methicillin-resistant *S. aureus*	AR0484	++	++
Methicillin-resistant *S. aureus*	AR0485	++	++
Methicillin-resistant *S. aureus*	AR0486	++	++
Methicillin-resistant *S. aureus*	AR0487	+	-/+
Methicillin-resistant *S. aureus*	AR0488	+	-/+
Methicillin-resistant *S. aureus*	AR0489	+	-
Methicillin-resistant *S. aureus*	AR0490	++	++
Methicillin-resistant *S. aureus*	AR0491	-/+	-
Methicillin-resistant *S. aureus*	AR0492	+	-

II. Variation in the demise – resurrection population dynamics presented in Figure 1B.
To explore the generality of the PYO^{Sa} – *S. aureus* Newman serial transfer results presented in Figure 1B, we performed the experiments with ten 2 ml and six 10ml cultures. Both had effectively the same initial densities of phage and bacteria. The dynamics of only one of the ten 2 ml samples serially transferred were similar to the demise–resurrection dynamics observed from Figure 1B. While all six 10 ml serial transfer cultures were turbid by the 5th transfer, some did not become turbid until the 4th transfer. We interpret this observation to be consistent with the hypothesis that the resurrection of these phage-exposed bacteria is a stochastic process in *S. aureus* and thus is more likely to occur in the 10ml populations because the total number of bacteria is 5-fold greater than that in 2 ml cultures.

Also consistent with this stochastic hypothesis is that the time before the recovery of the *S. aureus* population due to the ascent of small colony variants, S, is variable. In Figure 1B, all three serial transfer populations started the recovery to high density by the second transfer. This was not the case for a repeat of this experiment. The recovery of one of the three populations (noted in red) started at the end of the first transfer (Figure S1A).

In Figure 1D, where the serial transfer cultures were initiated with *S. aureus* isolated at the end of serial transfer experiments with the PYO^{Sa}, the evolved bacteria, the phage were maintained in one of three cultures and were lost in two. In the repeat of this experiment with three independently evolved strains, the phage were lost in all three (Figure S1B).

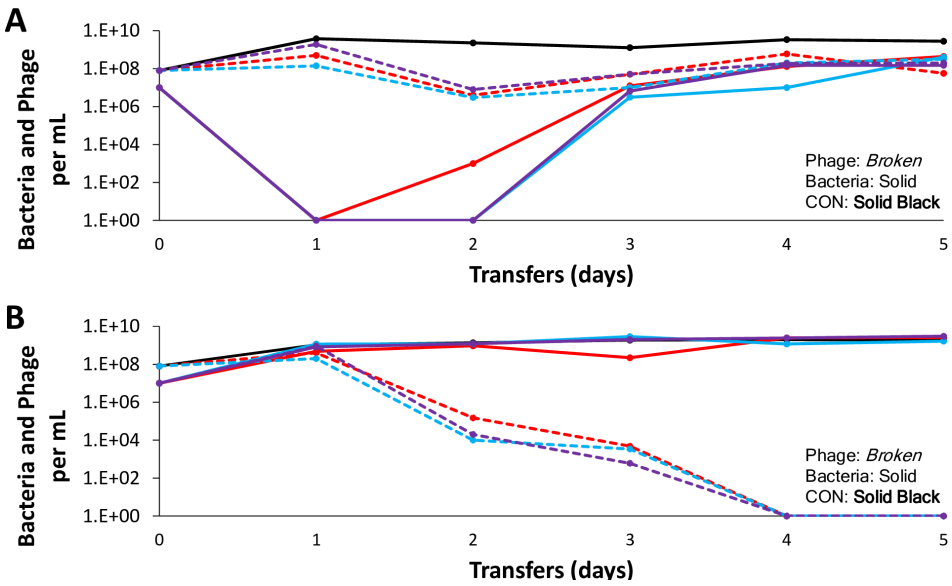

Figure S1 Changes in the densities of bacteria and phage in (1/100) 10 ml serial transfer cultures of *S. aureus* Newman and PYO^{Sa} and a phage-free control, CON. **A)** Three independent cultures initiated with *S. aureus* Newman (W) that had not previously been exposed to PYO^{Sa}. **B)** Three independent cultures initiated with *S. aureus* Newman (E) obtained from the 5th transfer of independent serial passage experiments with *S. aureus* Newman mixed with PYO^{Sa}.

III. Genotypes and phenotypes of *S. aureus* mutants selected in the presence of PYO^{Sa}

Table S2. Small colony variant (S) mutants selected by exposure to PYO^{Sa} in experiments of the type shown in Fig. 1*B*.

Gene	Mutation
femA	G>T (nt -9)
femA	G>A (nt -12)
femA	M1I
femA	Δ G124 - A126
femA	I171AN
femA	Y327D
femA	72 bp Dup (nt 987-1058)
femA	G331D
femA	G368V

Mutations in *femA* were identified after whole genome sequencing of small colony mutants.

Table S3. Genotypes of evolved (E) mutants from 5 different small colony variant (S) mutants.

Primary Mutation	Compensatory mutation(s)	Annotated function of compensatory mutation(s)
femA nt(-9) G>T	*mgrA* W48*	HTH-type transcriptional regulator
femA Met1Ile	*cshA* Δ258bp	DEAD-box ATP-dependent RNA helicase
femA Met1Ile	*mgrA* Δnt 160-216	HTH-type transcriptional regulator
femA Met1Ile	*rpsL* G126D	30S ribosomal protein
femA Tyr327Asp	*clpX* G177fs	ATP-dependent Clp protease
femA Tyr327Asp	CNH36_00075 H326Q	Cyclic-di-AMP phosphodiesterase
femA Tyr327Asp	CNH36_06865 Δnt 1011-1058	2-oxoacid:acceptor oxidoreductase subunit alpha
femA Tyr327Asp	CNH36_12385 W117L	Lysostaphin
femA Tyr327Asp	*dacA* V78G	Diadenylate cyclase
femA Tyr327Asp	*femA* Q346R	*femA* internal suppressor
femA Tyr327Asp	*lpdA* E245*	Dihydrolipoyl dehydrogenase
femA Tyr327Asp	*nrnA* Q303*	Bifunctional oligoribonuclease PAP phosphatase
femA Tyr327Asp	*sarA* K72::IS1181	Transcriptional regulator
femA Tyr327Asp	*sarA* R84C, CNH36_04445 N3S	Transcriptional regulator, uncharacterized protein
femA Tyr327Asp	*sarA* Y51*	Transcriptional regulator
femA Gly331Asp	*lipL* V94fs	Octanoyl-[GcvH]:protein N-octanoyltransferase
femA Gly331Asp	*sarA* Inv (nt -51 to +78)	Transcriptional regulator
femA Gly331Asp	*sarA* R90K	Transcriptional regulator
femA Gly368Val	*rpoD* G77fs	RNA polymerase sigma factor

Secondary compensatory mutations associated with the evolved state were identified by whole genome sequencing.

Table S4. Relative growth rates and MICs of wild-type (W), small colony variant (S), and evolved (E) compensated mutants.

Genotype / Mutations		Growth Rate	MIC mg/L	
Primary	**Compensatory**	**Relative**	**Lysostaphin**	**Oxacillin**
Wild-type		1	≤ 0.008	2
femA M1I		0.53	1	0.25
femA I171N		0.48	4	0.125
femA Y327D		0.59	>4	0.125
femA Y327D	*femA* Q346R	0.76	0.125	0.063
femA Y327D	*sarA* K72::IS1181	0.93	>4	0.125
femA G331D		0.53	>4	0.063
femA G331D	*sarA* INV (nt -51-+78)	0.94	>4	0.125

Doubling time for wild-type is 25.8 min.

IV. A hypothesis to account for the population and evolution dynamics of the *S. aureus* and PYO^{Sa}.

In our article we postulated that the dynamics presented in Figure 1B can be attributed to the generation of small colonies from the wild-type *S. aureus* Newman and their ascent due to selection mediated by PYO^{Sa} phage. While the PYO^{Sa} phage adsorb to these small colony

variants, they do not kill them, and the infecting phage are lost. The phage are maintained because the small colony variants are of low fitness and continually produce normal or near normal colony variants, an "evolved" state, that can support the replication of the phage. These evolved cells are not stable and continually produce small colonies. This hypothesis is illustrated in Figure S2.

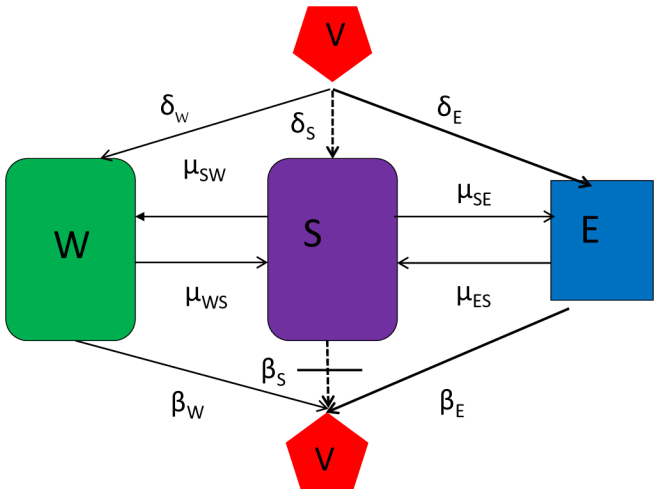

Figure S2 – A population dynamic model to account for the observed changes in the densities of bacteria and phage in Figure 1B. The variables, W, S and E are, respectively the wildtype *S. aureus* Newman, small colonies, and the evolved bacteria, cells per ml, and V the PYO^{Sa} phage, particles per ml. The parameters δ_W, δ_S and δ_E are the adsorption rate constants per cell per ml. The parameters β_W, β_S and β_E are the number of phage particles produced per infected cell burst. We assume that $\beta_S=0$, the S are infected by PYO^{Sa} but the phage but do not replicate or kill these bacteria. The parameters μ_{SW}, μ_{WS}, μ_{SE} and μ_{ES} are the transition rates, per cell per hour, between the different states.

The population is in liquid culture in which there is a limiting resource of concentration, r, μg/ml. The net growth rate of the bacteria is proportional to its maximum rate of growth and a hyperbolic function of concentration of the limiting resource, r , μg/ml and a constant, k_i which is the concentration of the resource when the growth rate is half its maximum value., where the subscript i is W, S or E (1). We assume there is no latent period and upon adsorption, W and E are killed by the phage and instantly produce, β_W and β_E phage particles per cell (2). Although the phage adsorb to the small colonies, they are not killed. As the bacteria grow the concentration of the resource declines at a rate proportional to the net growth rates of the bacteria, and a conversion efficiency parameter, e_i μg/ml, the conversion efficiency, which is the amount of resource needed to produce a new cell of that type (3). To account for the declining physiological state of the bacteria as the bacteria approach stationary phase, r=0, we assume that the rates of phage adsorption and transitions between states is proportional to the net growth rate of that cell line. With these definitions and assumptions, the rates of change in the densities of the bacteria of

different states, the density of free phage, and the concentration of the resource are given by the below set of coupled differential equations.

$$\frac{dr}{dt} = -v_W \bullet \psi_W(r) \bullet e_W \bullet W \; - v_S \bullet \psi_S(r) \bullet e_S \bullet S \; - v_E \bullet \psi_E(r) \bullet e_E \bullet E$$

$$\frac{dW}{dt} = v_W \bullet \psi_W(r) \bullet W - \delta_W \bullet W \bullet V \bullet \psi_W(r) + \mu_{SW} \bullet E \bullet \psi_S(r) - \mu_{WS} \bullet W \bullet \psi_W(r)$$

$$\frac{dS}{dt} = v_S \bullet \psi_S(r) \bullet S + \mu_{WS} \bullet W \bullet \psi_W(r) \; - \mu_{SW} \bullet S \bullet \psi_S(r)$$

$$\frac{dE}{dt} = v_E \bullet \psi_S(r) \bullet E \; - \delta_E \bullet E \bullet V \bullet \psi_E(r) \; + \mu_{SE} \bullet S \bullet \psi_S(r) \; - \mu_{ES} \bullet E \bullet \psi_E(r)$$

$$\frac{dV}{dt} = \delta_W \bullet W \bullet V \bullet \psi_W(r) \bullet \beta_W \; + \delta_E \bullet E \bullet V \bullet \psi_E(r) \; - \delta_S \bullet V \bullet S \bullet \psi_S \bullet (r)$$

$$where \; \psi_W(r) = \frac{r}{(r + k_W)} \, , \psi_S(r) = \frac{r}{(r + k_S)} \, , and \; \psi_E(r) = \frac{r}{(r + k_E)}$$

Numerical Solutions – Simulations: To solve this set of coupled differential equations and those for the models that follow we use Berkeley Madonna. The population growth and phage infection parameters employed for these numerical solutions, simulations, are of the range estimated for PYO^{Sa} and *S. aureus* Newman in MHII medium. To simulate a serial transfer mode of population maintenance every 24 hours there is a 100-fold reduction in densities of the bacteria and phage and the resource concentration is restored to its maximum level of 1000μg per ml. For copies of the program and instructions for it use write to blevin@emory.edu.

Simulation Results: In Figure S3, we follow the changes in the densities of the bacteria and phage in simulated serial populations. As observed in Figure 1B, the model predicts, as seen in Figure S3A, that upon the first encounter with the phage, V, the density of susceptible cells, W, and the total cell density of the bacteria, NT, will declines whilst that of the phage increase. In subsequent transfers, the population of bacteria recovers and becomes dominated by small colonies. As a consequence of the transitions between the different states of bacteria, the phage and all three bacterial populations are maintained with small colonies dominating the bacterial community. If we make the small colonies less efficient in the use of the resource, $e_c = 5x10^{-6}$ rather than $5x10^{-7}$, the total density of the small colony population is lower (Figure S3A). When cultures of just small colonies are started without the phage, they are ultimately diluted out due to their lower growth rate and low production rate in the absence of the phage and are overtaken by the evolved population being generated through reversion from small colonies (Figure S3B). When the evolved cells and phage are mixed, as observed in Figure 1D, the population recovers to full density more rapidly than when sensitive cells and phage are mixed (Figure S3C). However, in these simulations, the phage continue to be maintained, which was the case for only one of the two parallel experiments in the main body of the paper and in none of the parallel experiments presented in supplemental Figure S1B. This model can also account for why the mixture of the ancestral *S. aureus* Newman and small colony variants can maintain the phage (Figure 1E) with little effect on the density of bacteria (Figure S3D).

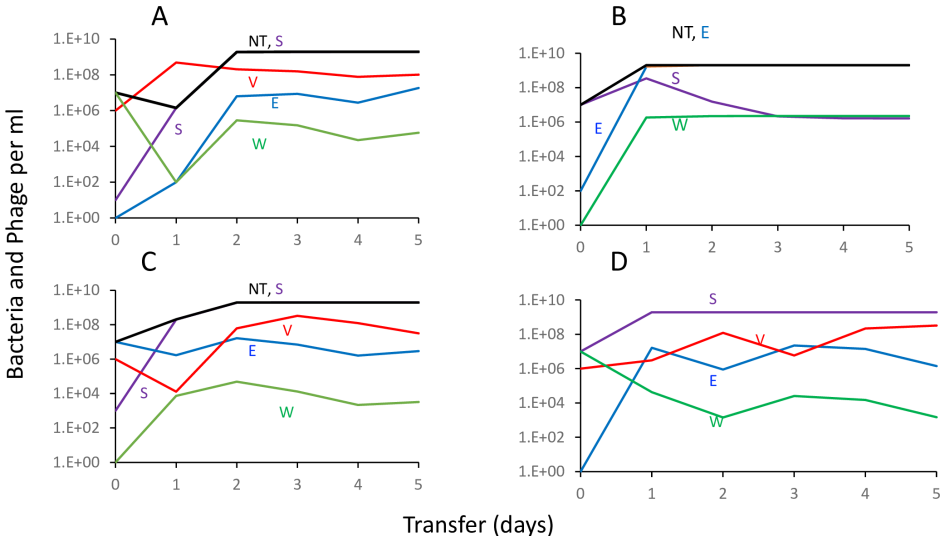

Figure S3 Simulated serial transfer populations. Changes in the densities of bacteria and phage in serial transfer culture. Standard parameters, $v_W = v_E = 1.7$, $v_S = 0.5$, $\delta_W = \delta_E = \delta_S = 2\times10^{-7}$, $\beta_W = \beta_E = 80$, $\beta_S = 0$, $e_w = e_e = 5\times10^{-7}$, $ec = 5Ex10^{-6}$, $C = 1000$, $k_W = k_E = 1$, $k_S = 10$, $\mu_{WS} = 10^{-6}$, $\mu_{SW} = 10^{-6}$, $\mu_{ES} = 10^{-3}$, $\mu_{SE} = 10^{-3}$. NT is the total density of bacteria. **A)** Serial transfer population with W and V and few S and E. **B)** Serial transfer population initiated with S and miniority populations of W and E, but no phage. **C)** Serial transfer population initiated with E and phage and minority populations of S and W. **D)** Serial transfer populations initiated with a mixture of W, S, and V and a minority of E.

V – The joint action of bactericidal antibiotics and phage.

In our article, we postulate that the reason bactericidal antibiotics in combination with phage do worse than phage alone (Figure 4) can be attributed to the antibiotics reducing the density of bacteria and thereby the capacity of the phage to replicate. We illustrate this with the following model of the joint action of antibiotics and a bactericidal antibiotic.

Model of the joint action of antibiotics and phage: There are two populations of bacteria enumerated in cells per ml. The populations are respectively W (wild-type, which are sensitive to antibiotics) and P (persisters which are sensitive to the phage but phenotypically resistant to antibiotics). There is a single antibiotic of concentration A in µg/ml, a lytic phage of density V particles per ml, and a limiting resource r in µg/ml. The sensitive bacteria grow at a maximum rate, v_N, with the net growth/death rate $\psi(A,r)$ being proportional to the concentration of the antibiotic and the limiting resource voila,

$$\psi(A,r) = \left[v_{WMAX} - \left[(v_{WMAX} - v_{WMIN}) \bullet \left(\frac{\left(\frac{A}{MIC}\right)^{\kappa}}{\left(\left(\frac{A}{MIC}\right)^{\kappa} - \left(\frac{v_{WMIN}}{v_{WMAX}}\right)\right)} \right) \right] \right] \bullet \frac{r}{(r+k)}$$

Where v_{WMAX} (>0) is the maximum growth rate, v_{WMIN} (<0) is the minimum growth rate/maximum kill rate, MIC the minimum inhibitory concentration of the antibiotic, and a shape parameter, κ, such that the greater the value of κ the more acute the function.

The persisters, P, are non-replicating bacteria that are resistant to the antibiotic. The phage adsorb to the persisters but do not replicate on them. The rate constant of adsorption of the phage to sensitive cells is δ_W and that to the persisters δ_P. Infections of phage to sensitive cells produce β phage particles per cell. With a rate y per cell per hour, sensitive cells produce persisters, W-->P, and with a rate x per cell per hour, persisters produce sensitive cells, P -->W. With these definitions and assumptions, the rates of change in the densities of phage and the concentration of the resources are given by

$$\frac{dr}{dt} = -\psi(A,r) \bullet W \bullet e - v_{MAXW} \bullet \psi(r) \bullet W \bullet e$$

$$\frac{dW}{dt} = \psi(A,r) \bullet W - \delta_W \bullet W \bullet V \bullet \psi(r) - y \bullet W + x \bullet P$$

$$\frac{dP}{dt} = y \bullet W - x \bullet P - \delta_P \bullet P \bullet V \bullet \psi(r)$$

$$\frac{dV}{dt} = \delta_W \bullet V \bullet W \bullet \beta \bullet \psi(r) - \delta_P \bullet P \bullet V \bullet \psi(r)$$

where $\psi(r) = \frac{r}{(r+k)}$ *and e μg is the amount of the* limiting *resource need to produce a new cell*

Numerical Solutions – Simulations: To solve these equations and simulate the dynamics, we use Berkeley Madonna. Copies of the programs are available from blevin@emory.edu.

Results

In Figure S4, we illustrate the rate of change in the total density of bacteria with a bactericidal antibiotic alone, with phage alone, and with the phage and antibiotic together. The rate of decline in the density of bacteria is lowest in the simulations where antibiotics are used alone. The highest rate of decline in the density of bacteria is obtained in the simulation where phage are used alone. When phage are used in combination with bactericidal antibiotics, they do not increase in density to the same extent that they do in the absence of the antibiotics. The leveling off in the density of bacteria is a consequence of persistence (4-6).

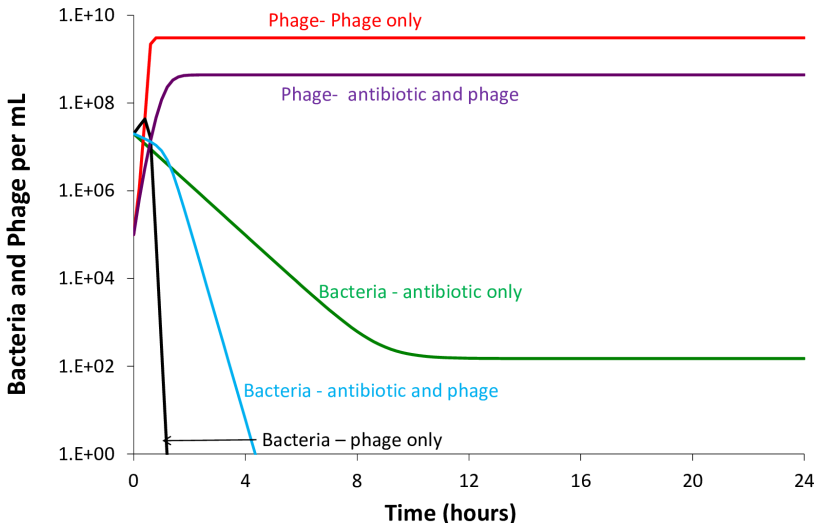

Figure S4 Simulation results: changes in the densities of bacteria and phage with different treatments. Parameter values. v_{SMAX}=2.0, v_{SMIN} -2.0, κ=1.0, MIC=1.0, e=5x10^{-7}, k=1.0, x=y=10^{-5}, δ_P=10^{-8}, δ_S=10^{-8}, β=50, A =2 μg/ml, r(0)=1000.

VI. Persistence and heteroresistance

In the absence of phage in serial transfer culture, the super MIC concentration of oxacillin does not eliminate the bacteria but rather maintains the density at levels markedly lower than the antibiotic-free controls (Figure 4 A center). We postulate that this can be attributed to persistence in serial transfer culture. In the absence of phage, ciprofloxacin initially reduces the density of *S. aureus* but in subsequent transfers, the density of these bacteria return to levels within the range anticipated for antibiotic-free controls (Figure 4A bottom). We postulated that these pharmacodynamics can be attributed to heteroresistance (7). In the following, we present the evidence for persistence for oxacillin-treated cultures and heteroresistance for ciprofloxacin-treated cultures. Using a mathematical - computer simulation model, we illustrate how these postulated mechanisms can account for the observed pharmacodynamics of these drugs with *S. aureus* Newman.

A model for antibiotics in serial transfer culture with persistence and heteroresistance: There are three populations of bacteria, wild-type, heteroresistant, and persisters with designations and densities, W, H, and P cells per ml; a single antibiotic of concentration, A μg/ml; and a limiting resource of concentration r μg/ml. As in our model of the joint action of antibiotics and phage (II), we assume that the rate of growth, of bacteria of each of these states, $\psi_i(A,r)$ is a product of a Hill function for the antibiotic (4), and a Monod function for the resource (1).

$$\psi_i(A,r) = \left[v_{iMAX} - \left[(v_{iMAX} - v_{iMIN}) \bullet \left(\frac{\left(\frac{A}{MIC_i}\right)^{\kappa}}{\left(\left(\frac{A}{MIC_i}\right)^{\kappa} - \left(\frac{v_{iMIN}}{v_{iMAX}}\right)\right)} \right) \right] \right] \bullet \frac{r}{(r+k)}$$

The subscript i represents the bacterial population, W, H, or P. For strain i, v_{iMAX} (>0) is the maximum growth rate, v_{iMIN} (<0) the minimum growth rate/maximum kill rate, and MIC_i the minimum inhibitory concentration of the antibiotic. Bacteria of the same state have the same Hill coefficient and Monod constant, respectively κ and k.

In these simulations, we assume the persisters can replicate, $v_{PMAX}>0$, and are resistant to the antibiotics, $v_{PMIN}=0$. As in (7), the MIC for the antibiotic of the heteroresistant cells is greater than the sensitive, $MIC_H > MIC_S$. There is a transition from W to P and from P to W respectively at rates, x and y per cell per hour, and a transition from W to H and H to W at rates, x_H, and y_H per cell per hour. Finally, we assume that the concentration of the antibiotic can decline at a rate d per hour. With these definitions and assumptions, the rates of change in the densities of the bacterial population and concentration of the antibiotic and resource are given by:

$$\frac{dr}{dt} = -\psi(r) \bullet e \bullet (W \bullet v_{MAXW} + H \bullet v_{MAXH})$$

$$\frac{dW}{dt} = \psi_W(A,r) \bullet W - x \bullet W + y \bullet P - x_H \bullet W + y_H \bullet H$$

$$\frac{dP}{dt} = v_{MAXP} \bullet \psi(r) \bullet P + x \bullet W - y \bullet P$$

$$\frac{dH}{dt} = \psi_H(A,r) \bullet H + x_H \bullet W - y_H \bullet H$$

$$\frac{dA}{dt} = -d \bullet A$$

$$where\ \psi(r) = \frac{r}{(r+k)}$$

Numerical Solutions: To solve these equations, we simulate the changes in the densities of the bacterial populations and changes in the concentration of the antibiotics with Berkeley Madonna. As in our experiments, in these simulations every 24 hours the density of the bacteria is reduced by a factor of 100, and new resources and antibiotics are added.

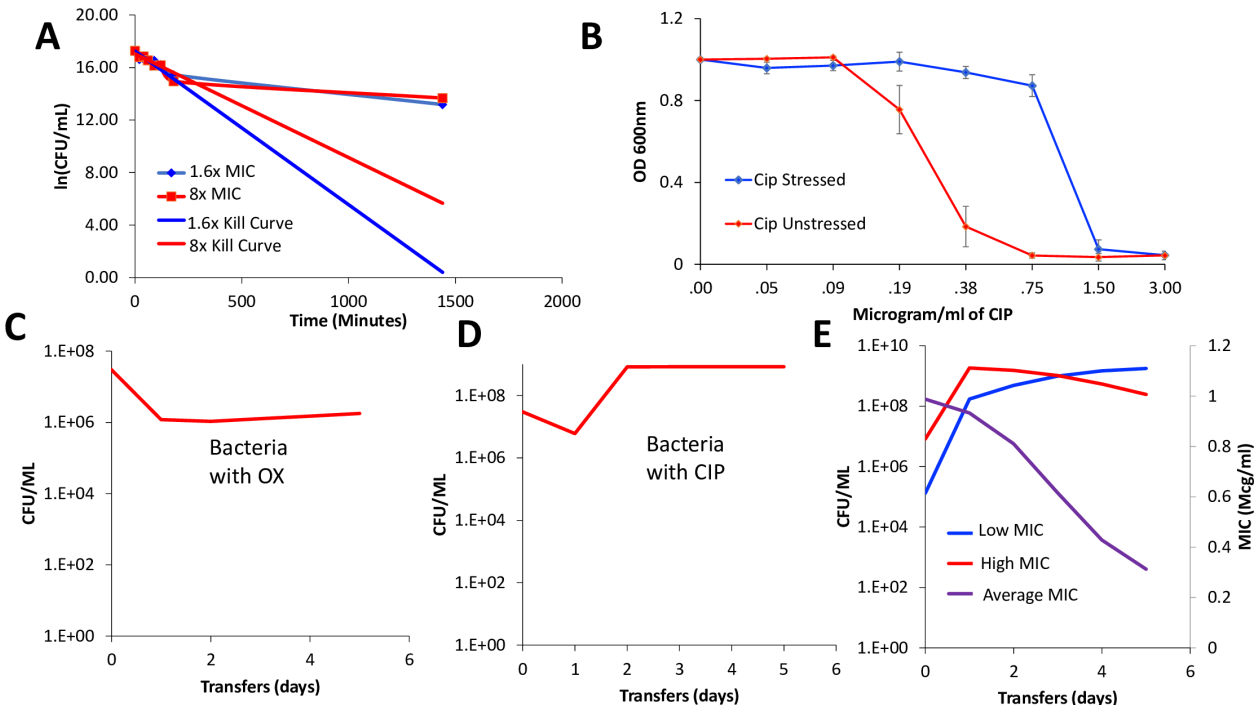

Figure S5 *S. aureus* antibiotic pharmacodynamics **A)** Natural log of the change in density of growing cultures of *S. aureus* Newman exposed to 1.6X and 8X MIC, low and high for 24 hours (1440 minutes). The green and purple lines are experimental changes in density estimated by plating; while the blue and red lines are the results of a linear regression of the changes in density estimated during the first 180 minutes of exposure. **B)** Changes in the optical density of experimental cultures of *S. aureus* Newman exposed to 5µg/ml ciprofloxacin, mean and standard error of the ODs for three replicas. In red are cells that were treated with ciprofloxacin and then transferred without the presence of this drug for seven days before the MIC determination was performed. In blue are cells that were treated with ciprofloxacin for one week before the MIC was performed. **C, D, E)** Simulation Results Common parameters: v_{MAXS}=1.5, v_{MINS}=-0.6 ,v_{MAXSR} = 1.2 , v_{MINSR}=-3 , ks=1 ,kh=1 ,k=1 ,e=5.0E-7 **C)** Changes in the densities of bacteria in serial transfer culture with persistence with parameters in the range estimated for *S. aureus* in oxacillin. Specific parameters: vp=0.2, MIC_S= 1.8, x_P=1.0e-4, y_P=1E-3, A_{MAX}=3. Exposure to oxacillin does not start until the second transfer (compare to Figure 4A center). **D) and E)** Heteroresistance for ciprofloxacin MIC_S= 0.218, MIC_H=1, x= 1E-6, y=1e-3, x_P=0, y_P=0, A_{MAX}=0.5 Changes in the densities of bacteria in serial transfer culture with heteroresistance with parameters in the range estimated for *S. aureus* in ciprofloxacin. Exposure to ciprofloxacin doesn't start until the second transfer (compare to Figure 4A bottom). **E)** Unique Parameters for E: MIC_S= 0.218, MIC_H=1, x=1E-6, 1E-3 x_P=0, y_P=0. Simulation of the changes in the densities of bacteria and average MIC of the antibiotic mixture of the low MIC sensitive and the high MIC heteroresistant populations in serial transfer culture in the absence of the antibiotics.

In Figure S5A, we present the results of time-kill experiments with *S. aureus* Newman exposed to oxacillin. The observation that the rate of decline in the viable density of sensitive cells during the first few hours of exposure considerably exceeds the rate of decline later in the experiment is what would be anticipated for persistence. In Figure S5B, we present the evidence for heteroresistance; higher concentrations of ciprofloxacin are needed to kill cultures initiated with bacteria exposed to ciprofloxacin than to kill bacteria cultured in the absence of this drug. If we allow for persistence with parameters in the range estimated for oxacillin the predicted changes in the density of *S. aureus* Newman in serial transfer culture with oxacillin are similar to those observed (compare Figures S4A and S4C). If we allow for heteroresistance with the parameter in the range estimated for *S. aureus* Newman in serial transfer culture with ciprofloxacin the results are similar to those observed (compare figures S5B and S5D). One property of heteroresistance is that when the bacteria are removed from the drug, the resistant population will decline in frequency, and the average MIC will decline to levels similar to that of the original sensitive population (7), see Figure S5E.

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