Antibiotic killing of diversely generated populations of non-replicating bacteria

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Running Title – Antibiotic killing of non-replicating bacteria

Abstract:

Non-replicating bacteria are known to be (or at least commonly thought to be) refractory to antibiotics to which they are genetically susceptible. Here, we explore the sensitivity to antibiotic-mediated killing of three classes of non-replicating populations of planktonic bacteria (1) stationary phase, when the concentration of resources and/or nutrients are too low to allow for population growth; (2) persisters, minority subpopulations of susceptible bacteria surviving exposure to bactericidal antibiotics; (3) antibiotic-static cells, bacteria exposed to antibiotics that prevent their replication but kill them slowly if at all, the so-called bacteriostatic drugs. Using experimental populations of *Staphylococcus aureus* Newman and *Escherichia coli* K12 and respectively 14 and 11 different antibiotics, we estimate the rates at which these drugs kill these different types of non-replicating bacteria. Contrary to the common belief that bacteria that are non-replicating are refractory to antibiotic-mediated killing, all three types of non-replicating populations of these Gram-positive and Gram-negative bacteria are consistently killed by aminoglycosides and the peptide antibiotics, daptomycin and colistin, respectively. This result indicates that non-replicating cells, irrespectively of why they do not replicate, have an almost identical response to the cidal activity of antibiotics. We discuss the implications of these results to our understanding of the mechanisms of action of antibiotics and the possibility of adding a short-course of aminoglycosides or peptide antibiotics to conventional therapy of bacterial infections.

Introduction

For therapeutic purposes, the relationship between the concentrations of antibiotics and the rates of growth and death of bacteria, pharmacodynamics, is almost exclusively studied *in-vitro* under conditions that are optimal for the action of these drugs; relatively low densities of bacteria growing exponentially in media and under culture conditions where all members of the exposed population have equal access to these drugs, resources, wastes and metabolites excreted into the environment. To be sure, in some sites and tissues in acutely infected hosts, relatively low densities of the target pathogens may be growing exponentially at their maximum rate and thus are under conditions that are optimal for the action of antibiotics. However, this situation is almost certainly uncommon in established, symptomatic and thereby treated infections where the offending bacteria are likely to be compartmentalized in different sites and tissues and confronting the host's immune defenses (1)

Infecting populations of bacteria may be non-replicating for different reasons and by different mechanisms. First, they may have exhausted the locally available resources; thus modified their environment so their populations are at or near stationary phase (2-6). Second, although local nutrients may be sufficient for their replication, for hosts treated with bactericidal drugs these bacteria may be minority populations of physiologically refractory survivors, the so-called "persisters" (7-9). Third, the offending bacteria may be non-replicating because of exposure to bacteriostatic antibiotics, a state we shall refer to as antibiotic-induce stasis. Fourth, infecting bacteria may be slowly replicating or at stationary phase inside phagocytes or other host cells (10, 11), or

attached to the surfaces of tissues or prosthetic devices and within polysaccharide matrices know as biofilms (12) and thereby not replicating for one or more of previously described reasons (4, 13).

The concept of antibiosis has been classically linked to the fight against of microbial active invasion of the host tissues, implying active replication. With some exceptions associated with permeability (14), the susceptibility of bacteria to killing by bactericidal antibiotics is related to their rate of replication. In fact, with beta-lactams, the rate at which bacteria are killed has been shown to be strictly proportional to the rate at which the population is growing (15, 16). The same trend seems to occur for other bactericidal agents as fluoroquinolones, aminoglycosides, glycopeptides, and lipopeptides (17-20). It is well known that exposure to bacteriostatic antibiotics markedly reduce the efficacy of beta-lactam drugs to kill bacteria (21-23). However, save for these cases of antagonism between bacteriostatic and bactericidal drugs and the now more than a quarter of century old classical studies by R. Eng and colleagues (24), despite the potential clinical implications, there is remarkable little information about the pharmacodynamics of antibiotics for non-replicating populations of bacteria.

In this investigation we address two fundamental questions about the pharmacodynamics of non-replicating bacteria. What antibiotics and to what extent do these drugs kill non-replicating bacteria? With respect to their susceptibility to antibiotic-mediated killing are bacteria entering non-replicating states physiologically similar, irrespectively of the reason responsible for their not replicating? To address these questions we compare the

activity of antibiotics on non-replicating bacterial populations obtained by different procedures. We present the results of experiments estimating the susceptibility of various non-replicating populations of *Staphylococcus aureus* and *E. coli* to killing by respectively 14 and 11 different antibiotics. We consider three types of non-replicating states of planktonic bacteria; (i) those at stationary phase in oligotrophic culture, (ii) the non-replicating survivors of exposure to bactericidal antibiotics, persisters, and (iii) bacteria exposed to bacteriostatic antibiotics, antibiotic-static populations. Contrary to the popular conception that antibiotics are ineffective at killing bacteria that are not replicating, (15, 25, 26), even at relatively low concentrations a number of existing bactericidal antibiotics can kill non-replicating bacteria of all three states. The results of our experiments indicate that the same classes of antibiotics, the aminoglycosides and the peptides, are particularly effective at killing non-replicating bacteria irrespective of the mechanism responsible for their failure to replication. In addition to being relevant clinically, these results are interesting mechanistically; they suggest non-replicating bacteria of different types share a common cell physiology with respect to their interactions with antibiotics.

Results

1- Antibiotic-mediated killing of exponentially growing bacteria: As a baseline for our consideration of the antibiotic susceptibility of non-replicating bacteria, we explore the response of exponentially growing populations *S. aureus* and *E coli* MG1655 to antibiotics. For these experiments, overnight cultures of these bacteria were added to broth at a ratio of 1:100 and incubated for 1.5 hours and the density of the cultures

116 estimated, N(0). Five mls of these cultures were then put into 6 well "macrotiter"
117 plates, CELLTREAT, and the antibiotics added. The results of these experiments are
118 presented in Figure 1,

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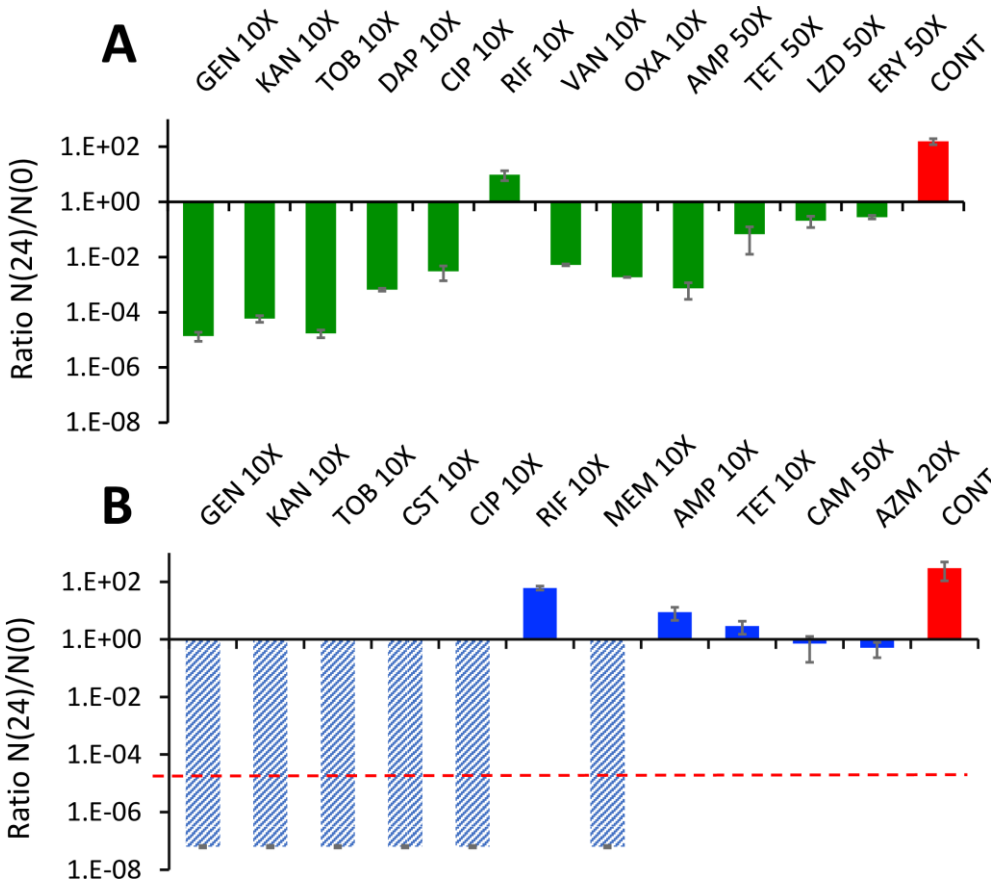

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123 **Figure 1.** Ratio of the viable 24 hour and initial viable cell densities, N(24)/N(0) of
124 exponentially growing broth cultures exposed to the concentrations of bactericidal and
125 bacteriostatic antibiotics (multiples of MIC values). A. *Staphylococcus aureus* Newman
126 in MHII. B. *E. coli* MG1655 in LB. Mean standard error of the N(24)/N(0) ratios of

three independent experiments each with three samples. The broken line is the limit of detection (10^2 cells per ml, hatched bars mean that this limit was surpassed in the assay). The initial, N(0) densities of *S. aureus* and *E. coli* in these experiments were, on average, $1.3 \pm 0.6 \times 10^7$ and $1.6 \pm 0.2 \times 10^7$ cells per ml, respectively.

For *S. aureus*, the aminoglycosides, GEN, KAN and TOB kill to the greatest extent, with reductions in viable cell density of more than 4 orders of magnitude. DAP, CIP, VAN OXA and AMP are clearly bactericidal and reduce the viable cell density by more than 3 orders of magnitude. The increase in the N(24)/N(0) ratio for rifampin can be attributed to the ascent of RIF-R mutants. Even at 50X MIC, TET, LZD, and ERY are effectively bacteriostatic. When exponentially growing cultures of *E. coli* are exposed to 10X MIC of GEN, KAN, TOB, CST, CIP and MEM, the viable cell density is below that which can be detected by plating. As with *S. aureus*, the failure of RIF to reduce the viable cell density can be attributed to the ascent of RIF-R mutants. For *E. coli* exposed to AMP, our results suggest that the decay in the effective concentration of this drug, probably due to the chromosomal beta-lactamase effect at high initial densities can account for the failure of this bactericidal antibiotic to reduce the viable cell density of *E. coli*. As evidence for this, when cell-free extracts of the 24-hour AMP cultures are spotted onto lawns of sensitive *E. coli* MG1655, there is no zone of inhibition as there is when we spot the original media containing 10XMIC AMP.

2- Antibiotic-mediated killing of stationary phase bacteria: For the stationary phase experiments with both *S. aureus* and *E. coli*, we used cultures that had been incubated

under optimal growth conditions for 48 hours. To estimate the amount of unconsumed, residual, resources in these 48-hour stationary phase cultures, and thereby the capacity for additional growth, we centrifuged and filtered (0.20 microns) 48-hour cultures of these bacteria. We then added, ~10^5 cells from overnight cultures to the cell-free filtrates, and estimated the viable cell density after 24 hours of incubation. The results of this experiment with 3 ort six replicas and three independent samples from each, suggest there little free nutrients available in these 48-hour stationary phase cultures. The viable cell densities of *S. aureus* and *E. coli* in these cell-free filtrates were respectively, 3.5 ± 0.8x10^6 and 4.8±0.8x10^6 cells per ml after 24 hours. The corresponding numbers for stationary phase densities fresh media were more than 500 fold greater.

At 48 hours, the viable cell densities of the stationary phase cultures were estimated, N(0). Following that, 5 ml aliquots were put into the wells of 6-well plates, the antibiotics added, and the cultures incubated with shaking for another 24 hours, at which time the viable cell densities were estimated. In Figure 2, we present the results of this stationary phase experiment.

In the absence of treatment (the control), there is no significant mortality between 48 and 72 hours for either *S. aureus* or *E. coli.* For *S. aureus*, only high concentrations of the aminoglycosides, GEN, KAN and TOB, and the cyclic peptide, DAP are effective in reducing the viable density of these 48-hours stationary phase culture (Figure 2A). For *E. coli*, the aminoglycosides, GEN, TOB, KAN are also effective for killing stationary

phase cells, as is high concentrations of CST. Save possibly for high concentrations (50XMIC) TET, there is no evidence for the other antibiotics tested (CIP, RIF, AZM, AMP, CAM) killing stationary phase *E. coli*.

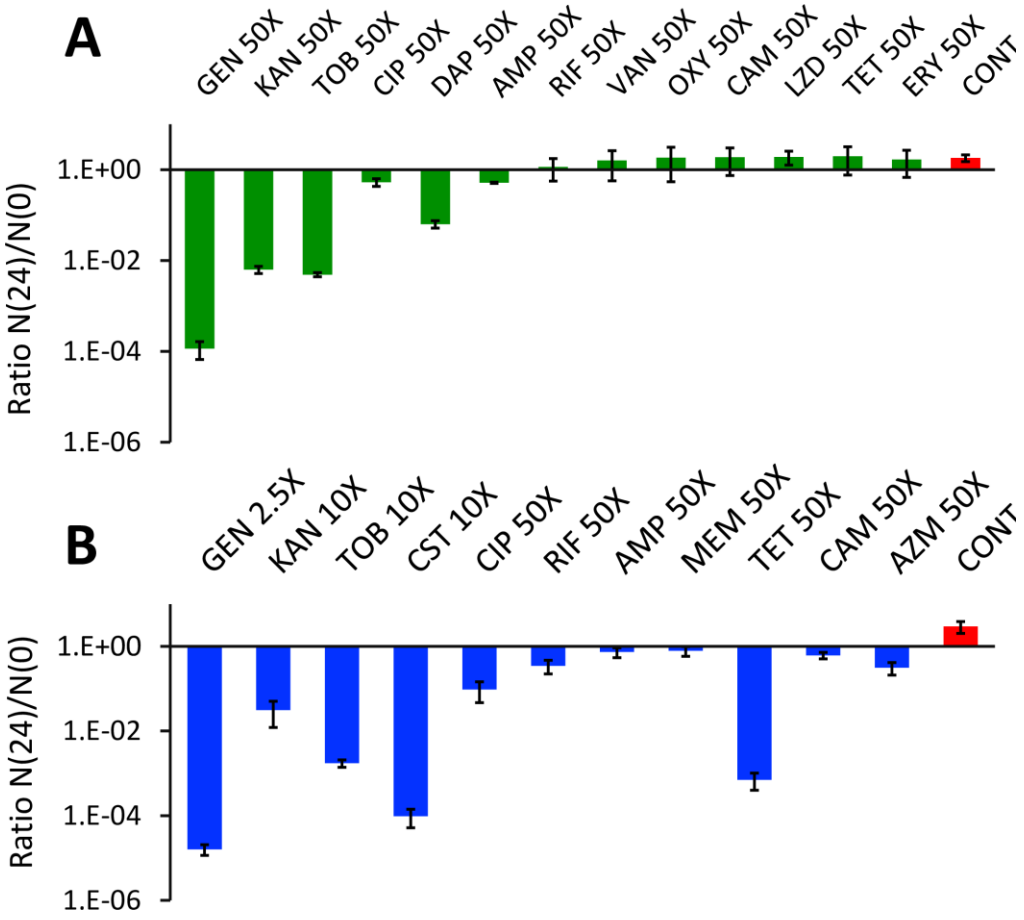

Figure 2—Antibiotic treatment of stationary phase bacteria. Ratio of the viable cell densities after and before 24 hours of exposure to antibiotics, N(24)/N(0). Forty-eight-hour stationary phase cultures of *S. aureus* (A) and *E. coli* (B) were treated with the noted multiples of MIC of different drugs and antibiotic free controls. Average viable N(0) densities were ~3.6×10^9 CFU/ml and ~8.8×10^8 CFU/ml for A and B, respectively. Mean and standard error of the N(24)/N(0) ratios of three or more independent experiments.

3) Antibiotic-mediated killing of persisters:

As can be seen Figure 1B, for *E. coli*, even when concentration ampicillin is 10X the MIC the density of surviving cells is too low to test for the susceptibility of these cells for killing by subsequent exposure to bactericidal antibiotics. To address this issue, we restricted our *E. coli* persister experiments to a *hipA7* (the Moyed mutant (27)) a construct that produces 10^3-10^4 times greater numbers of persisters than wild type due to an increase in the basal level of (p)ppGpp synthesis (28). This is illustrated in Figure 3, where we compare the dynamics of formation and the relative densities of persisters for *E. coli* MG1655 and the *hipA7* construct exposed to 10X MIC ampicillin.

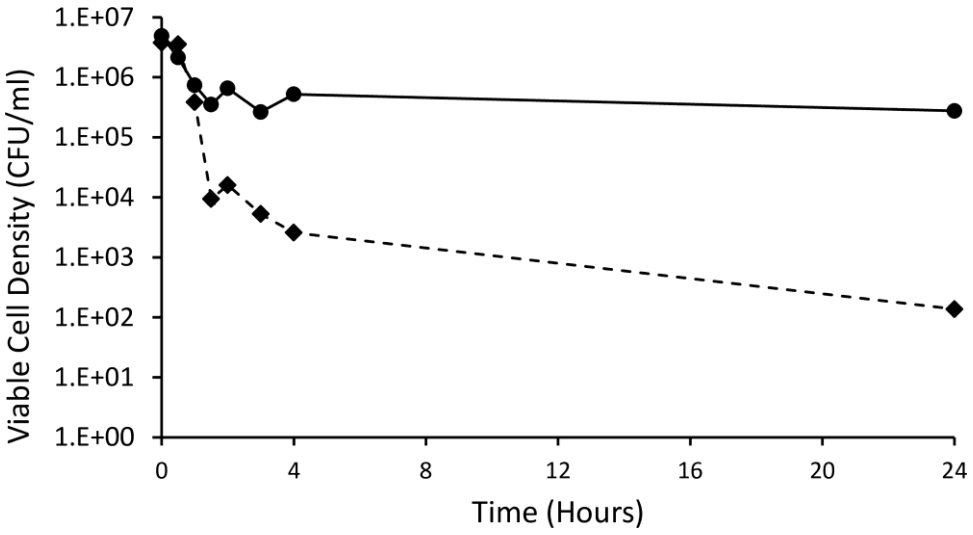

Figure 3—Dynamics of *hipA7* persister formation. Changes in viable cell density of exponentially growing *E. coli* MG1655 (broken lines) and a *hipA7* mutant of this strain (solid lines) exposed to 10X MIC Ampicillin. All data points represent the mean and standard error three independent measurements. Initial densities *hipA7* $4.9 \pm 0.3 \times 10^6$, MG1655 $3.8 \pm 0.6 \times 10^6$.

To generate the *Staphylococcus aureus* persisters, we used a protocol similar to that employed by (29). Overnight MHII cultures of *S. aureus* Newman were diluted 1/10 in fresh MHII and 25XMIC ampicillin added immediately. After 24 hours, the viable cell densities were estimated, N(0). Five mls of these ampicillin treated cultures were put into the wells of 6 well plates and the second antibiotic(s) added and the cultures incubated with shaking for another 24 hours. The results of these experiments are presented in Figure 4A. The extent to which these ampicillin-exposed *S. aureus* die in the absence of subsequent treatment is noted in by the N(24)/N(0) ratios of the controls, CON. These *S. aureus* persisters are refractory to killing at 50XC concentrations of the bactericidal antibiotics CIP, VAN, OXA and the three bacteriostatic drugs, ERY, TET and LZD. This is not the case for the aminoglycosides, 5XMIC GEN, and 10XMIC TOB and KAN reduce the viable cell densities of these persisters by nearly three orders of magnitude. Albeit to an extent less than these aminoglycosides, at 20XMIC, the cyclic peptide daptomycin also kills these ampicillin-generated S. *aureus* persisters.

The *hipA7* persisters were prepared with a protocol similar to that employed by Keren and colleagues(30). Exponentially growing LB cultures of *E. coli hipA7* were exposed to10X MIC ciprofloxacin or ampicillin for 4 hours, N(0) at which time they were treated with the second antibiotic for another 24 hours, N(24). The results of these experiments are presented in Figure 4B.

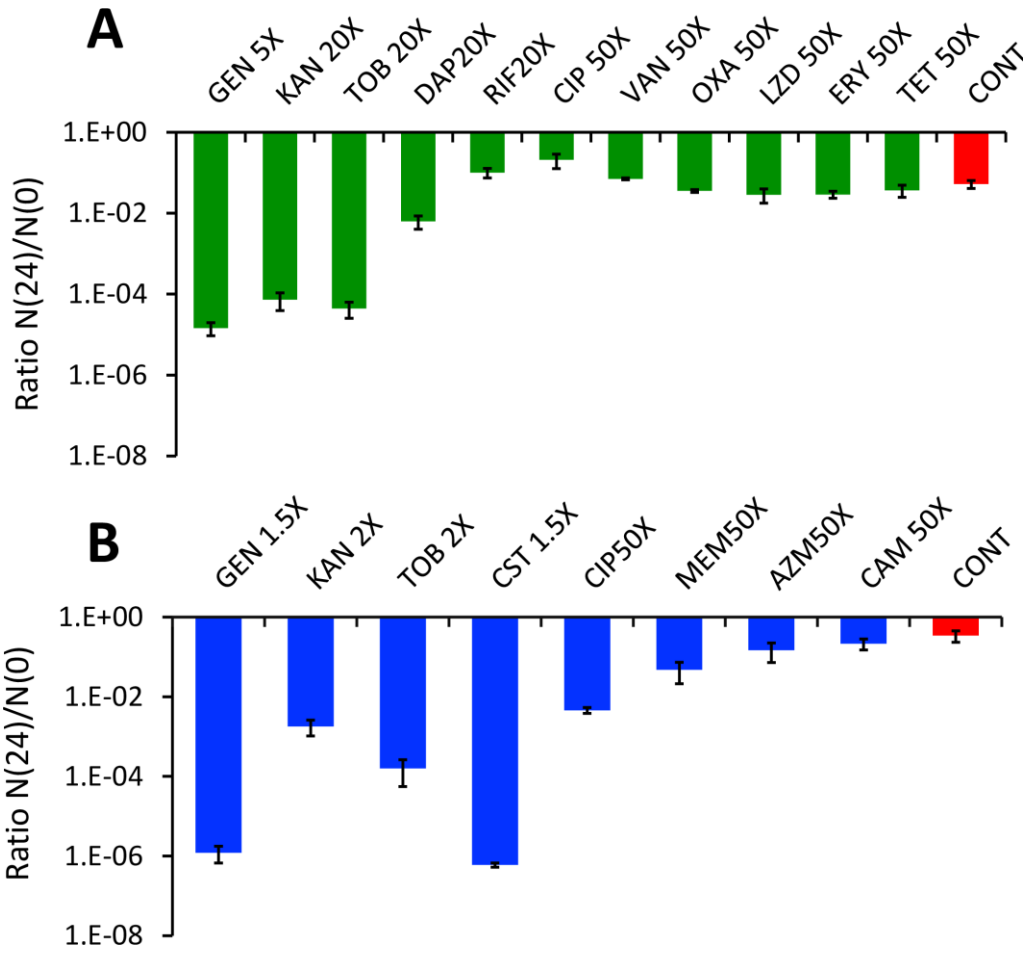

Figure 4—Ratio of viable cell density of persisters after 24 hours exposure to the noted concentrations of exposure to the second antibiotic N(24)/N(0). A. *S. aureus* treated with 25XMIC Ampicillin to generate the persisters N(0). B. *E. coli hipA7* treated with 10 XMIC Ampicillin to generate the persisters. Mean and standard error of the N(24)/N(0) ratios from 3 or more independent experiments

Relative to the controls, low, but super-MIC concentrations of the aminoglycosides, GEN, KAN and TOB and also the peptide CST reduce the viable cell density of the *E. coli hipA7* persisters by three to six orders of magnitude. Even at 50XMIC the other

236 antibiotics have little or no effect in reducing the viable cell density of the *E. coli hipA7*
237 persisters
238
239 **4- Antibiotic-mediated killing of antibiotic-induced static populations:** Antibiotic
240 induced static populations were generated by exposing exponentially growing *S. aureus*
241 and *E. coli* to bacteriostatic drugs for 24 hours, at which time the viable cell density was
242 estimated N(0). The culture was divided into 5ml aliquots in 6 well macrotiter plates
243 and the second antibiotic added. The cultures were maintained for another 24 hours and
244 the viable cell densities estimated N(24). The results of these experiments are presented
245 in Figure 5.
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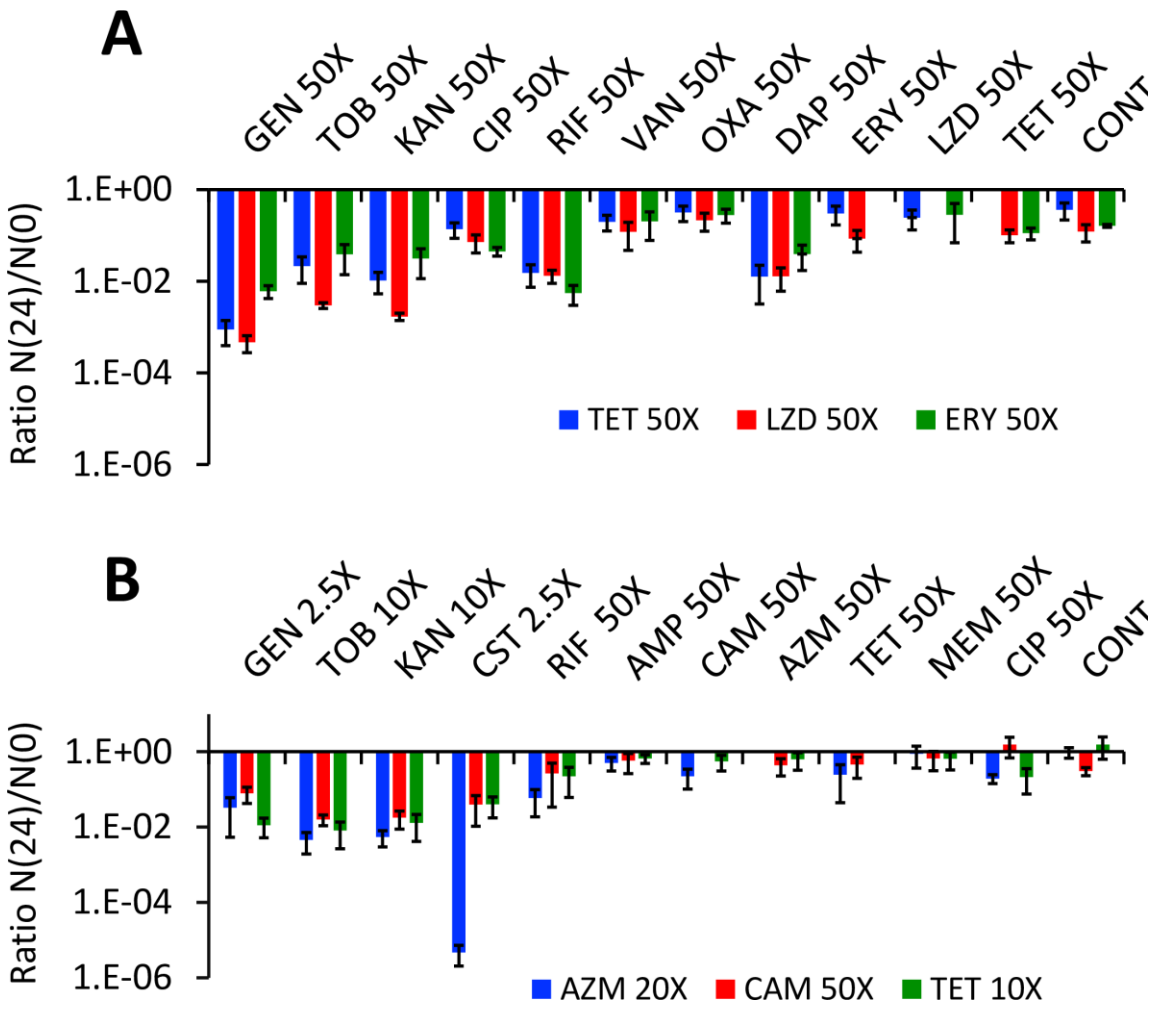

Figure 5—Antibiotic-mediated killing of antibiotic-static populations. Mean and standard error of the N(24)/N(0) ratios (A) *S. aureus*, (B) *E. coli*. The concentrations of the antibiotics as multiples of MICs used to generate the static populations and for subsequent treatment are noted in the figure. The densities of the cultures used to generate the *S. aureus* persisters were between $3x10^6$ and $1x10^8$, and for between $6x10^6$ and $7x10^7$ cells per ml for *E. coli* .

The *S. aureus* antibiotic-static populations surviving exposure to tetracycline, erythromycin and linezolid are killed by the aminoglycosides and by high concentrations

of rifampin. The *E. coli* antibiotic-static populations surviving exposure to AZM, CAM and TET are readily killed by the aminoglycosides and colistin and marginally if at all so by high concentrations of RIF. As noted in Figure 1A and 1C, even in the absences of super-treatment, the *S. aureus* antibiotic-static populations die at higher rate than those of *E. coli*.

Discussion

During the course of an infection, several different conditions contribute to the non-replicating status of initially growing populations of the infecting bacteria. Among these conditions are stationary phase resulting from a dearth of the nutrients and resources in the infected tissues, the formation of biofilms, and the immune defenses, primarily engulfment by professional and amateur phagocytic host cells. Therapy with antibiotics will also result in the production of non-replicating populations of bacteria; persisters surviving exposure to bactericidal antibiotics, and stasis induced by bacteriostatic drugs. We postulate that although they are generated in different ways, common mechanisms are responsible for the failure of these bacteria to replicate and why the same classes of antibiotics can used to kill them. These mechanisms involve the general stringent response, a strongly regulated process governed by the alternative sigma factor RpoS (30)up-regulated by the accumulation of hyperphosphorylated guanine nucleotides, as the “alarmone” (p)ppGpp) (31-33).

The contribution of the stringent response to antibiotic-induced stress is not well understood. A number of observations suggest that antibiotic exposure can trigger a RpoS-stationary response and the generation of non-replicating populations. Sub-inhibitory concentrations of beta-lactams induce the stringent response (34, 35). Response to stress by bacteriostatic antibiotics acting on the ribosome (as macrolides, chloramphenicol, or tetracyclines) is probably interfered by the reduction in protein synthesis, which might reduce the building-up of (p)ppGpp, but that might be over-compensated by reduced degradation of this nucleotide (36, 37). Proteome analysis of erythromycin-exposed "permeable" E. coli suggests a RpoS-regulated profile (38). In fact, sub-inhibitory concentrations of bacteriostatic antibiotics induce the stringent response, leading to beta-lactam tolerance (39)

Cationic peptides, including polymyxins, do not elicit RpoS response, but rather increase the permeability of the cell membranes and thereby act as rapid "external killers" (40, 41). Whether the aminoglycosides can elicit an RpoS response is unclear at this time. As is the case of other protein synthesis inhibitors, this machinery might be less effective in the presence of the antibiotic, than in the absence; the same occur with oxidative stress responses (37). The rapid killing observed in our work (see Figure 1) may well be because the stringent response has no time to develop when exposed to these drugs. However, if aminoglycosides are able to induce the RpoS response, that might favor aminoglycoside transport into cells that results in membrane-damage and killing of non-growing cells (42).

303 The cellular immune response might also responsible for generating non-replicating
304 populations? Salmonella RpoS-dependent genes are activated into the intracellular
305 environment of eukaryotic cells (43). An RpoS active system contributes to survival of
306 *E. coli* and Salmonella to the phagocyte oxidase-mediated stress resistance (44, 45). and
307 influences intracellular survival of *Legionella* macrophages and *Acanthamoeba* cells
308 (46-48). Intracellular survival is also related with the overexpression of heat-shock stress
309 proteins, promoting non-replication (49, 50).
310
311 These studies suggest that non-replicating bacteria might also arise by the stringent
312 response derived from professional and/or non-professional phagocytosis. Of course,
313 aminoglycosides and peptide antibiotics do not enter in eukaryotic cells, but non-
314 replicating bacteria can be released from phagocytes lysed by bacteria-induced
315 programmed necrosis, contributing to the chronification of the infection (51). Antibody-
316 antibiotic conjugates might improve therapy of phagocytized bacteria where
317 aminoglycosides and peptides are excluded (11).
318
319 We postulate that essentially the same set of cellular responses occurs in the different
320 stress-inducing conditions bacteria encounter during the infection process, and different
321 classes of antibiotics have similar pharmacodynamic effects on these different types of
322 non-replicating cells. These effects probably are expressed as an hormetic (dose-
323 dependent) stress response(52, Davies, 2006 #416). Of course, non-growth is a complex
324 mechanism in which the final antibiotic effects are influenced by those resulting from

different coexisting stresses, for instance, non-growth resulting from ribosome hibernation could facilitate some degree of gentamicin tolerance (53).

The pharmacodynamic data presented in this study are inconsistent with what seems to be the common conviction that stationary phase populations of bacteria are refractory to killing by antibiotics (15, 25, 26). We show how aminoglycosides and peptide antimicrobials kill non-replicating populations of *E. coli* and *S aureus*. This observation is not new; it has been known for some time that the aminoglycosides and the cyclic peptides, daptomycin and colistin are capable of killing stationary phase bacteria (20, 53-55). Less seems to be known about the susceptibility to killing by bactericidal antibiotics of other non-replicating states of bacteria considered here.

Although there have been many studies of persistence, relatively little is known about the susceptibility of these non-replicating bacteria to antibiotics other than those employed to generate them. One exception to this is a study by Allison and colleagues, (56) that demonstrates that by adding metabolites, *E. coli* and *S. aureus* persisters in the form of biofilms become susceptible to killing by aminoglycosides. Our results with the ampicillin-generated planktonic *S. aureus* persisters are fully consistent with these observations. Results with the *hip*A7 persisters used in this work also suggest that *E. coli* "natural" persisters are sensitive to killing by even low concentrations of the aminoglycosides and the peptide colistin.

There is also a relative dearth of quantitative information about the susceptibility to antibiotic-mediated killing of non-replicating bacteria induced by exposure to bacteriostatic antibiotics. To be sure, within the first decade after the discovery of antibiotic, there was evidence for exposure to bacteriostatic drugs reducing the efficacy of bactericidal (57) and these observations were corroborated more recently (23). Early observations concerning the lower efficacy of penicillin in static cells produced by chloramphenicol or tetracycline's have engendered what some may see as an immutable law in the practice of antibiotic therapy, don't mix bacteriostatic and bactericidal drugs. However, we show that some existing bactericidal antibiotics are quite effective in killing bacteria that are not replicating because exposure to bacteriostatic antibiotics, what we refer to as antibiotic-static populations.

In summary, stationary-phase *S. aureus* populations are killed at a substantial rate by the aminoglycosides and to a lesser extent by daptomycin. *S. aureus* persisters generated by exposure to ampicillin exposure are also killed aminoglycosides and the lipopeptide daptomycin, but not the other drugs tested. Antibiotic-induced static populations of S. *aureus* maintain the same killing profile, with aminoglycosides and daptomycin as the sole killing agents, with the exception, in this case of rifampicin. The same antibiotics are also effective at killing stationary phase *E. coli*, whilst the other antibiotics tested were not. This is also the case for the *hip*A7 E. coli persister and *E. coli* "suffering" from the stasis induced by ribosome-targeting bacteriostatic antibiotics.

Potential clinical implications

In recent years there has been a great of deal of interest in discovering and developing drugs to treat non-replicating populations of bacteria, particularly those associated with biofilms. A prime example of this is Kim Lewis and colleague's (58) use of a novel antibiotic, acyldepsipeptide (ADEP4). Despite growing efforts in the endeavor to find new antibiotics to kill non-replicating bacteria, the results presented here suggest that existing antimicrobials may well be up to that task. A well-warranted concern is that the antibiotics with this virtue are among the more toxic drugs, aminoglycosides and the peptides(59-61). It should be noted, however, relatively low, and possible non-toxic concentrations of the aminoglycosides and the peptide, colistin can kill *E. coli* antibiotic-static and hipA7 persisters. Most importantly, as has been the case with cancer chemotherapy, there are conditions under which some toxic side-effects of systemic treatment are more then made up for by the sometimes life-saving benefits of that treatment, (62). Of course, inhaled therapy, providing very high local concentrations of aminoglycosides or peptidic antibiotics, has proven its efficacy in mostly non-growing populations of *Pseudomonas aeruginosa* and *Staphylococcus aureus* involved in chronic lung colonization in cystic fibrosis patients (63). Also, high local concentrations of these antibiotics have been useful in intravesical therapy of recurrent urinary tract infections (64) or in catheter locking solutions to treat catheter-related bloodstream infections (65).

How important persisters are clinically is, at this juncture, not all that clear. Persisters remain susceptible to phagocytosis and other elements of the innate immune system, the

main factor influencing control of infections, can be attributed to the innate immune system, and they would play little or no little role in reducing the efficacy of antibiotic therapy (66). Consistent with this yet-to-be tested (but testable) hypothesis in experimental animals and patients is observation that for immune competent patients, bacteriostatic drugs are as effective for treatment as highly bactericidal agents (67, 68).

As intriguing as they may be scientifically, planktonic persisters surviving exposure to bactericidal antibiotics are not the majority of the non-replicating bacteria present during the infection process. Stationary phase bacteria resulting from local shortage of nutrients, non-growing populations induced by bacteriostatic agents, biofilm populations and phagocytosed bacteria (eventually released by the lysis of the engulfing phagocytes) are likely to be the majority of phenotypically antibiotic resistant bacteria in an established infection. They are certainly involved in chronification of infections, and subsequent reactivations and relapses. It may well be that along with the standard therapy, the addition of a short-course administration of antibiotics that kill these non-replicating bacteria, like the aminoglycosides and peptides, may well accelerate the course of treatment and increase the likelihood of its success.

Materials & Methods

Bacteria, culture and sampling media and procedure

Bacteria: *Staphylococcus aureus* Newman (ATCC 25904), *E. coli* K12 MG1655 and the high frequency persister strain of E. coli K12, *hipA7* constructed by Moyed and

Betrand(27)

Liquid culture: For the *S. aureus* Mueller-Hinton Broth (MHII) (275710, BD™) and for *E. coli* Lysogeny Broth (LB) (244620, Difco).

Sampling Bacterial Densities: The densities of bacteria were estimated by serial dilution in 0.85% saline and plating on LB (1.6%) agar plates.

Antibiotics and their sources: Ampicillin, chloramphenicol, colistin, gentamicin, kanamycin, oxacillin, tetracycline, and vancomycin – SIGMA, azithromycin and tobramycin, TOCRIS, daptomycin, TCI, erythromycin, MP BIOCHEMICALS, ciprofloxacin and rifampin, APPLICHEM, meropenem- COMBI-BLOCKS, linezolid – CHEM IMPEX INT'L

Minimum Inhibitory Concentrations (MICs): *For* both *S. aureus* Newman and *E. coli* MG1655 the MICs of the antibiotic were estimated using the two-fold micro dilution procedure (69). Two different initial concentrations of antibiotics were used to obtain accurate measurements from the two-fold micro dilution procedure. The estimated MICs of each of the antibiotic bacteria combination are listed in Table 1.

Table 1 Minimum Inhibitory Concentrations in µg/ml.

Antibiotic	*S. aureus Newman*	*E. coli MG1655*	*E. coli MG1655 hipA7*
Ampicillin (AMP)	4.7	4.7	4.7
Azithromycin (AZM)	2.3	19.0	38.0
Chloramphenicol (CAM)	13	4.7	4.7
Ciprofloxacin (CIP)	0.2	0.6	0.6
Colistin (CST)	**N/A**	1.1	1.1
Daptomycin (DAP)	1.6	**N/A**	**N/A**
Erythromycin (ERY)	0.6	**N/A**	**N/A**
Gentamicin (GEN)	0.8	9.3	9.3
Kanamycin (KAN)	2.3	4.7	4.7
Linezolid (LZD)	1.1	**N/A**	**N/A**
Meropenem (MEM)	**N/A**	1.2	**0.6**
Oxacillin (OXA)	0.3	**N/A**	**N/A**
Rifampin (RIF)	0.002	9.4	9.4
Tetracycline (TET)	0.6	2.3	75.0
Tobramycin (TOB)	0.3	1.2	2.3
Vancomycin (VAN)	1.6	**N/A**	**N/A**

N(24)/N(0) Ratios**:** As our measure of the efficacy of the different antibiotics to killing the exposed bacteria, we use the ratio of the viable cell density after 24 hours of exposure to the drug and the initial density estimated prior to exposure, N(24)/N(0). For each experiment, we estimated the N(0) and N(24) densities (CFUs) with three independent serial dilutions. For each antibiotic - bacteria combination, unless otherwise stated, we ran at least three independent experiments and calculated the mean and standard error of the N(24)/N(0) ratios.

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