Widening the Spaces of Selection: Evolution along Sublethal Antimicrobial Gradients

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ABSTRACT The work of Gullberg et al. (E. Gullberg, L. M. Albrecht, C. Karlsson, L. Sandegren, D. I. Andersson, mBio 5:e01918-14, 2014) indicates that extremely low concentrations of antibiotics and heavy metals are able to compensate for the cost of harboring a plasmid encoding resistances to these inhibitors. Therefore, the "spaces of selection" for plasmids encoding antibiotic or metal resistance along gradients of antimicrobial agents might be huge, and in wide spaces a high number of bacterial cells are exposed to the selective effects. These spaces are even broader if several inhibitors are simultaneously present. Probably very small inhibitor concentrations in the environment, including in sewage and other water bodies, are sufficient to ensure the maintenance and spread of this kind of multiresistance plasmid.

Bacterial plasmids harboring multiple resistance genes frequently impose fitness costs on the host cells, so that their maintenance in bacterial populations depends on the advantages they might produce. Long-term maintenance of multiresistance plasmids certainly contributes to the spread of resistance genes to other microbial populations and communities. The advantage of harboring resistance genes is dependent on the bacterial risk of being exposed to selective concentrations of antibacterial substances such as antibiotics and heavy metals. The question is, how large might the ecological compartment where these antibacterials exert effects reducing bacterial fitness be? The traditional view was that this compartment was mostly confined in the case of antibiotics to hospitals and farms, where they were concentrated, and to individuals undergoing therapy and in the case of heavy metals to water and soil that had been exposed to nearby industrial pollution. Delineating the real size of the compartment where antibiotic resistance plasmids might be maintained and spread requires knowledge of the antibiotic and heavy metal concentrations able to negatively influence bacterial physiology and ultimately growth. In a recent article in *mBio*, Gullberg et al. (1) demonstrated that sublethal concentrations of antibiotics and heavy metals, nearly 150 times lower than those required for inhibiting visible growth in cultures, are able to cause enough bacterial harm to make the maintenance of multiresistance plasmids profitable. This finding illustrates the possibility of a significant expansion in the size of the compartment, the selective space where these mobile genetic elements might evolve and spread.

Sublethal concentrations of harmful molecules acting on microorganisms are frequently found as the result of the diffusion from sites in which they are intensively released. From these source sites a gradient of concentrations is produced, eventually reaching the limit of no-biological-effect concentrations (Fig. 1). Note that bacterial cells located in the active-concentration compartment might be exposed to different levels of the antimicrobial agent. An important concept to be considered here is that the different concentrations along the gradient might result in discrete qualitative effects, such as the selection of particular antibiotic-resistant mutants at particular segments of the gradient acting as selective compartments, also called "resistance-selective environments" or sanctuaries (2–4). Antibiotic resistance frequently results from a sequence of mutational events which is favored by the independent selection of each of them along the gradient. Very low antibiotic concentrations might select a high diversity of resistant mutants (5) frequently with low fitness cost. Such selection of low-level resistance variants will facilitate further steps in the evolution of resistance. As Lenski and Mittler pointed out in a classic article, if subtle selection for some particular variants may occur only at very precise compartments, then that might explain how highly effective double mutants may in some cases reach high frequency without invoking the notion of "directed mutation" (6). The influence of such spatial heterogeneity on the development of antibiotic resistance and other source-sink dynamics ecologies (4) applies to the selection of particular novel host-plasmid combinations and/or plasmid modular rearrangements providing small advantages in terms of antibiotic or metal resistance to the recipient cell, which might help explain the high diversity of plasmid variants in natural populations.

Gottfried Wilhelm Leibniz, the person who contributed most to the understanding of a continuum gradient as composed of a multiplicity of "differential" units of activity, could certainly have posed a pertinent question (10). The question is, how small might the selective spaces be along the gradient to produce effects on the bacterial population structure? Of course, that depends not only on the steepness of the gradient and the mode of action of the selective agent but also on the bacterial organism. Phenotypic plasticity of bacteria, the ability to display a variety of noninheritable phenotypes to adapt to relatively small environmental changes around the optimum conditions, including so-called physiological or metabolic adaptation, might locally abolish the effects of the gradient. The resulting "physiological" occupation of small segments of the gradient might favor the emergence of efficient mutants (7) or the acquisition of adaptive traits by lateral genetic transfer, thus facilitating the climb up the inhibitory gradient.

The smaller the changes in antimicrobial concentrations that are able to produce changes in the bacterial population structure

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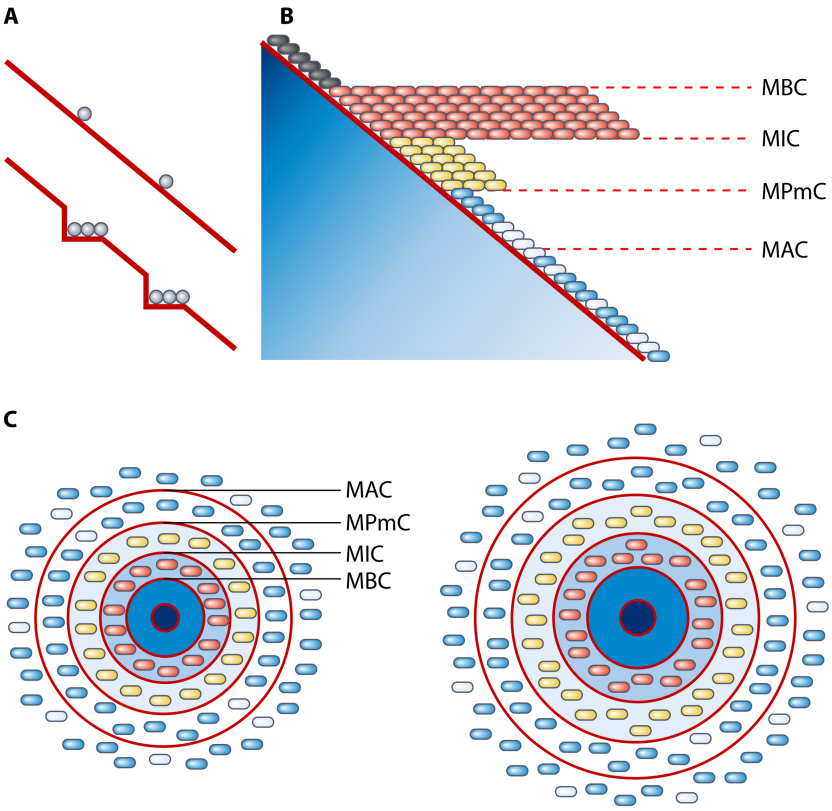

FIG 1 Bacterial populations on antimicrobial gradients. Lines represent the selective effects of a gradient of antibiotic or metal concentrations, diffusing up to down. (A) When bacteria are exposed to particular (stressful) points of the gradient (top), they may adapt to different neighbor concentrations without any genetic change (phenotypic adaptation), in a way deconstructing segments of the gradient locally (down) which facilitates local replication, and eventually inheritable adaptation. (B) Down in the gradient, the fitness of bacteria carrying a resistance-encoding plasmid exposed to subminimal antibiotic concentrations (MAC) is not affected by the antimicrobial (blue ovals), and therefore the plasmid is of no benefit, imposing only cost for the host cell, resulting in no selection for maintenance and plasmid loss (blue ovals move to white ovals). Bacterial fitness decreases when MAC is slightly surpassed, and harboring a plasmid might impose an extra fitness cost, eventually resulting in even more plasmid loss (more white ovals near the MAC). Up in the gradient, the antimicrobial imposes increased fitness costs, and at a particular concentration, the MPmC (minimal plasmid maintenance concentration, named MSC in the article by Gullberg et al. [1]), the extra cost of harboring the plasmid starts to be compensated for by the advantages provided in terms of resistance to antimicrobials (antibiotics and/or metals), and the plasmid-carrying population starts to be selected (yellow ovals; the number of piled ovals represents selection). Beyond the MIC of the plasmid-free population, selection of plasmid-bearing cells reaches a maximum (red ovals) and the relative cost of harboring the plasmid reaches a minimum. At very high antimicrobial concentrations, over the minimal bactericidal concentration (MBC), the bacterial population is extinguished (black ovals). (C) Concentric circles represent an apical view of the gradient. (Left) Spaces of selection of bacteria maintaining the plasmid when exposed to a single antimicrobial. (Right) The spaces of selection are broadened when the gradient involves two antimicrobials (e.g., metals plus antibiotics), resulting in an absolute increase in cells harboring a multiresistance plasmid.

(for instance, increasing the relative frequency of plasmid-bearing cells, or inhibitor resistance at large), the bigger the size of the selective compartment. The work of Gullberg et al. (1) indicates that the "space of selection" for plasmids encoding resistance to different chemical compounds might be huge, and certainly in wide spaces a high number of bacterial cells are exposed to the selective effects. These spaces might be located inside the human body (the bacterium-overpopulated large intestine has a surface of about 50 square meters) or in the free environment. The cost of harboring certain types of bacterial plasmids encoding resistance to antibiotics and metals has been considered one of the factors that might contribute to leveling off of antibiotic resistance. It can be suggested that under circumstances imposing an extra cost for the bacterial organism, plasmid loss might be facilitated. However, the work by Gullberg et al. (1) suggests that the plasmid might "pay the fee" of being maintained even when bacteria are confronted with concentrations of the inhibitor very close to the minimal concentration producing any effect on bacterial cells (minimal antibiotic concentration [MAC]). Of course, the "pay-off line" depends on the intrinsic cost of plasmid and the effects of the inhibitor. If the plasmid encodes multiple resistances (e.g., resistance to antibiotics and metals, as is the case for the plasmid pUUH239.2 in the study by Gullberg et al.), and if the bacterial population is exposed to various agents, the cost of harboring a plasmid becomes negligible. Therefore, if the population harboring a multiresistance plasmid is present in the wide selective space resulting from very low antibiotic concentrations along the gradient (8, 9), the plasmid will be maintained even if the effects of these concentrations on the host bacteria are minimal ones. Indeed, the plasmid will be maintained even in the absence of novel transfer events. However, the maintenance of a resistance plasmid might favor the transfer of such genetic element to compatible plasmid-free bacteria of the same or any other recipient population, particularly if sublethal concentrations trigger conjugation events.

In summary, the work of Gullberg et al. (1) suggests that the selective spaces for multiresistance plasmids could be huge and alerts us to the need to prevent the release of antimicrobial agents (antibiotics and heavy metals) in the environment (9).

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