

## Microbiology

# Bacteria under stress: cheating to survive

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This Break was edited by Max Caine, *Editor-in-chief* - TheScienceBreaker

### ABSTRACT

*Social bacteria growing in microbial communities cheat their resistant neighbors and survive antibiotic treatment. Understanding the social lifestyles of bacteria will help combat antibiotic resistance.*



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As scientists, we often think of bacteria as free-swimming, lone cells, growing in test tubes. Indeed, the majority of experiments are performed in this kind of environment. However, in reality, bacteria often grow in groups of cells, attached to surfaces. These cellular communities are social; cells are forced together and have no option but to interact.

Bacteria growing in this way can be up to 1000-times as resistant to antibiotic. Some bacterial resistance mechanisms involve the secretion of enzymes that break down antibiotic and detoxify the local environment. Resistance is costly for the resistant cell as it must divert resources to achieve resistance. However, a susceptible cell, that neighbours a resistant cell, may be able to cheat; benefiting from this local detoxification, without paying an

associated cost. We hypothesised that there might be cases where this can reduce selection for resistance, and favour cheating susceptible cells. We further hypothesised that this would only occur in environments in which bacteria were densely packed, as is the case when they grow together on surfaces.

In order to test whether this might be the case we mixed susceptible and resistant bacteria together in a 1:1 ratio and allowed them to grow on agar plates containing either no antibiotic or low concentrations (less than required to kill the susceptible strain, but enough to inhibit its growth) of two different antibiotics. Resistance to one of these antibiotics was due to a resistance mechanism that operated inside of the cell and the other antibiotic resistance

mechanism detoxified the antibiotic in the surrounding environment. We found that when resistant strains with the external resistance mechanism were grown side by side with susceptible cells, these susceptible cells fared better on antibiotic than when they were grown alone. This indicates that the susceptible strains were able to cheat – benefiting from the detoxification of the local environment by resistant cells, without paying an associated cost. This did not occur in the case of the resistant bacteria with an internal resistance mechanism. Furthermore, this result only occurred when bacteria were grown together in cellular communities, on agar plates. When the experiment was repeated and performed in the liquid environment of a test tube, susceptible cells were not able to cheat.

One result however puzzled us, susceptible cells didn't just grow better than resistant cells on the antibiotic; they grew even better relative to the resistant strain than when no antibiotic was present. Somehow, low levels of antibiotic were helping susceptible strains to outcompete resistant strains; the opposite of what we would expect. Initially we considered the simplest explanations; that the antibiotic was inducing the susceptible strains to grow or that the resistant strain was inhibited by low doses of antibiotic. However, we were unable to find evidence for any of these effects.

Still intrigued by this unexpected benefit for susceptible cells grown on antibiotic, we put the growing colonies under the microscope and saw the presence of the antibiotic was causing the

susceptible cells to form long filaments. This effect is well documented – the antibiotic attacks the cell wall and, in response, cells continue to divide without separating from the mother cell, generating filaments. This is also a stress response in many bacteria, as delaying division can provide the cell with the opportunity to repair DNA damage if it is under attack. Using a combination of microscopy and mathematical modelling we showed that these long cells align with surface of the agar plate and move ahead of the resistant cells. During this type of growth on a surface, reaching the colony edge is key to secure access to nutrients and space ahead of neighbouring cells. The elongated shape of the susceptible cells was allowing them to move ahead and outcompete the resistant cells for the edge. Thus we showed that what had been considered the response of a sick cell to antibiotic could also have this important evolutionary impact.

In the lab, bacteria are commonly studied in test tubes – growing as free-swimming entities, which have limited interactions with one another. Our research shows how important microbial interactions are to the way bacteria evolve. In the real world, whether growing in an infected burn wound or on a cowpat, bacteria are forced to interact with one another to ensure their survival. These interactions are social and the ability of bacteria to cooperate, or cheat, can have a profound effect on their evolution. Continuing to explore these processes will enhance our ability to understand and address the rising tide of antibiotic resistance.