The chemical topology of a bacterial swarm

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Microbes respond to antibiotics by initiating a suite of defense mechanisms, including the production of small-molecule effectors. However, it is not well-known how these defenses vary according to the particular effector or antibiotic and bacterial state, due in part to the challenges of monitoring small molecules in complex environments. A new study uses state-of-the-art imaging techniques to track the location of secreted small molecules produced by a bacterial swarm in response to different antibiotics, providing unexpected insights into the spatial heterogeneity of bacterial stress responses.

Bacteria occupy a wide array of diverse niches, where they may encounter numerous external stresses. As a result, they have evolved a variety of mechanisms to counter distinct stresses, often by coordinating community-wide responses that may protect at least a subset of the bacterial population. These mechanisms can be mediated by secreted small molecules; compounds discovered thus far have been shown to play roles in the cell-to-cell communication known as quorum-sensing, as well as virulence, aggregation and biofilm formation, antibiotic tolerance, the stress response, and interspecies interactions. Our understanding of the roles of specific small-molecule effectors in mediating these behavioral responses has been established mostly by population level studies. However, a more detailed understanding of where, when, and why these molecules are produced has been difficult to unravel. A new study by Morales-Soto et al. (1) sheds light on this topic, using advanced analytical strategies to directly image the alkyl quinolones produced by swarming Pseudomonas aeruginosa in response to two different antibiotics. Their results redefine the role of one well-known quorum-sensing molecule and demonstrate the geographical precision with which bacteria can control their communities.

P. aeruginosa is an opportunistic pathogen that has the ability to become tolerant as well as resistant to several antibiotics. It has also become an important model system for studying biofilm formation and quorum-sensing. It secretes more than 50 different small molecules, referred to as 2-alkyl-4(1H)-quinolones, or alkyl quinolones (2). Two of these molecules—2-heptyl-3-hydroxyquinolone (also termed Pseudomonas quinolone signal, PQS) and 2-heptyl-4-hydroxyquinoline N-oxide (HQNO)—are well-studied with the former serving as a quorum-sensing signal (3), whereas the latter possesses antimicrobial activity (4), in addition to various other biological roles. Previous work, including our own, has shown spatial variability in the production of these molecules and others at the macro scale within lungs of humans and pigs infected with P. aeruginosa (5–7). However, the spatial distribution of individual alkyl quinolones at the cellular level is unknown. Furthermore, the interplay between environmental cues and bacterial responses remains poorly understood during the “swarming” state, a term for bacterial translocation across a solid (or semisolid) substrate that has been suggested to be a pre-biofilm state. Swarming bacteria have been shown to be more resistant to some antimicrobials; thus, it may have importance in vivo. In addition, the swarming phenotype may be important for finding favorable conditions in vivo prior to biofilm formation. Understanding how bacteria spread on surfaces and how these processes are affected during stress response is crucial to creating effective treatment strategies.

Morales-Soto et al. began their study by confirming that both the ribosome-binding tobramycin and the cell-wall biosynthesis–disrupting carbenicillin inhibit swarming of P. aeruginosa, resulting in different cellular morphologies. The authors then used confocal Raman microscopy, which is able to distinguish PQS and HQNO families of compounds, to monitor the chemical outcome of antibiotic treatment. In contrast to untreated swarms, in which PQS-like molecules were localized in the center of the swarms, these compounds were found at the edge of the swarm after addition of a higher dose of tobramycin (Fig. 1). HQNO-like compounds, which were uniformly distributed in untreated swarms, were also found at the swarm periphery upon challenge with the same dose of tobramycin, but at the opposite side of the swarm. Surprisingly, PQS-like compounds could not be detected after carbenicillin treatment. Tests of bacterial strains deficient in the PQS or HQNO biosynthetic pathways confirmed a unique relationship between PQS and tobramycin though, paradoxically, swarms of cells deficient in PQS production were actually less susceptible to tobramycin.

To gain further insights, the authors turned to MS-based imaging. This technique can theoretically distinguish individual compounds, but traditionally has relied on the detection of

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2 The abbreviations used are: PQS, Pseudomonas quinolone signal; HQNO, 2-heptyl-4-hydroxyquinoline N-oxide.
antibiotic dosages. Are such conditions present in a clinical setting? Previous work, including our own, has demonstrated regional diversification in antibiotic resistance, antibiotic penetration, and the properties of \textit{P. aeruginosa} in patient lungs and biofilms (5–7). Thus, microbial communities are exposed to and respond to differential antibiotic stresses, even within the same organ. But, previous to the paper by Morales-Soto \textit{et al.} (1), a direct correlation between antibiotic exposure and spatial differences in specific alkyl quinolone levels was not known. In this regard, Morales-Soto \textit{et al.} narrowed our gap in the understanding of individual functions of alkyl quinolones and how they contribute to antibiotic tolerance. Future investigations into the role of alkyl quinolone signaling in \textit{Pseudomonas} clinical isolates from patients with prior exposure to antibiotics will reveal how these pathways evolve in complex infection environments such as cystic fibrosis. Such studies are necessary to explore the efficacy and timing of administration and development of new therapeutics.

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