Swimming bacteria promote dispersal of non-motile staphylococcal species

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Swimming motility is considered a beneficial trait among bacterial species as it enables movement across fluid environments and augments invasion of tissues within the host. However, non-swimming bacteria also flourish in fluid habitats, but how they effectively spread and colonize distant ecological niches remains unclear. We show that non-motile staphylococci can gain motility by hitchhiking on swimming bacteria, leading to extended and directed motion with increased velocity. This phoretic interaction was observed between Staphylococcus aureus and Pseudomonas aeruginosa, Staphylococcus epidermidis and P. aeruginosa, as well as S. aureus and Escherichia coli, suggesting hitchhiking as a general translocation mechanism for non-motile staphylococcal species. By leveraging the motility of swimming bacteria, it was observed that staphylococci can colonize new niches that are less available in the absence of swimming carriers. This work highlights the importance of considering interactions between species within polymicrobial communities, in which bacteria can utilize each other as resources.

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Bacteria often exist as polymicrobial communities in a multitude of environments (Fernandez et al., 2000; Hosni et al., 2011; Burmølle et al., 2014). Here we investigate the ways in which bacteria in the same community may affect each other’s motility in liquid environments. Swimming motility offers a considerable advantage for bacteria by enabling movement toward environments of favorable conditions (Stocker et al., 2008; Dennis et al., 2013), and movement away from toxins or predators (Adler, 1966; Berg, 1975). Non-flagellated bacteria do not have the capacity to independently translocate with this mechanism. The genus Staphylococcus, for example, is classically considered non-motile in fluid environments due to the lack of flagella (Kloos and Bannerman, 1994; Freney et al., 1999). Despite their limitations in motility, staphylococcal species effectively reach and thrive in their preferred ecological niches.

We tested if non-motile species may benefit from the swimming motility of flagellated bacteria. To address this, we studied two human pathogens that are found in the same ecological habitats, but rely on different mechanisms for translocation. Staphylococcus aureus is a Gram-positive, non-motile cocci species and Pseudomonas aeruginosa is a Gram-negative, flagellated rod species capable of swimming motility. Using a standardized biofilm assay (Ceri et al., 1999), a vertical insert was placed into a microwell containing bacterial inoculum. The insert does not touch the bottom or sides of the microwell, thereby creating two distinct niches for potential colonization: the first at the bottom of the well, and the second at the top of the inoculum (air–liquid interface) on the lateral surface of the insert (Figure 1a). We expected non-motile S. aureus to settle and form a biofilm at the bottom of the microwell and motile P. aeruginosa to build a biofilm at the air-liquid interface, which requires upward swimming. Figures 1b and c show that indeed, P. aeruginosa readily colonized the air-liquid interface while S. aureus was largely absent from this location in monoculture. However, when the two species were co-cultured in the same microwell, significantly more S. aureus cells were isolated from the air-liquid interface. On average, there were 6-fold more S. aureus cells isolated 30 seconds after initiation of biofilm formation, and 16-fold more after 16 hours (Figure 1b). Together, these results indicate that colonization of this niche was strongly enhanced by the presence of P. aeruginosa. A similar trend was observed with a 100 times higher inoculation density of S. aureus cells, with 30-fold more S. aureus cells at the air-
liquid interface after 30 seconds, and 3-fold more cells at the same location 16 hours after inoculation (Supplementary Figure 1A). For comparison, *P. aeruginosa* cell numbers at the air-liquid interface were largely unaffected by the presence of *S. aureus* (Figure 1c; Supplementary Figure 1B). Scanning electron micrographs of cells from vertical inserts confirmed co-localization of *P. aeruginosa* and *S. aureus* in the biofilm at the air-liquid interface after 2 h (Figures 1d and e). Taken together, these data suggest *S. aureus* has acquired, through *P. aeruginosa*, an increased capacity to travel longer...
distances, allowing it to colonize niches that are relatively inaccessible in the absence of swimming carrier bacteria.

We hypothesized that the increased colonization of the air-liquid interface by *S. aureus* may be due to hitchhiking of *S. aureus* on *P. aeruginosa*. To evaluate this possibility, fluorescently labeled *P. aeruginosa* and *S. aureus* were combined at equal numbers and placed in a microchamber (Supplementary Figure 2) for observation by live confocal

**Figure 2** For caption see in next page.
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microscopy. When imaged concurrently, S. aureus cells were observed associated to P. aeruginosa cells (Figure 2a) and moving together for a period of time (Supplementary Video 1).

To further quantify the motility of S. aureus in the absence and presence of P. aeruginosa, single cell tracking was employed on videos obtained of the cells in single and dual species cultures. For tracking experiments, we used P. aeruginosa PAO1-eGFP and S. aureus stained with hexidium iodide. From the resultant cell trajectories, we calculated persistence length (defined as the length scale of decay for angular autocorrelation of a trajectory, which provides a measurement of how linear a trajectory is), velocity and mean squared displacement (MSD). S. aureus showed random motion patterns (Supplementary Video 2) with MSDs similar to a sphere undergoing Brownian motion in liquid medium (Supplementary Figure 3). For comparison, P. aeruginosa exhibited a run-reverse motility pattern consistent with previous accounts of swimming motility among this species (Supplementary Video 3). P. aeruginosa also exhibited greater velocities and persistence lengths than non-motile S. aureus (Figure 2b; Supplementary Figure 4). S. aureus trajectories changed distinctly when P. aeruginosa was present, with persistence lengths increased by an order of magnitude compared to values obtained for S. aureus in monoculture (Figures 2c and d; Supplementary Video 4). Cell trajectories set to start at coordinate (0,0), plotted for S. aureus alone (Figure 2d; blue) and S. aureus mixed with P. aeruginosa (Figure 2d; red) illustrate the extended and directed motion of S. aureus when P. aeruginosa was present. Comparing the MSD of S. aureus in the absence (Figure 2e; blue) and presence (Figure 2e; red) of P. aeruginosa, a shift was observed toward increased directed motility for S. aureus when P. aeruginosa was present. These S. aureus trajectories are superdiffusive (Supplementary Figure 5), which has previously been suggested for bacteria undergoing flagellar motility (Matthäus et al., 2009). For comparison, the trajectories of S. aureus in the presence of non-motile P. aeruginosa mutants, PAO1ΔmotABCD and PAO1ΔfgE, did not measurably deviate from those of S. aureus alone (Figures 2f–i; Supplementary Figure 6; Supplementary Videos 5 and 6), further suggesting that the extended motility of S. aureus is dependent on the swimming motility of P. aeruginosa.

It was also observed that P. aeruginosa can carry another staphylococcal cargo, Staphylococcus epidermidis. S. epidermidis trajectories in the presence of P. aeruginosa were characterized by longer persistence lengths and increased velocity compared with S. epidermidis alone (Figures 2j–m; Supplementary Figures 5B and E; Supplementary Video 7). Moreover, P. aeruginosa was capable of transporting carboxylated polystyrene beads (Supplementary Videos 8–11). Together, these results suggest that P. aeruginosa can engage with a variety of cargos with distinct biochemistries, presumably through different types of chemical interactions. Another motile carrier species, E. coli EMG2 also has the ability to carry staphylococci as cargo (Figures 2n–q; Supplementary Figures 5C and F; Supplementary Video 12). Collectively, these data may support a generalized mechanism for translocation among staphylococcal species via interaction with flagellated bacteria.

The findings presented here quantitatively indicate that staphylococcal species, classically defined as non-motile, have altered motility patterns in the presence of flagellated P. aeruginosa and E. coli. Specifically, P. aeruginosa and E. coli may function as microbial carriers for staphylococcal species and result in enhanced dispersal range in fluid environments. The carrier-dependent movement described here appears mechanistically distinct from previously described spreading of S. aureus on surfaces, which occurs independent of a second, swimming bacterium (Kaito and Sekimizu, 2007; Pollitt et al., 2015).

While there exists a wealth of information regarding flagella-mediated bacterial self-propulsion...
(Lauga and Powers, 2009), in most natural environments, bacteria are part of polymicrobial communities (Sibley et al., 2008; Consortium, 2012) and the contributions of interspecies phoretic interactions to bacterial dispersal in aqueous environments are not well understood. The ability of non-motile bacterial species to leverage motility from other bacteria has been observed to occur between microbes on the surface of plants, and in soil (Hagai et al., 2014; Finkelshtein et al., 2015). The primary motivation of this work is to highlight the impact of microbial hitchhiking on translocation and distribution of non-motile bacteria in liquid. We found that this phoretic mobility could directly change the localization patterns of staphylococci and open new niches for colonization, as observed in biofilm formation at the air-liquid interface. Looking forward, such behavior could influence community diversity, microbial dispersal, and perhaps enhance transmission of non-motile pathogenic strains. From a clinical perspective, our observations may have important implications on how non-motile pathogens disseminate.

Conflict of Interest
The authors declare no conflict of interest.

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