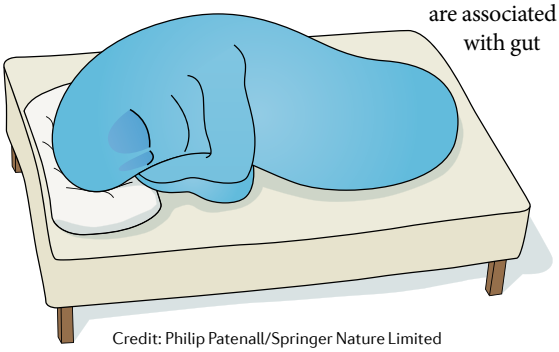
ANTIMICROBIALS

Spreading resistance in *Salmonella*'s sleep

> tissue-resident *S.* Typhimurium persister cells can function as a reservoir to promote the spread of conjugative plasmids in the gut

Plasmids carrying antimicrobial resistance (AMR) genes have a major role in the spread of AMR as they are often transmissible between bacteria. Understanding the factors that promote the spread of resistance-conferring plasmids is therefore crucial for combatting AMR. In a recent study, Bakkeren et al. investigated whether persister cells contribute to the spread of AMR plasmids. Bacterial persister cells are a subpopulation of cells that can survive intensive antibiotic treatment without being resistant. Bakkeren et al. found that persister cells represent a long-lasting reservoir of AMR plasmid donors or recipients, fostering the spread of AMR.

The enteric pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium can colonize the gut lumen and is eliminated by antibiotic treatment within hours. By contrast, *S.* Typhimurium persisters survive within host tissues, even after treatment with antibiotics. After antibiotic treatment, tissue-resident *S.* Typhimurium can migrate back to the gut lumen and resume growth (re-seeding). The authors hypothesized that persister cells increase the chance of plasmid donors co-occurring with gut-luminal recipients, thereby increasing the spread of AMR in vivo. To test this, they first investigated whether *S.* Typhimurium persister cells that are associated with gut tissue are a reservoir for conjugative plasmids. After orally infecting mice with a ciprofloxacin-sensitive *S.* Typhimurium strain harbouring the P2 conjugative plasmid, the mice were treated with ciprofloxacin, which cleared the luminal bacteria but not the persister revoir in host tissues. A *S.* Typhimurium recipient lacking P2 was then orally introduced into the infected mice. Within 1–3 days of donor re-seeding, transconjugants (that is, recipients of P2) outnumbered the non-recipients 100-fold. Control experiments confirmed that this observation was owing to plasmid conjugation and that P2 transfer occurs within the gut lumen. The importance of the persister reservoir in systemic tissues was verified using an intravenous infection model. Persister *S.* Typhimurium P2 cells formed in the spleen and liver before migrating to the gut lumen and producing a significant number of transconjugants within 1–3 days of re-seeding, which suggests that tissue-resident *S.* Typhimurium persister cells can function as a reservoir to promote the spread of conjugative plasmids in the gut.



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Next, the authors investigated plasmid transfer dynamics to understand how transconjugant populations arise from the persister donor population. DNA-tagged P2 plasmid variants were used to estimate the rate at which persisters re-enter the gut lumen, the rate of plasmid conjugation and the relative growth rate of transconjugants compared to recipients. Remarkably, most transconjugant populations contained only one or two of the tagged plasmids, which suggests that transconjugative populations arise from a small number of donor-to-recipient events, which are followed by further transconjugant-to-recipient spread. Mathematical modelling confirmed that low-frequency re-seeding events could explain high levels of conjugation in the gut, and suggested that reducing the rate of re-seeding and donor-to-recipient conjugation 100-fold (for instance, through vaccination) would halt conjugation and plasmid spread. Consistent with this, vaccinated mice had a 10–500-fold reduction in persister cell reservoirs, and vaccination prevented plasmid transconjugation in most cases.

Next, the authors tested whether commensal *Escherichia coli* (a common member of the human microbiota) could acquire conjugative plasmids. They observed that a P2 plasmid in *E. coli* could efficiently transfer to a P2-deficient strain of *E. coli* and to tissue-associated *S.* Typhimurium persisters in an oral mouse model. In an intravenous model, *S.* Typhimurium P2 persister cells re-seeded the gut lumen and P2 was transferred to *E. coli*. These findings indicate that tissue-associated *S.* Typhimurium persister cells can function as both donors and recipients of plasmid transfer between various species of Enterobacteriaceae.

Importantly, *S.* Typhimurium persister cells were also shown to be a reservoir for several clinically relevant AMR plasmids, which highlights the need for new strategies to reduce bacterial persistence.

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ORIGINAL ARTICLE Bakkeren, E. et al. *Salmonella* persisters promote the spread of antibiotic resistance plasmids in the gut. *Nature* **573**, 276–280 (2019)
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