Salmonella persisters promote the spread of antibiotic resistance plasmids in the gut

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The emergence of antibiotic-resistant bacteria through mutations or the acquisition of genetic material such as resistance plasmids represents a major public health issue1,2. Persisters are subpopulations of bacteria that survive antibiotics by reversibly adapting their physiology3–10, and can promote the emergence of antibiotic-resistant mutants11,12. We investigated whether persisters can also promote the spread of resistance plasmids. In contrast to mutations, the transfer of resistance plasmids requires the co-occurrence of both a donor and a recipient bacterial strain. For our experiments, we chose the facultative intracellular entero-pathogen Salmonella enterica serovar Typhimurium (S. Typhimurium) and Escherichia coli, a common member of the microbiota13. S. Typhimurium forms persisters that survive antibiotic therapy in several host tissues. Here we show that tissue-associated S. Typhimurium persisters represent long-lived reservoirs of plasmid donors or recipients. The formation of reservoirs of S. Typhimurium persisters requires Salmonella pathogenicity island (SPI)-1 and/or SPI-2 in gut-associated tissues, or SPI-2 at systemic sites. The re-seeding of these persistor bacteria into the gut lumen enables the co-occurrence of donors with gut-resident recipients, and thereby favours plasmid transfer between various strains of Enterobacteriaceae. We observe up to 99% transconjugants within two to three days of re-seeding. Mathematical modelling shows that rare re-seeding events may suffice for a high frequency of conjugation. Vaccination reduces the formation of reservoirs of persisters after oral infection with S. Typhimurium, as well as subsequent plasmid transfer. We conclude that—even without selection for plasmid-encoded resistance genes—small reservoirs of pathogen persisters can foster the spread of promiscuous resistance plasmids in the gut.

S. enterica and E. coli strains contain numerous resistance plasmids13,14, and different strains frequently colonize the same host15–19. High cell densities in the gut lumen allow high rates of plasmid transfer within and between species20–22. S. Typhimurium strains can colonize the gut lumen and survive within the tissues of their host for prolonged periods5–7,15–17,23. In the absence of suitable resistance genes, the gut-luminal population of S. Typhimurium is eliminated by antibiotics within a few hours, whereas tissue-associated S. Typhimurium persisters cells survive for more than ten days5–8,24 (Supplementary Discussion). After withdrawal of antibiotics, surviving cells may migrate to the gut lumen and resume growth24. We hypothesized that this promotes the co-occurrence of donors with gut-luminal recipients, and thereby fuels the transfer of resistance plasmids in vivo (Extended Data Fig. 1a).

Wild-type S. Typhimurium SL1344 naturally carries P2, a well-characterized conjugative plasmid of the IncI1 incompatibility group25 in Enterobacteriaceae20–22 (Extended Data Fig. 1b); this strain of S. Typhimurium served as the donor in our initial experiments. We labelled P2 with a chloramphenicol-resistance marker (P2cat) to monitor plasmid transfer by plating (Extended Data Fig. 1c, d). Controls excluded cross-resistance for any of the resistance markers used in this study (Extended Data Fig. 2). In the ‘oral model’, the donor (that is, S. Typhimurium P2cat) initially colonized the gut lumen and invaded host tissues (Fig. 1a, b, Extended Data Fig. 3). Ciprofloxacin cleared the gut-luminal bacteria, whereas tissue-associated S. Typhimurium P2cat persisters survived5–8. The recipient S. Typhimurium ATCC 14028S strain, which naturally lacks P2, was introduced at day 85–22. Transconjugants (that is, recipients that obtained the plasmid) replaced the recipients within one to three days of donor re-seeding (>99% median; day 11–12 of the experiment) (Fig. 1c, Extended Data Fig. 3a). Control experiments refuted that the replacement by transconjugants is attributable to P2-mediated fitness benefits over the recipients (Extended Data Fig. 3d, e). Further control experiments verified that P2cat transfer occurs in the gut lumen (Extended Data Fig. 3f, g) and that P2cat spreads by conjugation (Extended Data Fig. 3h, i). Non-invasive donor mutants indicated that the donor cells originated from reservoirs of tissue-associated S. Typhimurium P2cat persisters (Fig. 1c–e, Extended Data Figs. 3b, 4). However, this cannot definitively rule out that rare persisters could also exist in the gut lumen.

To verify the importance of reservoirs of tissue-associated S. Typhimurium persisters, we used an ‘intravenous infection model’ (Fig. 2a, b). Consistent with previous work23, S. Typhimurium P2cat formed large populations of persisters that survived in the spleen and the liver after intraperitoneal treatment with ceftriaxone (Fig. 2c, d, Extended Data Fig. 5a). In contrast to the oral model, gut-luminal colonization by S. Typhimurium P2cat is observed only very rarely by day 5 (Extended Data Fig. 5). Nevertheless, when donors were detected in the gut lumen, transconjugants were formed with high efficiency within one to three days (Fig. 2e, Extended Data Fig. 5b). Donors that lacked a key virulence factor that promotes both systemic growth of pathogens in vivo26 and the survival of persisters27 yielded much smaller reservoirs of persisters than did wild-type S. Typhimurium P2cat (Fig. 2d), and did not produce transconjugants (Fig. 2e, Extended Data Fig. 5c). We conclude that tissue-associated S. Typhimurium persisters can serve as reservoirs that promote the spread of conjugative plasmids.

Next we identified the rate-limiting process in transconjugant formation in vivo, focusing on the oral model. Three key parameters may dictate plasmid transfer dynamics: (1) the rate at which plasmid-carrying donors re-enter the gut lumen and perform the initial conjugation event (that is, re-seeding), which is dependent on the size of the reservoir of persisters; (2) the rate of plasmid transfer from transconjugants to recipients; and (3) the relative growth rate of transconjugants compared to recipients. To estimate these rates, we used donor mixtures that carried five variants of DNA-tagged P2cat (S. Typhimurium P2cat-tag) (Extended Data Fig. 6). Whereas all tags were present in roughly equivalent abundance in the inoculum, the faeces (on day 1) and the mucosa (on day 15), most transconjugant populations contained just
Fig. 1 | S. Typhimurium persisters associated with gut tissue are a reservoir for conjugative plasmids. a. Plasmid-bearing S. Typhimurium (S. Tm) (blue lozenge) form persisters (shown as smaller lozenges) in gut tissues and the mesenteric lymph nodes (mLN). These can survive antibiotic treatment, re-seed the gut lumen and transfer their plasmid to recipients (green). Transconjugants (red) can further amplify the spread of plasmids. b. Oral infection mouse model. Col., colonization. c. Donor mucosa invasion is required for persister-promoted plasmid transfer. Invasive (streptomycin-resistant (SmR) and CmR) or non-invasive S. Typhimurium SL1344 P2 cat tag strain (TTSS-1-negative, SmR and CmR) was used as a donor, and S. Typhimurium 14028S P2 cat tag (free of P2) (KanR and AmpR) was used as a recipient, as shown in b. Transconjugant proportions in faeces from experiments with invasive (solid black circles; grey bars indicate median; n = 15 mice; 5 independent experiments; n = 9 mice pooled from data in Fig. 3) or non-invasive donors (open black circles; white bars indicate median; n = 6 mice, 2 independent experiments). Dashed lines connect data points from the same mouse. d. Donor reservoirs in the caecal mucosa from mice, as infected in c, were quantified by tissue gentamicin protection. Solid blue circles represent invasive donors; open blue circles represent non-invasive donors; open circles indicate median. Statistics were performed using a two-tailed Mann–Whitney U-test. NS, not significant (P > 0.05), *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Dotted line denotes the detection limit. e. Persisters in the caecal lamina propria (LP) (oral model, day 8). S. Typhimurium (yellow), anti-LPS O5 and anti-LPS O12 staining, nuclei (blue), actin (green) and lamina propria (red, ICAM-1) are stained. Ep, epithelium; Lu, lumen. Scale bars, 20 μm. White arrows highlight S. Typhimurium (magnified in inset). Representative of three independent experiments. Quantification is provided in Extended Data Fig. 4.

Fig. 2 | Persisters at systemic sites are a reservoir for plasmid transfer in the gut. a. As in Fig. 1a, but donors are introduced by intravenous infection. b. Intravenous (i.v.) infection mouse model. c. Persisters in the spleens and livers. In two independent experiments, n = 8 mice (grey circles) were infected intravenously with an equal mix of five S. Typhimurium P2 cat tag strains (SL1344 strain; SmR and CmR). Black circles indicate control mice (n = 8) injected for 5 days without ceftriaxone treatment. Extended Data Figure 5a shows counts for additional organs. d. e, SPI-2 promotes donor reservoir formation at systemic sites. In two independent experiments, mice were infected as described in b with donors; that is, with mixtures of five wild-type (n = 6; closed circles) or SPI-2-deficient (n = 6; open circles) S. Typhimurium P2 cat tag strains (SmR and CmR). d. Donor populations in internal organs from mice infected with wild-type (solid blue) or SPI-2-deficient donors (open blue). Lines indicate the median. e. Faecal populations from mice from d. Proportions of transconjugants in faeces, from experiments with wild-type (solid black circles; grey bars indicate median) or non-invasive donors (open black circles; white bars indicate median). Dashed lines connect data points from the same mice. Statistics were performed using a two-tailed Mann–Whitney U-test. NS, not significant (P > 0.05), *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Dotted lines denote detection limits.

To quantify the relative contribution of donor re-seeding and plasmid conjugation, we developed a mathematical model (Extended Data Fig. 6g). This model assessed the interdependence of the rate of donor re-seeding (r), the sum of donor re-seeding plus the donor-to-recipient conjugation (γ), and the rate of transconjugant-to-recipient conjugation (γ) (Supplementary Information). Fitting our mathematical model to the distributions of the plasmid tags confirmed that r is rate-limiting, and identified the most likely parameters; these were per-recipient rates of n = 3.16 × 10−10 re-seeding events per day and γ = 3.16 × 10−8 conjugation events per colony-forming unit (CFU) per gram of...
facilities per day (red in Fig. 3c; marginal posterior densities are listed in Supplementary Table 4). This corresponds to approximately 1.6 donor re-seeding events (plus the initial plasmid transfer) per day into the gut lumen. By contrast, transconjugant-to-recipient plasmid conjugation rates were ≥32 events per day for the initial conjugations, and increased exponentially thereafter (Supplementary Information).

We then used our mathematical model to predict the effect of reducing the rate of donor re-seeding. The transconjugant-to-recipient conjugation parameter was fixed at \( \gamma = 3.16 \times 10^{-8} \) events per CFU per gram of faeces per day (Fig. 3c), and we evaluated the probability of conjugation events occurring within the course of our 15-day experiment (Fig. 3d). Reducing \( \gamma \) by more than 100-fold diminished conjugation (Fig. 3d). To test this prediction, we used oral vaccination with killed S. Typhimurium cells, a procedure that is known to reduce S. Typhimurium invasion of the gut tissue. Consistent with previous work, vaccinated mice contained 10–500-fold smaller reservoirs of persisters than did naive mice (Fig. 3e). Indeed, this vaccination prevented plasmid transfer in the vast majority of mice (Fig. 3f).

We next addressed whether strains of the microbiota could also acquire these conjugative plasmids. First, we analysed P2\textsuperscript{cat} transfer to the commensal E. coli strain B1878\textsuperscript{9,22}. For this purpose, we created an ampicillin-resistant derivative of E. coli B1878 that was free of the P2 plasmid\textsuperscript{9}, and used it as a recipient in the oral model (Fig. 1b). This yielded efficient plasmid transfer (Fig. 4a, b, Extended Data Fig. 8a). We tested whether tissue-associated S. Typhimurium persisters could also serve as recipients. In the oral model (Fig. 1b), mice were first infected with a P2-cured variant of S. Typhimurium SL1344 (kanamycin-resistant (Kan\textsuperscript{R})) Then, we treated the mice with ciprofloxacin and ampicillin, and introduced the donor (E. coli B1878 P2\textsuperscript{cat} ampicillin-resistant (Amp\textsuperscript{R}) and chloramphenicol-resistant (Cm\textsuperscript{R})). S. Typhimurium re-seeded the gut lumen and transconjugants were formed (>38% median) (Extended Data Fig. 8b–d). Thus, tissue-associated S. Typhimurium persisters can serve as donors or as recipients of plasmid transfer between different species of Enterobacteriaceae.

To assess whether mucosa-associated S. Typhimurium can also serve as a reservoir for clinically relevant plasmids that carry genes of the extended-spectrum \( \beta \)-lactamase (ESBL) family, we used pESBL15 (Extended Data Fig. 1b). S. Typhimurium SL1344 pESBL15 was used as a donor. We modified our oral model from Fig. 1b in two ways: by replacing ampicillin with kanamycin in the drinking water, and by using an ampicillin-sensitive (Amp\textsuperscript{S}) variant of our standard recipient strain (S. Typhimurium 14028S pK17 (Kan\textsuperscript{S} and Amp\textsuperscript{S})). We observed tissue-associated persisters, re-seeding of the gut lumen and efficient plasmid transfer (Fig. 4c, d, Extended Data Fig. 8e). Thus, our findings apply to clinically important resistance plasmids. The choice of the antibiotics used for the different phases of our oral model had little effect on these fast plasmid-transfer kinetics (Extended Data Fig. 9a–d).

Finally, we validated the transfer of antibiotic-resistance plasmids to E. coli in the intravenous model. Intravenous infection of S. Typhimurium P2\textsuperscript{cat} led to populations of persisters in the internal organs (Fig. 4e); these populations could re-seed the gut lumen and transferred the plasmid into the luminal recipient population, forming >25% E. coli transconjugants in the absence of antibiotic selection (Fig. 4f).

Our results uncover a mechanism by which antibiotic persistence promotes the spread of antibiotic-resistance plasmids, specifically by promoting the co-occurrence of donors and recipient bacteria in the gut-luminal niche. This reveals a role for persistence in clinical bacterial infection: not only can bacterial persistence lead to relapse of disease in chronic infections, but also it can facilitate the spread of antibiotic resistance. In the case discussed here, the transfer rate is independent...
Fig. 4 | Tissue-associated persisters promote the transfer of resistance plasmids between different species of Enterobacteriaceae. a, B. Plasmid transfer to E. coli. Five mice (one experiment) were infected orally as described in Fig. 1b (except that ampicillin was removed from the drinking water on day 8), with donors (S. Typhimurium P2Δid SL1344 strain, SmR and CmR) and recipients (E. coli 8178 P2cured strain, AmpR). Mucosa-associated donors and faecal transconjugants were enumerated by plating. Median indicated by solid line or bars. c, d, Transfer of plasmids containing ESBL genes between Salmonella spp. The experiment used S. Typhimurium SL1344 PESBL15 (SmR and AmpR) as the donor and S. Typhimurium 14028S apHT (KanR) as the recipient (n = 5 mice, 1 experiment). This experiment was performed and evaluated as in a, b, except that kanamycin was used in the drinking water from day 3 to day 8 (instead of ampicillin, as PESBL15 confers ampicillin resistance). e, f, Plasmid transfer from S. Typhimurium to E. coli using the intravenous model. Six mice (two independent experiments) were infected as described in Fig. 2b, with donors (S. Typhimurium P2Δid SL1344 strain [SmR and CmR], intravenous) and recipients (E. coli 8178 P2cured [AmpR], oral). Tissue-associated donors and faecal transconjugants were enumerated by plating. Median indicated by solid line or bars. a–f, Dotted lines indicate detection limit by selective plating. Oral E. coli recipient

Online content

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment. This was necessary to avoid cross-contamination of pathogens and plasmid donors between the different experimental groups as far as possible.

Strains and plasmids used in this study. Supplementary Table 1 contains all strains and plasmids used in this study. For cultivation of bacteria, lysogeny broth (LB) medium containing the appropriate antibiotics (50 μg/ml streptomycin (AppliChem), 6 μg/ml chloramphenicol (AppliChem), 50 μg/ml kanamycin (AppliChem) or 100 μg/ml ampicillin (AppliChem)) were used. To create P2 Δtag strains (using previously published neutral genetic barcodes34) or gene-deletion mutants (for example, oriT::aphT on P2), the λ red system was used, as previously described35. If desired, antibiotic-resistance cassettes were removed using the temperature-inducible FLP recombinase encoded on pCP20. Mutations or sequence tags coupled to antibiotic-resistance cassettes were transferred into the desired genetic background using transduction with P22 HT105/1 int-2036. Primers used for strain construction or verification of genetic background are listed in Supplementary Table 2.

In vitro plasmid-transfer kinetics. Overnight cultures with appropriate antibiotics of donor (SL1344 P2 ΔoriT::aphT) and recipient (140285 apfT) strains carrying pMW975 to confer ampicillin resistance were subcultured 1:20 in LB without antibiotics, and grown for 4 h. Approximately 10^6 CFU of each were added (sequentially as 25-ml volumes) to 450 μl LB with 100 μg/ml ampicillin. Samples were incubated at 37°C mixing at 1,000 r.p.m. for 24 h. Aliquots (10 μl) were taken every hour, diluted, and plated on selective MacConkey agar for enumeration.

In vitro antibiotic-resistance profiling. Flat-bottom transparent 96-well plates were filled with 100 μl of LB containing twofold dilutions of the specified antibiotic (streptomycin (AppliChem), chloramphenicol (AppliChem), kanamycin (AppliChem), ampicillin (AppliChem), ciprofloxacin (ciprofloxacin hydrochloride monohydrate, Sigma-Aldrich), gentamicin (AppliChem) or ceftriaxone (ceftriaxone disodium salt hemi(heptahydrate), Sigma-Aldrich). Each plate contained eleven twofold dilution steps of each antibiotic, plus a no-antibiotic control and the appropriate antibiotics, subcultured (1:20 dilution) for 4 h at 37°C in LB without antibiotics, and diluted in PBS. Cells were seeded in each well at a final density of 10^5 CFU/ml. Ninety-six-well plates were incubated at 37°C at 120 r.p.m. for 16 h and the optical density at 600 nm (OD600 nm) was measured. The no-bacteria sterility control was used for background subtraction.

Infection experiments. Oral infection model. For in vivo plasmid-transfer experiments, 8–12-week-old 129/SvEv mice were used. These mice are Nramp1+/− (Nramp1 is also known as Slil11a1) and therefore allow for long-term S. Typhimurium infections37. They carry a complex specified-pathogen-free microflora. The experiment was conducted at the beginning of the experiment. The two competitor strains were cultured separately in LB containing the appropriate antibiotics, subcultured and washed with PBS, as above. Strains were mixed at a 1:1 ratio immediately before gavaging 5 × 10^4 CFU of the mixture into the ampicillin-pre-treated mice. Faeces were monitored daily, homogenized and enumerated by selective plating. The competitive index was calculated using the ratio of population sizes of competitors at the indicated time. Mice were euthanized after seven days.

All mouse infection experiments were approved by the responsible authority (Tierversuchskommission, Kantonales Veterinäramt Zürich, license 193/2016). Sample size was not predetermined. Mouse age and gender were matched between treatment groups and mice were randomly distributed among groups. In four cases, data from mice were excluded because the mice needed to be euthanized prematurely, owing to organ failure or symptom severity.

Confocal microscopy. To visualize persisters in the caecum lamina propria, caecum tissues were fixed in PBS/4% paraformaldehyde, saturated in PBS/20% sucrose and embedded in optimal cutting temperature medium (OCT, Tissue-Tek) before being flash-frozen in liquid nitrogen. Ten-micrometre cryosections were air-dried, rehydrated with PBS, permeabilized with PBS/0.1% Triton X-100 and blocked with PBS/1% goat serum. Anti-S. Typhimurium LPS O5 (Difco), anti-S. Typhimurium LPS O12 (STAS, ref. 35), anti-ICAM-1/CDSF (BD Biosciences), appropriate secondary antibodies, DAPI (Sigma-Aldrich) and Alexa-Fluor-488-conjugated phallolidin (Santa Cruz) were used for the staining. A Zeiss Axiovert 200 laser microscope with 10–100× objectives, a spinning-disc confocal-laser user (Visirotom), and two Evolve 512 EMCCD cameras (Photometrics) were used for acquiring images. Images were processed using Visiview (Visirot). LPS-positive (O5-positive and/or O12-positive) S. Typhimurium was manually enumerated blindly in 8–12 consecutive sections per mouse. Phallolidin and ICAM-1 staining were used to differentiate the lamina propria and epithelium. All data represent averages per section.

Mucosal fixation and staining. Caeal tissue samples were fixed with freshly prepared Methacarn solution (60% methanol, 30% chloroform and 10% glacial acetic acid) for 24 h at room temperature39. The samples were transferred to methanol for 24 h and processed over night with a Logol tissue processor (Milestone) using the following program: 30 min, 37°C EtOH; 30 min, 37°C EtOH; 60 min, 37°C EtOH; 60 min, 40°C isopropanol, 15 min for heating up; 60 min, 45°C isopropanol, 20 min for heating up; 180 min, 68°C isopropanol, 20 min for heating up; and 240 min, 82°C paraffin, 75 min for heating up.

The paraffinized tissue was then embedded as paraffin blocks for further storage. Ten-micrometre sections were deparaffinized in Xylene substitute solution (Sigma-Aldrich) for 20 min. The sections were rehydrated in sequential baths of 100%, 95%, 70%, 50% and 30% ethanol for 5 min each, and subsequently incubated for 10 min in PBS. For mucus visualization, the sections were stained with DAPI, phallolidin–FITC, wheat germ agglutinin AFE67 conjugate (Invitrogen, cat. no. W32466), rabbit polyclonal anti-Salmonella O3 (Becton Dickinson, cat. no. 226600) and goat polyclonal anti-rabbit Fab Cy3 conjugate (Jackson ImmunoResearch Laboratories, cat. no. 111-167-003; BRID: AB_2313593) antibodies.

Analysis of plasmid-transfer dynamics. Mice were orally or intravenously infected with a mixture of 5 isogenic SL1344 P2 Δtag strains (tags at a 1:1:1:1:1
ratio; inoculum made of approximately 10^7 CFU of each tagged strain for oral infection and 2 × 10^5 CFU of each tagged strain for intravenous infection) using the model protocol described in ‘Oral infection model’ and ‘Intravenous infection model’ (Figs. 1b, 2b). On day 8 (oral model), mice were gavaged with a mixture of 5 recipient strains (14028TS frag, Kan^R and Amp^R; tags at 1:1:1:1:1 ratio; inoculum approximately 10^5 CFU for each tag). Barcode analysis of the recipient chromosome tags could not be performed because of technical issues with kanamycin enrichments and subsequent qPCR. In the intravenous model, the recipient used was not tagged, but was introduced on day 7 (10^6 CFU by oral gavage). The plasmid and recipient tags can be easily distinguished, as the primer pairs used for qPCR are unique (that is, WITXSX-R paired with Cat_internal for P2 tags and WITXSX-R paired with Kan internal for recipient tags; in the intravenous model in which recipient tags were not used, WITXSX was paired with ydgA for qPCR as there was no need for antibiotic-resistance-specific qPCR primers). For the oral model, the inocula, faeces at days 1, 9 and 15, as well as caecal tissue after gentamicin treatment, was enriched overnight in 5 ml LB containing the appropriate antibiotics (donors = streptomycin + chloramphenicol; recipients = kanamycin; and transconjugants = chloramphenicol + kanamycin) in parallel with selective plating to enumerate bacterial population sizes. For the intravenous model, the donor inoculum, as well as faeces, gall bladder, liver, spleen, mesenteric lymph nodes and caecal tissue at euthanasia (day 25) were enriched in LB with the appropriate antibiotics.

Enrichments were concentrated and genomic DNA was extracted using a QiAamp DNA Mini Kit (Qiagen). qPCR analysis was performed using temperature conditions as previously described. qPCR primers are listed in Supplementary Table 2. For each sample, 5 primer pairs were used to amplify P2 tags (in the oral model WITXSX-R with Cat internal, in which X denotes 2, 11, 13, 19 or 21; in the intravenous model WITXSX with ydgA in which X denotes 2, 11, 13, 19 or 21). Primers were modified from the original, previously described WITS primers; tag loci remain the same as previously described. Relative proportion was determined by dividing the DNA copy number (calculated from the Ct value; a dilution standard of purified chromosomal DNA allowed for a correlation between DNA copy number and Ct value) of each tag detected by the sum of all tags in the sample. The detection limit was determined by the Ct value of the most-diluted DNA standard (linearity of the standard curve decreases markedly below this value) for each qPCR run. The least-precise detection limit constitutes the conservative detection limit used for fitting our mathematical model (2.9 × 10^3). Plasmid tags were ranked according to frequency (Extended Data Fig. 6c) as the strains are isogenic (except for the tag), and each mouse can be treated as an independent realization of the stochastic population dynamics. The data of plasmid-tag frequencies and the bacterial disease.}

**Data availability**

The genome and plasmid sequence of E. coli ESBL 15 have been deposited in the National Center for Biotechnology Information (Accession numbers CP041678–CP041681). Numerical Source Data for all figures are provided with the paper. Source images are available upon request to the corresponding authors.

**Code availability**

Code for the stochastic simulation of plasmid-transfer dynamics and parameter estimation from the experimental data are provided with the paper. The R code follows the notation used in the Supplementary Information.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Supplementary information is available for this paper at https://doi.org/10.1038/s41586-019-1521-8.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Emergence and spread of antibiotic resistance in bacteria using P2 as a model for conjugation. a, Antibiotic resistance in bacteria can emerge through mutation, or be acquired via horizontal gene transfer. Plasmid transfer is an important driver of the spread of antibiotic resistance. Tolerance increases the abundance of bacteria that survive antibiotic exposure, allowing for a higher probability of the emergence of mutations that lead to resistance. We hypothesize that antibiotic resistance can also spread through the formation of reservoirs of persisters that contain plasmids; here, we hypothesize that the gut mucosa of the host can serve as a reservoir for persisters. The formation of long-term reservoirs, followed by re-seeding of bacteria from this reservoir into a niche occupied by other bacteria (for example, the gut lumen occupied by the microbiota following antibiotic therapy) increases the chance that two different strains interact with each other, leading to plasmid transfer (that is, increased strain co-occurrence). The representation in the bottom right panel is an example of donor persisters boosting co-occurrence. Note that tissue-associated recipient persisters may also increase co-occurrence. b, P2 shares homology with resistance plasmids. An alignment is shown between S. Typhimurium SL1344 P2 (GenBank sequence identifier HE654725.1), S. Typhimurium plasmid R64 (GenBank sequence identifier AP005147.1), and pESBL15 of E. coli Z2115 (strain isolated from a rectal swab of a patient at the University Hospital Basel) using the Artemis comparison tool (https://www.sanger.ac.uk/science/tools/artemis-comparison-tool-act). Red fill indicates high sequence identity (>85% sequence identity), blue fill indicates inversions and no fill indicates no sequence identity. For each plasmid, open reading frames (in each of the six translational frames) are shown by white regions (detected by the Artemis comparison tool). Antibiotic resistances (for example, streptomycin and tetracycline resistances on R64 and CTX-M-1 on pESBL15) are labelled, shown by light-blue directed rectangles (found by a Basic Local Alignment Search Tool (NCBI) search against the ResFinder antibiotic-resistance-gene database). In P2, the locus for insertion of the chloramphenicol-resistance cassette and neutral sequence tags is shown. For each alignment, the percentage of the sequence that aligns to P2 is shown, as well as the average sequence similarity for these regions. c, Model strains for addressing evolution by conjugation in S. Typhimurium. SL1344 contains P2cat (chloramphenicol-resistance cassette (cat) that allows the enumeration of plasmid-bearing strains by selective plating) that can be conjugated to 14028S (kanamycin-resistance cassette (aphT) used for selective plating) to form a transconjugant (CmR and KanR). Transconjugants can then transfer P2cat to additional recipients. d, P2cat transfer kinetics in vitro. P2cat transfer is dependent on the density of donors and recipients. Donor and recipient strains were inoculated into LB (n = 2, 1 experiment) at a 1:1 ratio and selective plating was performed every hour. CFUs per millilitre are reported for each population (donors in blue, recipients in green and transconjugants in red). Solid lines connect medians. The dotted line indicates the detection limit by selective plating.
Antibiotic susceptibility testing was performed in LB in 96-well plates. Six strains (indicated on the figure axes) were tested against seven antibiotics, grown at 37 °C at 120 r.p.m. for 16 h, at which point the OD\textsubscript{600 nm} was measured. For each antibiotic, the highest concentration used was based on the working concentration in this study. For example, the concentration of antibiotic used for selective plating in the case of streptomycin, kanamycin, ampicillin and chloramphenicol (the highest concentration of chloramphenicol was fivefold-higher than the concentration used for selective plating, because this was already close to the minimum inhibitory concentration), or the concentration of antibiotic used for the gentamicin protection assay. Importantly, a very low minimum inhibitory concentration was observed for antibiotics used in vivo to enrich for persisters (that is, ciprofloxacin and ceftriaxone). The mean of three experiments is presented on a blue–white colour gradient, in which blue indicates a large amount of bacterial growth. This is calculated by subtracting the OD\textsubscript{600 nm} measured for each sample well from the background generated by the medium.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 Controls for conjugation after antibiotic treatment in the oral infection model. a–c, Faecal bacterial-population sizes and inflammatory status of mice in Fig. 1c, d (comparison of invasive versus non-invasive donors in the oral model). Faecal loads of donors (blue, SmR and CmR), recipients (green, KanR), and transconjugants (red, CmR and KanR) were determined by selective plating on MacConkey agar. Black dotted line indicates detection limits for donors and transconjugants. Green dotted line indicates detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10^8 CFU per gram of faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit. Blue lines connect medians of donor populations; red lines connect medians of transconjugant populations. a, Mice infected with invasive S. Typhimurium donors (solid circles; n = 15 singly housed mice from 5 independent experiments). b, Mice infected with non-invasive S. Typhimurium donors (open circles; n = 6 singly housed mice from 2 independent experiments). c, Inflammatory status was determined by lipocalin-2 ELISA. Statistics were performed using a two-tailed Mann–Whitney U-test. NS, not significant (P > 0.05), ****P < 0.0001, comparing mice infected with an invasive donor (solid black circles; n = 15 singly housed mice from 5 independent experiments) to mice infected with a non-invasive donor (open black circles; n = 6 singly housed mice from 2 independent experiments) at each time point. Medians are shown (solid red line for invasive donors; dotted red line for non-invasive donors). Dotted line indicates the detection limit.

d, e, Carrying P2cat does not lead to a measurable fitness cost or benefit. d, A 'locked' transconjugant (14028S P2catΔoriT (KanR and AmpR)), in which conjugation is blocked by removing the origin of transfer) was competed against a recipient (14028S cat (CmR and AmpR)). e, To ensure that removing the origin of transfer did not affect fitness, the locked transconjugant was competed against a transconjugant with a normal P2cat plasmid (that is, mobile transconjugant) (14028S P2cat (CmR and AmpR)). In d, e, both strains were introduced at a 1:1 ratio (total inoculum size about 5 x 10^7 CFU per os) and faeces were monitored daily by selective plating (n = 6 singly housed mice from 2 independent experiments for both experiments). The competitive index is calculated by dividing the population size of one competitor by the other. Lines indicate medians.

The dotted line indicates no competitive advantage for either strain. This is consistent with previously published data. These data indicate that it is the plasmid-encoded conjugation efficiency (not the effects of the plasmid on host bacterial fitness) that drives the rise of transconjugants (Fig. 1c). e, g, Plasmid transfer in the oral model does not require an invasive recipient. Invasive donors (SL1344 P2cat (SmR and CmR)) were orally infected into pre-treated mice. After ciprofloxacin treatment, a non-invasive mutant of S. Typhimurium 14028S was used as a recipient (non-invasive 14028S apkT (KanR and AmpR)), n = 5 mice. f, Selective plating determined faecal loads of donors (blue, SmR and CmR), recipients (green, KanR), and transconjugants (red, CmR and KanR). Black dotted line indicates the detection limit for donors and transconjugants. Green dotted line indicates the detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10^8 CFU per gram of faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit. Blue lines connect medians of donor populations; red lines connect medians of transconjugant populations. g, Donor populations enumerated after a gentamicin protection assay on caecal tissue of mice shown in f. Median indicated by solid line. Dotted line indicates the detection limit. h, i, Conjugation is required for plasmid transfer after antibiotic treatment. Mice were infected with invasive S. Typhimurium that lacks the origin of transfer in P2cat (SL1344 P2catΔoriT (SmR and CmR)) as a donor, and 14028S apkT (KanR and AmpR) as a recipient, after antibiotic treatment. n = 5 mice. h, Selective plating determined the faecal loads of donors (blue, SmR and CmR), recipients (green, KanR) and transconjugants (red, CmR and KanR). Black dotted line indicates the detection limit for donors and transconjugants. Green dotted line indicates detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10^8 CFU per gram of faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit. Blue lines connect medians of donor populations; red lines connect medians of transconjugant populations. i, Donor populations enumerated after a gentamicin protection assay on caecal tissue of mice shown in h. Median indicated by solid line. Dotted line indicates the detection limit.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Quantification and localization of \textit{S}. \textit{Typhimurium} in the host mucosa after antibiotic treatments in the oral model. \textit{a–d}, Mice were orally infected with either an invasive (SL1344 P2\textsuperscript{cat}) (blue solid circles, \(n = 7\) mice) or non-invasive (non-invasive SL1344 P2\textsuperscript{cat}, TTSS-1 negative) (blue open circles, \(n = 7\) mice) donor and treated with antibiotics. Mice were euthanized at day 8 after infection (at which point recipients were normally added), and organs were analysed. Dotted lines indicate the detection limits. \textit{a}, Faecal populations were monitored daily by selective plating on MacConkey agar. Blue lines connect medians. \textit{b}, Organ loads were determined by selective plating. \textit{c}, Population size of donors in the caecal mucosa determined by selective plating after a gentamicin protection assay, or microscopy of tissue sections (same mice for each quantification method). Each data point is the average of 12 sections (10-\(\mu\)m thick). \textit{b, c}, Statistics were performed using a two-tailed Mann–Whitney \(U\)-test. NS, not significant (\(P \geq 0.05\)), \(*P < 0.05\), \(*\!*P < 0.01\), \(*\!*\!*\!*P < 0.001\), \(*\!*\!*\!*\!*\!*P < 0.0001\), comparing mice infected with invasive or non-invasive donors for each organ. \textit{d}, Localization of \textit{S}. \textit{Typhimurium} detected in the caecal tissue by microscopy, reported as a percentage of bacteria detected in \(c\) in either the lamina propria (l.p.) or epithelium (ep.). Bar indicates the median from five mice. \textit{e–h}, Analysis of reservoirs of persisters in Carnoy-fixed caecal tissue sections. Mice were orally infected with an invasive (SL1344 P2\textsuperscript{cat}, \(n = 5\) mice) donor and treated with antibiotics. Mice were euthanized at day 8 after infection (at which point recipients are normally added) and organs were analysed. Dotted lines indicate the detection limits. \textit{e}, Faecal populations were monitored daily by selective plating on MacConkey agar. Blue lines connect medians. \textit{f}, Organ loads were determined by selective plating. Line indicates median. \textit{g}, A Carnoy fixation was performed on caeca of mice to preserve the mucus structure. Ten-micrometre sections were stained to visualize \textit{S}. \textit{Typhimurium} (yellow, anti-LPS O5), actin (green, phalloidin–FITC), the mucus (red, wheat germ agglutinin AF647 conjugate) and nuclei (blue, DAPI). Mu., mucus. Scale bars, 20 \(\mu\)m. White arrows highlight \textit{S}. \textit{Typhimurium} (magnified in inset). Representative images shown from two independent experiments. \textit{h}, Localization of \textit{S}. \textit{Typhimurium} detected in the caecal tissue by microscopy, reported as a percentage of bacteria detected each section (12 sections per mouse caecum) in the lamina propria, epithelium, mucus or lumen. Bar indicates the median from five mice.
Extended Data Fig. 5 | See next page for caption.
**Extended Data Fig. 5 | Determination of reservoirs of tissue-associated persisters after intravenous infection, and subsequent plasmid transfer.**

a, An equal mix of five SL1344 P2^cat^Pl strains (Sm^R^ and Cm^R^) were intravenously infected into mice (10^3 CFU). Treatments were as described in Fig. 2b (grey circles, 3 intraperitoneal doses of ceftriaxone; n = 8 singly housed mice from 2 independent experiments) or mice were left untreated (black circles; n = 8 singly housed mice from 2 independent experiments). Mice were euthanized on day 5 of the experiment (after the final dose of ceftriaxone was given). The tissue-associated populations in organs were enumerated by selective plating. Detection limit by selective plating is shown as a dotted line. Lines indicate median.

b, c, Faecal bacterial population sizes of mice in Fig. 2d, e (comparison of wild-type and SPI-2-negative donors in the intravenous model). Faecal loads of donors (blue, Sm^R^ and Cm^R^), recipients (green, Kan^R^) and transconjugants (red, Cm^R^ and Kan^R^) were determined by selective plating on MacConkey agar. Black dotted line indicates the detection limits for donors and transconjugants. Green dotted line indicates the detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10^8 CFU per gram of faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit. Blue lines connect medians of donor populations; red lines connect medians of transconjugant populations. b, Mice infected with wild-type S. Typhimurium donors (solid circles). c, Mice infected with SPI-2-deficient S. Typhimurium donors (open circles).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Experimental strategy for assessing population dynamics and tag frequencies. Relates to Fig. 3a–d. a–c, Tags introduced in the oral model. a, Tags coupled to a chloramphenicol-resistance cassette were introduced in P2. qPCR primers are specific to the chloramphenicol-resistance cassette and the specific tag (shown as one-sided arrows). Five tagged donors were pooled and orally infected as a 1:1:1:1:1 mix into mice. b, Relative plasmid-tag proportion detected by qPCR in the initial donor population, the donor population persisting in the caecal mucosa and the transconjugant population is shown for eight mice (three independent inocula). Dotted line indicates the detection limit. Each tag is given a unique colour. c, Scheme illustrating how tags were sorted and recoloured to yield the plots in Fig. 3a, b. Two mice are shown as examples. For the mucosa-associated donor populations (top) and for the faecal transconjugant populations (bottom), tags were sorted according to relative frequency. These tags were re-coloured (darker colour indicates higher frequency) to visualize the trends shown in Fig. 3a, b. These re-ordered tags were used as the experimental data for fitting the mathematical model. d–f, Experimental strategy to assess plasmid-transfer dynamics in the intravenous model. d, Tags coupled to a chloramphenicol-resistance cassette were introduced in P2. qPCR primers are specific to ydgA (a pseudogene flanking the specific tags) and the specific tag (shown as one-sided arrows). Five tagged donors were pooled and intravenously infected as a 1:1:1:1:1 mix into mice. e, Tags from the faecal transconjugant population at day 25 are sorted by abundance. Note that each abundance rank can consist of any tag (ranking and re-colouring scheme in c and raw tag data in f). n = 6 singly housed mice from 2 independent experiments. Dotted line indicates the conservative detection limit by qPCR. This detection limit refers to the most-conservative detection limit of any qPCR run (2.9 × 10⁻³; that is, tags can appear below this detection limit if the qPCR run yielded a lower detection limit). Line indicates the mean; error bars indicate the s.d. f, Relative plasmid-tag proportion detected by qPCR in the inoculum, the donor population persisting in the internal organs and the transconjugant population in the faeces is shown for six mice (three independent inocula; faeces and organ population data in Fig. 2d). Dotted line indicates the detection limit. Each tag is given a unique colour. g, Representation of key parameters in the mathematical model. Donors form a reservoir of persisters in host tissues. These cells can interact with other bacteria that colonize the gut lumen (that is, recipients), and transfer plasmids. Our mathematical model summarizes these plasmid-transfer dynamics using a few key parameters. Donors re-seed the gut lumen and transfer plasmids at rate $\eta$, transconjugants transfer plasmids to recipients (without a plasmid) at rate $\gamma$ and the turn-over of each bacterial population is given by $r - c$, in which $r$ is the birth rate and $c$ is the clearance rate.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Faecal bacterial-population sizes and inflammatory status of mice (comparison of vaccinated versus naive mice in the oral model), and validation by mixtures of invasive and non-invasive donors. Relates to Fig. 3e, f. a, b, Faecal loads of donors (blue, SmR and CmR), recipients (green, KanR), and transconjugants (red, CmR and KanR) were determined by selective plating on MacConkey agar. Barcode analysis of the recipient chromosome tags could not be performed because of technical issues with kanamycin enrichments and subsequent qPCR. Black dotted lines indicate the detection limits for donors and transconjugants. Green dotted line indicates the detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10^8 CFU per gram of faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit. Blue lines connect medians of donor populations; red lines connect medians of transconjugant populations. a, Naive mice infected with invasive S. Typhimurium donors (solid circles). b, Vaccinated mice infected with invasive S. Typhimurium donors (open circles with light-grey fill). c, Inflammatory status to determine the success of vaccination was determined by lipocalin-2 ELISA. Statistics were performed using a two-tailed Mann–Whitney U-test. NS, not significant (P ≥ 0.05), *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, comparing naive mice (black circles, n = 9 singly housed mice from 3 independent experiments) to vaccinated mice (open circles, n = 14 singly housed mice from 4 independent experiments) at each time point. Medians are shown (solid red line for naive mice and dotted red line for vaccinated mice). Dotted line indicates detection limit. d–g, Experimentally reducing the population size of mucosa-associated persisters by mixing invasive and non-invasive donors reduces plasmid transfer in the oral model. Mice were orally infected with a mixture of invasive (SL1344 P2cat, SmR and CmR) and non-invasive (non-invasive SL1344 P2cat, SmR and CmR) donors at two ratios: 1:10 (n = 6 singly housed mice) or 1:500 (n = 5 singly housed mice). In both cases, an excess of non-invasive donors was used to experimentally reduce the number of cells that can establish a reservoir of persisters in the intestinal mucosa (two independent experiments). S. Typhimurium 14028S aphT (KanR and AmpR) was used as a recipient after antibiotic treatment. d, Donor populations enumerated after a gentamicin protection assay on caecal tissue of mice infected with a 1:10 ratio (blue circles with dark grey fill; line indicates median) or 1:500 ratio (blue circles with light grey fill; line indicates median) of invasive to non-invasive donors. Statistics were performed using a two-tailed Mann–Whitney U-test. **P < 0.01. e, Proportion of transconjugants (transconjugant population size divided by the sum of recipients and transconjugants) in the faeces is shown for each day, for mice infected with a 1:10 ratio (black circles with dark grey fill, grey bars indicate median) or a 1:500 ratio (black circles with light grey fill, light grey bars indicate median) of invasive to non-invasive donors. f, g, Faecal loads of donors (blue, SmR and CmR), recipients (green, KanR) and transconjugants (red, CmR and KanR) were determined by selective plating on MacConkey agar. f, Mice infected with a 1:10 ratio of invasive to non-invasive donors. g, Mice infected with a 1:500 ratio of invasive to non-invasive donors. In d–g, the black dotted line indicates the detection limit for donors and transconjugants. Green dotted line indicates the detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10^8 CFU per gram of faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Faecal bacterial-population sizes of mice and the role of persistence and conjugation in host tissues if the recipient constitutes the reservoir. Relates to Fig. 4. a, Faecal loads of donors (blue, Sm<sup>a</sup> and Cm<sup>b</sup>), recipients (green, Amp<sup>b</sup>), and transconjugants (red, Cm<sup>b</sup> and Amp<sup>b</sup>) were determined by selective plating on MacConkey agar (same mice as in Fig. 4a, b; S. Typhimurium donor and E. coli recipient in the oral model). Black dotted line indicates the detection limit for donors and transconjugants. Green dotted line indicates the detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10<sup>8</sup> CFU per gram of faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit. Blue lines connect medians of donor populations; red lines connect medians of transconjugant populations.

b–d, Persistence in host tissues also promotes plasmid transfer if the recipient constitutes the reservoir. Pre-treated mice were orally infected with a S. Typhimurium recipient (SL1344 P<sup>2</sup>cured aph<sup>T</sup>, Sm<sup>b</sup> and Kan<sup>b</sup>) and treated with ciprofloxacin and ampicillin as in the oral model (Fig. 1b). On day 8, ampicillin was removed from the drinking water and an E. coli donor was introduced orally (E. coli 8178 P<sup>2</sup>cat, Cm<sup>b</sup> and Amp<sup>b</sup>). b, Recipient populations enumerated after a gentamicin protection assay on caecal tissue (n = 5 singly housed mice from 2 independent experiments). Line indicates median. c, Proportion of transconjugants (transconjugant population size divided by sum of recipients and transconjugants) in the faeces is shown for each day. Grey bars indicate median. d, Faecal loads of recipients (green, Sm<sup>b</sup> and Kan<sup>b</sup>), donors (blue, Cm<sup>b</sup> and Amp<sup>b</sup>), and transconjugants (red, Sm<sup>b</sup>, Cm<sup>b</sup> and Kan<sup>b</sup>) were determined by selective plating on MacConkey agar. In b–d, dotted lines indicate detection limits by selective plating.

e, Faecal bacterial-populations sizes of mice in Fig. 4c, d (S. Typhimurium ESBL donor in the oral model). Faecal loads of donors (blue, Sm<sup>a</sup> and Amp<sup>b</sup>), recipients (green, Kan<sup>b</sup>) and transconjugants (red, Kan<sup>b</sup> and Amp<sup>b</sup>) were determined by selective plating on MacConkey agar. Black dotted line indicates the detection limit for donors and transconjugants. Green dotted line indicates the detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10<sup>8</sup> CFU per gram of faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit. Blue lines connect medians of donor populations; red lines connect medians of transconjugant populations.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Exchanging ampicillin for kanamycin to limit gut-luminal growth of the donor does not affect the overall plasmid-transfer kinetics, and faecal bacteria-population sizes of intravenously infected mice. Relates to Fig. 4. a–d. Mice were orally infected with SL1344 P2cat (SmR and CmR) as a donor, and 14028S aphT (KanR and AmpR) as a recipient after antibiotic treatment. Mice were either treated with ampicillin in the drinking water until day 15 (normal protocol as in Fig. 1b), day 8 (when the recipient is added) or kanamycin until day 8. a. Donor populations enumerated after a gentamicin protection assay on caecal tissue of mice, in which ampicillin is maintained until day 15 (solid blue circles, n = 15 singly housed mice from 5 independent experiments; data taken from Fig. 1d), ampicillin is removed on day 8 (blue circles with pink fill, n = 3 singly housed mice from 1 experiment) or kanamycin is used until day 8 (blue circles with yellow fill, n = 3 singly housed mice from 1 experiment). Median indicated by solid line. b. Proportion of transconjugants (transconjugant population size divided by the sum of recipients and transconjugants) is shown for the groups receiving ampicillin treatment until day 15 (solid black circles, grey bars indicate median; n = 15 singly housed mice from 5 independent experiments; data taken from Fig. 1c), ampicillin treatment until day 8 (black circles with pink fill, pink bars indicate median; n = 3 singly housed mice from 1 experiment), and kanamycin treatment until day 8 (black circles with yellow fill, yellow bars indicate median; n = 3 singly housed mice from 1 experiment). c. d. Faecal loads of donors (blue, SmR and CmR), recipients (green, KanR) and transconjugants (red, CmR and KanR) were determined by selective plating on MacConkey agar. c. Mice treated until day 8 with ampicillin. d. Mice treated until day 8 with kanamycin. In a–d, the black dotted line indicates the detection limit for donors and transconjugants. Green dotted line indicates the detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10⁸ CFU per gram of faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit. e. Faecal bacterial-populations sizes of mice in Fig. 4e, f (S. Typhimurium donor and E. coli recipient in the intravenous model). Faecal loads of donors (blue, SmR and AmpR), recipients (green, KanR) and transconjugants (red, KanR and AmpR) were determined by selective plating on MacConkey agar. Black dotted line indicates the detection limit for donors and transconjugants. Green dotted line indicates the detection limit for recipients. The detection limit is higher for recipients once transconjugants reach a density of >10⁸ CFU per gram faeces. Before this happens, recipients can be found below the detection limit; the black dotted line should then be considered as the detection limit. Blue lines connect medians of donor populations; red lines connect medians of transconjugant populations.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Increasing growth rate at carrying capacity to model inflammation, or running simulations on a finer parameter grid, does not affect overall simulation trends. a, b, Simulations were run with identical parameters to Fig. 3c, d, but an increased birth and death rate at carrying capacity to simulate cases in which inflammation is present (Supplementary Information). The trends of the simulations remain the same as in Fig. 3c, d, a. Likelihood of the model as a function of the donor re-seeding (including donor-to-recipient conjugation) rate, and the rate of transconjugant-to-recipient plasmid transfer. All other parameter values are given in the Supplementary Information. The most-likely set of parameters is shown in red (\(\eta = 1 \times 10^{-9}\) events per recipient per day; \(\gamma = 3.16 \times 10^{-8}\) events per recipient per CFU per gram of faeces per day). b, The fraction of simulations with plasmid re-seeding (defined as a final transconjugant population size above \(5 \times 10^8\) CFU per gram of faeces) is shown as a function of \(\eta\). Here, \(\gamma\) is fixed at its most-likely value of \(3.16 \times 10^{-8}\) events per recipient per CFU per gram of faeces per day. The black vertical dotted line at \(\eta = 1 \times 10^{-9}\) events per recipient per day indicates the estimated most-likely value from a. The red vertical dotted line at \(\eta = 1 \times 10^{-12}\) events per recipient per day indicates a hypothetical 100-fold decrease of \(\eta\) (shown by a red arrow) (as might be caused, for example, by vaccination). c–f, Running simulations on a finer parameter grid does not affect the overall simulation trends. Supplementary Table 4 provides details of the differences between specific simulation results. c, d, Simulations were run on a grid of \(\eta = 10^{-12}\) to \(10^{-8}\) and \(\gamma = 10^{-10}\) to \(10^{-4}\) with 0.25-log increments, instead of the \(\eta = 10^{-12}\) to \(10^{-4}\) and \(\gamma = 10^{-12}\) to \(10^{-4}\) with 0.5-log increments used in Fig. 3c, d. e, f, Simulations were run with parameters identical to a, b, but on a grid of \(\eta = 10^{-12}\) to \(10^{-8}\) and \(\gamma = 10^{-10}\) to \(10^{-4}\) with 0.25-log increments, instead of \(\eta = 10^{-12}\) to \(10^{-4}\) and \(\gamma = 10^{-12}\) to \(10^{-7}\) with 0.5-log increments. c, e, Likelihood of the model as a function of the donor re-seeding (including donor-to-recipient conjugation) rate, and the rate of transconjugant-to-recipient plasmid transfer. All other parameter values are given in the Supplementary Information. The most-likely set of parameters is shown in red. d, f, The fraction of simulations with plasmid re-seeding (defined as a final transconjugant population size above \(5 \times 10^8\) CFU per gram of faeces) is shown as a function of \(\eta\). Here \(\gamma\) is fixed at its most likely value. The black vertical dotted line indicates the estimated most-likely value (from c or e). The red vertical dotted line indicates a hypothetical 100-fold decrease of \(\eta\) (shown by a red arrow) (as might be caused, for example, by vaccination).
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Software and code

Policy information about availability of computer code

Data collection

Data collection is described in detail in Materials and Methods. The mathematical model is described in detail in the supplementary materials. All code needed to reproduce the mathematical modelling is available as supplementary data. The custom code was written in R, and tested on MacOS 10.13.4 with R 3.4.4, as well as a computing cluster running CentOS 7 with R 3.4.0. There is an essential dependence on the R package adaptivetau (v2.2.1); and optional dependencies on the packages ggplot2 (v2.2.1), reshape2 (v1.4.3), gridExtra (v2.3), wesanderson (v0.3.6), parallel (v3.4.4), and tictoc (v1.0).

Data analysis

Statistical analysis and computation of the plots was performed using Graph Pad Prism, version 7; Fitting of the mathematical model to experimental values was performed using an Approximate Bayesian Computation (ABC) approach (the reference Marjoram et al. 2003, PNAS describes this in detail).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data from experiments and from the mathematical model calculations are shown. The sequence data is available at the indicated repositories (accession number is provided in the data availability statement).

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Study description

We performed mouse infection experiments. The numbers of mice per group are indicated in each figure. In figures where statistical comparisons were performed, we used a minimum of 5 mice that were singly housed per group (to ensure that each mouse is truly independent) per group from at least two independent experiments. In other experiments, we used a minimum of three singly housed mice per group. We sampled feces at the indicated times post infection and analyzed pathogens loads in tissues by plating and by fluorescence microscopy.

Research sample

We used defined clones of the indicated S. Typhimurium and E. coli strains. 8-12 weeks old 129 SvEv (Nramp+/+) inbred mice were used. The Nramp+/+ genotype confers resistance against long-term S. Typhimurium infection. The mice harbor a specified pathogen free microbiota. This resident microbiota does not feature any E. coli or Salmonella strains.

Sampling strategy

We sampled feces at the indicated times post infection and analyzed pathogens loads in tissues by plating and by fluorescence microscopy. Sample sizes were not predetermined. As large differences were expected, 5-7 mice were used in general for statistical comparisons. In some cases where the underlying data was central to the main hypothesis, we used as many as 15 mice to ensure claims were accurate and that we encompassed as large variability as possible.

Data collection

We sampled feces at the indicated times post infection and analyzed pathogens loads in tissues by plating and by fluorescence microscopy. Data was recorded by the experimenter (indicated in the author contributions section).

Timing and spatial scale

The time course of the sampling is shown in detail in Figure 1B and Figure 2B. Mice were infected and feces were monitored daily for either 5, 7, 8, 15, or 25 days (indicated for each figure specifically). Antibiotic treatments were given on days 2, 3, and 4 (where applicable). Organs were collected at defined endpoints in the experiment.

Data exclusions

All experimental data from all repeat of each experiment is shown. We did not exclude any data, except in the case of four mice where animals needed to be sacrificed prematurely due to disease or symptom severity.

Reproducibility

The number of animals used in the independent experiments are indicated in the figure legends. Mice were singly housed to ensure that each sample is completely independent. Experiments were very reproducible in each independently housed mouse. In addition, in the bulk majority of experiments, at least two independent experiments (with separate inocula) were used to validate reproducibility.

Randomization

Mice were randomly assigned to the different experimental groups.

Blinding

The data analysis was not performed in a blinded fashion. This is explained by the objective nature of the plating data (colony present = yes/no), which excludes enumeration bias. For microscopy, slides were blinded prior to analysis.

Did the study involve field work?  ☐ Yes  ☑ No

Reporting for specific materials, systems and methods
Materials & experimental systems

Methods

| n/a | Involved in the study |
|-----|-----------------------|
|     |                       |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

All materials are freely available from public repositories or commercial suppliers. The unique strains and plasmid constructs created for the plasmid transfer experiments are available from the corresponding author (W.D. Hardt).

Antibodies

| Antibodies used |
|-----------------|
| α-S.Tn LPS O12 monoclonal antibody | STA5 | (reference: Moor et al., 2017, Nature, 31:100)), α-ICAM-1/CD54 antibody | IBD Biosciences, Cat#539250, 1:100), DAPI (Sigma Aldrich, Cat#D9542, 0.1 μg/ml), Alexa Fluor 488 conjugated phalloidin (Santa Cruz, sc-336791, 1:100), wheat germ agglutinin (WGA) AF647 conjugate | Invitrogen, Cat#W32466, 1:1:200, Rabbit polyclonal anti-Salmonella | E5 | (Becton Dickinson, Cat#22601, 1:100) and Goat polyclonal anti-Rabbit Cy3 conjugate | Jackson ImmunoResearch Labs, Cat#111-165-144, 1:200, Goat polyclonal anti-Human Cy3 conjugate | (1:200), Goat polyclonal anti-Hamster Cy5 conjugate | Jackson ImmunoResearch Labs, Cat#127-175-160, 1:200, were used for the staining. |

Antibodies used

Validation

The employed α-S.Tn LPS antibodies have been validated in references Moor et al., 2017, Nature, 31:100, and Sellin et al., 2014, Cell Host Microbe. The α-ICAM-1/CD54 antibody was used in reference Sellin et al., 2014, Cell Host Microbe to differentiate the intestinal epithelium from the lamina propria. WGA AF647 specificity was validated on Carnoy-fixed, paraffin-embedded colon sections from untreated C57BL/6 mice. In the study, we used sex and age-matched experimental groups.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

- 8-12 week-old 129Sv/Evy mice from Jackson laboratories; Specified pathogen free status; Animals were bred in a SPF barrier facility and were used sex and age-matched experimental groups.

Wild animals

- The study did not involve wild animals.

Field-collected samples

- The study did not involve field-collected samples.