Escherichia coli limits Salmonella Typhimurium infections after diet shifts and fat-mediated microbiota perturbation in mice

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The microbiota confers colonization resistance, which blocks Salmonella gut colonization. As diet affects microbiota composition, we studied whether food composition shifts enhance susceptibility to infection. Shifting mice to diets with reduced fibre or elevated fat content for 24 h boosted Salmonella Typhimurium or Escherichia coli gut colonization and plasmid transfer. Here, we studied the effect of dietary fat. Colonization resistance was restored within 48 h of return to maintenance diet. Salmonella gut colonization was also boosted by two oral doses of oleic acid or bile salts. These pathogen blooms required Salmonella’s AcrAB/TolC-dependent bile resistance. Our data indicate that fat-elicited bile promoted Salmonella gut colonization. Both E. coli and Salmonella show much higher bile resistance than the microbiota. Correspondingly, competitive E. coli can be protective in the fat-challenged gut. Diet shifts and fat-elicited bile promote S. Typhimurium gut infections in mice lacking E. coli in their microbiota. This mouse model may be useful for studying pathogen-microbiota-host interactions, the protective effect of E. coli, to analyse the spread of resistance plasmids and assess the impact of food components on the infection process.

We hypothesized that shifts in diet composition might affect colonization resistance, as 24–48 h on diets with elevated fat or reduced fibre content suffice to alter microbiota compositions, and fibre deprivation accelerates murine Citrobacter rodentium infections. We initially analysed a typical high-fat Western-type diet without fibre (WD; Fig. 1a). C57BL/6 mice harbouring an unper- turbated, complex, E. coli-free microbiota (CON) were reraired on a standard plant-based maintenance diet (MD). Mice remaining on MD had limited gut luminal Salmonella Typhimurium (S.Tm) strain SL1344 growth (Fig. 1b). In spite of possible batch-to-batch variations, this colonization resistance has been consistent over many years. Another group was shifted to WD 24 h before per-oral inoculation with S.Tm. S.Tm blooms were also observed after shifts to a fibre-containing high-fat diet, but not the low-fat control. Thus, fat might be involved. As a fibre-free low-fat diet also enhanced S.Tm colonization, we conclude that gut luminal growth of S.Tm and other Enterobacteriaceae is promoted not only by fibre deprivation, as reported previously, but also by fat and possibly other unidentified food constituents.

We decided to focus on the role of fat, as associated microbiota changes and disease phenotypes hinted at this nutrient being one possible trigger of Enterobacteriaceal blooms. First, we supplemented MD with additional lard. Shifts to this diet promoted S.Tm gut colonization (Supplementary Fig. 2f) and gut pathogen loads (Fig. 1c,d, Supplementary Figs. 2a–d and Supplementary Table 1). C57BL/6 mice carrying another complex microbiota exhibited a 104-fold higher S.Tm colonization (Supplementary Fig. 4), we conclude that gut luminal growth of S.Tm-inflicted disease (Fig. 1a–d, Supplementary Figs. 2a,b and 5). Thus, short exposures to fat or oleic acid can promote S.Tm blooms, at least in E. coli-free mice.
Dysbiosis-mediated Enterobacteriaceal blooms can fuel the spread of resistance plasmids. To test if WD-shift or oleic acid-promoted blooms have a similar effect, we studied transfer rates of plasmid PII from S.Tm SL1344 (that is, PIICmR carrying chloramphenicol resistance). PII belongs to an IncI1-family of antibiotic-resistant and colicin plasmids commonly found in Enterobacteriaceae.

Mice were sequentially infected with the recipient strain (S.Tm ATCC14028k<sup>kan</sup>) and 15 min later with the donor (S.Tm SL1344, PIIC<sup>cmR</sup>). Indeed, WD-shift and oleic acid boosted plasmid transfer by 10<sup>2–10<sup>6</sup></sup>-fold compared to controls (Fig. 1e and Supplementary Fig. 6).

It remains unclear if this is solely attributable to increased donor/recipient densities.
The microbiota composition changed 12–24 h after WD-shift or oleic acid gavage (Supplementary Fig. 7). This provided a hint that microbiota disturbance might explain the elevated susceptibility to S.Tm. The alleviation of colonization resistance was transient. After return to MD, the mice regained full colonization resistance within 24–44 h (Fig. 1g and Supplementary Fig. 8). Therefore, our subsequent mechanistic analysis focused on the time window of 0–24 h after the dietary perturbation.

Next, we tested if bile salts might be involved. Fat digestion necessitates bile salt release into the gut lumen. Although most bile is resorbed before passage into the large intestine, faecal concentrations can reach ~0.3% (w/w) after fat consumption. Indeed, bile salts were elevated in the caecal contents after the WD-shift or oleic acid gavage (Supplementary Fig. 9). Cholate, a primary bile salt, reached concentrations of up to 0.1% (Fig. 2a). These data hinted at bile salt-mediated alleviation of colonization resistance, as bile salts are well-known inhibitors for numerous bacterial species, but not Salmonella or E. coli spp.. This has been utilized in stool diagnostics, which traditionally employs bile supplements to culture Salmonella or E. coli spp. while suppressing unwanted microorganisms. To establish the role of bile salts in alleviating colonization resistance, we administered cholate by oral gavage at 1 h before diagnostics, which traditionally employs bile supplements to culture Salmonella or E. coli spp. while suppressing unwanted microorganisms. We observed that bile salts were elevated in the caecal contents after the WD-shift or oleic acid gavage (Supplementary Fig. 9). Cholate, a primary bile salt, reached concentrations of up to 0.1% (Fig. 2a). These data hinted at bile salt-mediated alleviation of colonization resistance, as bile salts are well-known inhibitors for numerous bacterial species, but not Salmonella or E. coli spp.. This has been utilized in stool diagnostics, which traditionally employs bile supplements to culture Salmonella or E. coli spp. while suppressing unwanted microorganisms. To establish the role of bile salts in alleviating colonization resistance, we administered cholate by oral gavage at 1 h before and 4 h after infection. Two doses of cholate (100 µl, 8%) promoted S.Tm gut colonization almost as efficiently as streptomycin (streptomycin S.Tm enterocolitis model; Fig. 2b,c), while gut transit times remained unaltered (Supplementary Fig. 10). Taurocholate, another primary bile salt, had a similar effect (Supplementary Fig. 11). Thus, bile salts alone can elicit S.Tm blooms independent of dietary fat.

The microbiota were more sensitive to bile salts than WT S.Tm or E. coli. This was established by live/dead-dye exclusion from cholate-exposed gut luminal bacteria (Fig. 2d) and by cholate inhibition of the anaerobic growth of 16 representative strains isolated from human and nine strains from murine stools (from Oligo-MM24 microbiota; Supplementary Table 3). Most microbiota strains were inhibited by ≥0.125% cholate (Fig. 2e). In contrast, WT S.Tm and the human commensal E. coli ED1a were approximately tenfold more resistant than the microbiota or mutants lacking the AcrAB/TolC efflux pump, a known determinant of Enterobacteriaceae bile resistance (Fig. 2d,e and Supplementary Tables 2, 4 and 5). Equivalent observations were made with taurocholate (Supplementary Fig. 12). Thus, bile salts are sufficient to recapitulate the loss of colonization resistance associated with WD-shift or oleic acid treatment.

To assess, quantitatively, if bile-inflicted differences in growth rates are sufficient to explain why fat promotes S.Tm blooms, we formulated a mathematical model and used it to generate explicit predictions. Specifically, we modelled the outcome of competition (for colonizing the caecum lumen) between S.Tm and a generic microbiota after 24 h, using the bile salt-dependent growth parameters derived from Fig. 2e (see Supplementary Information on the model for details; Supplementary Table 6 and Supplementary Figs. 13–15). This recapitulated the behaviour of our system. WT S.Tm outcompeted the microbiota at bile salt concentrations realistic in the fat-exposed caecum, whereas the microbiota stayed dominant at bile salt concentrations below 0.15% (Fig. 3a). In contrast, an acrAB mutant could not efficiently outcompete the microbiota, even at high bile salt concentrations (S.TmacrAB; Fig. 3b). Thus, bile salt-mediated growth inhibition is sufficient to explain why WD, dietary fat and oleic acid promote gut infection.

This was verified by competitive infection experiments of WT S.Tm versus acr mutants that feature a similar bile salt sensitivity as most Bacteroidetes or Firmicutes strains (Fig. 2e). When WD-shift, oleic acid or cholate were applied (but not in the MD controls), WT S.Tm outcompeted the acr mutants (Fig. 3c–d and Supplementary Figs. 16 and 17). In keeping, S.TmacrAB yielded lower gut luminal densities than WT S.Tm after WD-shifts. To further assess the colonization defect of S.TmacrAB, we infected mice after WD-shifts with one S.Tm strain at a time (Fig. 3e). These data establish bile resistance as a key factor promoting Enterobacteriaceae blooms in the fat-exposed gut.

Control experiments assessed if pathogen growth on fatty acids may fuel S.Tm blooms in the fat-exposed gut. However, caecal long-chain fatty acid (LCFA) concentrations remained unchanged after WD-shift or oleic acid gavage (Supplementary Fig. 18a,b). Moreover, S.TmacrAB, which is deficient in LCFA uptake (ΔfadL), showed only a minor gut luminal growth attenuation (~twofold; Supplementary Fig. 18e). This defect was much smaller than the 10⁻² to 10⁻⁵-fold growth difference of S.TmWT between WD- or oleic acid-exposed mice and animals on MD (Supplementary Fig. 18e). Similarly, we could exclude cross-feeding on products from other microbiota, like short-chain fatty acids (SCFAs; acetate, propionate, butyrate; Supplementary Fig. 18f–i). In conclusion, fatty acid utilization by S.Tm is not necessary for pathogen growth in the fat-exposed gut.

A second set of controls assessed if fat exposure might elicit gut inflammation and thereby enhance pathogen growth. However, within 24 h, neither WD-shift, nor gavage with oleic acid or cholate, affected cardinal mucosal inflammation marker expression, Treg or phagocyte numbers implicated in disease-fueled Salmonella growth (Supplementary Figs. 19–21). Thus, sub-acute inflammatory responses are dispensable for the alleviation of colonization resistance. Although interleukin-22 (IL-22) is dispensable in our model (Supplementary Fig. 22), it may contribute in other situations, as some animals featured elevated il-22 and reg-IIIβ, an antimicrobial peptide favouring Enterobacteriaceae blooms (Supplementary Fig. 19).

Further controls verified that expression of SPI-1, an inflammation-triggering virulence factor, was unaffected upon WD-shift or oleic acid gavage (Supplementary Fig. 23). Finally, competitive infections verified that nitrate respiration (as needed for S. Tm growth in the inflamed gut) was dispensable for accelerating growth in mice subjected to WD-shift or oleic acid (competitive index (CI) ~1; Supplementary Fig. 24). Thus, mucosal inflammation is not required for fat-mediated promotion of S.Tm gut colonization (Supplementary Fig. 1, step 1b).

Finally, we assessed if E. coli may limit S.Tm infections after diet shifts. WT E. coli strains are common members of animal microbiota, encode AcrAB/TolC, are bile-resistant and bloom after WD-shift (Fig. 2e and Supplementary Figs. 2e and 12). Moreover, some, but not all, E. coli strains are capable of outcompeting Salmonella spp. in the gut of mice and chickens. Correspondingly, S.Tm blooms were ~10⁻² to 1,000-fold smaller when the microbiota includes a Salmonella-competitive E. coli (compare Fig. 1b versus Supplementary Fig. 2c; Supplementary Table 1). Therefore, competitive E. coli might limit fat-promoted pathogen blooms. CON5 mice were gavaged with oleic acid or shifted to WD as in Fig. 1a and co-infected with WT S.Tm and a mixture of three E. coli strains (5 × 10⁵ c.f.u., by gavage; Supplementary Table 2 and Fig. 4a,b) capable of growth in 2% cholate. E. coli 8178 and CFT073 can outcompete S.Tm in antibiotic-treated mice and E. coli Z1324 is a recent isolate from a healthy human volunteer. Indeed, the E. coli mix colonized the murine gut, suppressed oleic acid- and WD-promoted S.Tm blooms by 10⁻² to 10⁻⁵-fold and prevented enteropathy (Fig. 4a–c).

Within the E. coli mix, E. coli 8178 achieved the highest gut luminal densities. Indeed, E. coli 8178 alone could limit gut luminal S.Tm blooms after a WD-shift for 72–96 h. It did not matter whether E. coli 8178 was applied in parallel or 48 h before the S. Tm infection (Fig. 4d and Supplementary Fig. 25). However, E. coli 8178 alone was less efficient than the E. coli mix. We conclude that competitive E. coli can preserve colonization resistance after fat-mediated disturbance.

This work identifies fat as one factor promoting Salmonella diarrhoea in mice. Fat can achieve this by eliciting bile salts which...
Fig. 2 | Primary bile salts can explain S.Tm blooms. a, Quantitative cholate mass spectrometry analysis in CONE mice (n = 5) 9 h after the indicated interventions. b, Cholate titration experiment. CONE mice (n = 5, 6 and 7) were gavaged, as indicated (100 μl), 1 h before and 4 h after S.Tm infection (5 × 10⁷ c.f.u.). c, Effect of the indicated interventions on gut luminal S.Tm densities (n = 8 and 9). Control, CONE mice pre-treated with streptomycin (20 mg by gavage; 24 h before infection; indicated by grey circles). d, Cholate sensitivity of gut luminal microbiota from ileum (n = 2), caecum (n = 3) and colon (n = 2) microbiota (isolated from CONE mice) and of WT S.Tm (black circles; n = 3) or indicated S.Tm mutants (n = 3). Analysis was performed by SYTOX green exclusion and flow cytometry (an example gating for S.Tm<sup>tolC</sup> is shown). The mean value of all experiments is shown (whiskers indicate range). e, Cholate sensitivity of individual microbiota strains as analysed in MGAM (2% H₂, 12% CO₂, 86% N₂; Supplementary Table 3; n = 3, analysis versus growth without inhibitor). Controls, WT E. coli ED1a, indicated S.Tm strains. Bars indicate median; two-way ANOVA on log-normalized data with Dunnett’s multiple comparison test. Dotted lines indicate detection limits. NS, not significant (P > 0.05); *P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001.
are well tolerated by \(S\).Tm or \(E\). coli but not by most Bacteroidetes or Firmicutes strains. Experimental data and modelling indicate that bile salts, which are released during fat digestion, can ablate colonization resistance, that is, when competitive \(E\). coli strains are missing. The high prevalence of \(E\). coli spp. may explain why Western-type diets are not associated with human \(Salmonella\) diarrhoea (Supplementary Discussion). 

\(E\). coli presence and their exact genetic make-up may determine whether colonization resistance is maintained during microbiota disturbance, or not. Future studies should assess the relevant competitive mechanisms (for example, colicins, type 6 secretion systems, oxygen- or iron-depletion\(^{5,14-38}\)). Such knowledge might help to identify individuals at risk, and to prevent infections by supplementing potent competitive \(E\). coli strains. However, colonization resistance can also be achieved without \(E\). coli, as indicated by our experiments in unperturbed CON\(E\) mice. Nevertheless, when WD-shifts or oleic acid exposure disturb the microbiota, \(E\). coli can promote resilience, invigorating colonization resistance after perturbations.

\(S\).Tm growth in the fat-digesting gut relies on AcrAB/TolC efflux pumps\(^{19,20,39,40}\). Most likely, this also holds true for other Enterobacteriaceae like \(Citrobacter\), \(Escherichia\), \(Salmonella\), \(Enterobacter\), \(Yersinia\), \(Klebsiella\) and \(Shigella\) spp. Of note, bile resistance is further modulated by additional factors\(^ {41}\); bile can promote pathogen germination\(^ {42}\) and distant \(acrAB\)-like genes are also present in other bacteria (Supplementary Fig. 26). Deciphering their contribution to fat/bile-elicited pathogen blooms will be an interesting topic for future work.

Colonization resistance is also compromised by mechanisms other than bile-salt-mediated microbiota perturbation. In cases of fat exposure, pathogen growth on long-chain fatty acids and
elicitation of IL-22-induced antimicrobial peptides might contribute in some cases, in particular in the long term. However, in most mice, pathogen growth in the fat-exposed gut begins in the absence of inflammation (Supplementary Fig. 1, step 1b). Fibre deprivation can also promote C. rodentium3 and S. Tm blooms (Supplementary Fig. 4). Most probably, additional factors can also alleviate colonization resistance and remain to be identified.

Finally, our findings impinge on the spread of antibiotic resistance plasmids, which is currently of major concern (WHO, 2017)43. As conjugation is contact-dependent and therefore promoted by
high donor and recipient densities\(^1\). Enterobacteriaceal blooms elicited by inflammation or antibiotics are known to fuel this process\(^6\). Our findings suggest that, under certain circumstances, diet shifts may also fuel such bloom-driven plasmid transfer.

We conclude that dietary shifts can be used to study the microbiota–pathogen–host interaction in mice. This may be useful for research on acute infections, protective \(E.\ coli\) strains and resistance to plasmid spread.

**Methods**

**Bacterial strains.** The \(S.\ enterica\) serovar Typhimurium strains used in this study are generally derivatives of the WT strain SL1344 (SB300)\(^4\). The recipient in PII conjugation experiments, a derivative of S. enterica, \(\Delta triA\) (Tc\(^C\) C10428). Deletions in the acetate and propionate metabolism (\(\Delta aucA + \Delta prePPBCDE\)), a key determinant of \(\Delta gcd\) (as described previously)\(^4\). The plasmid pM972 (p\(\Delta sicA\) gfp\(\Delta gfp\)) was used to quantify \(\Delta sicA\) promoter (p\(\Delta sicA\) ) thereby controls the expression of the chromosomal \(sicAspBCDA\) operon, which encodes key parts of the SPI-1 type 3 secretion system (T3SS-1). In addition, the mouse commensal \(E.\ coli\) B37588 (ref. \(^\ast\)) was used in infection experiments. All constructs were verified by PCR and all strains and their corresponding genotype are listed in Supplementary Table 2.

**Ex vivo growth conditions.** For infection experiments, bacteria were grown for 12 h at 37 °C, shaking (160 rpm) in lysogenic broth (LB) broth and the appropriate antibiotics, sub-cultured for 4 h in LB broth containing 0.3 M NaCl without antibiotics using the same growth conditions and re-suspended in PBS as described previously\(^7\).

For the minimal inhibitory concentration assays, bacteria were grown overnight at 37 °C, shaking (160 rpm) in LB broth containing the appropriate antibiotics. Cultures were adjusted to an OD\(_{600}\) of 1 and further diluted 1:2,000 in LB broth to reach 10\(^4\) c.f.u. per tested condition.

**Animals.** Male and female 8- to 12-week-old mice were used. The mouse lines used, their microbiota and the presence of \(E.\ coli\) were summarized in Supplementary Table 1. All mice were bred under specific (CEBOS) and pathogen-free (SPF, SOPF) conditions in individually ventilated cages in the ETH Phenomics Centre (EPIC). 129Sv/Ev mice (CONX) were bred in full barrier SOPF and bred in full barrier conditions in individually ventilated cages in the ETH Phenomics Centre (EPIC). 129Sv/Ev mice (CONX) were bred in full barrier SOPF and bred in full barrier conditions in individually ventilated cages in the ETH Phenomics Centre (EPIC). All mice were maintained on the mouse maintenance diet (MD; in the figures, filled black circles; \(\text{Kliba Nafag, 3537; autoclaved; per weight: 4.5\% fat, 18.5\% protein, 62.6\% carbohydrates, 3.7\% fibre}\)) which were fed ad libitum.

**Data analysis.** All data were analyzed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA) or R (R Foundation for Statistical Computing, Vienna, Austria). Differences between groups were analyzed using non-parametric (Mann-Whitney or Kruskal-Wallis) tests and p-values were two-tailed except when otherwise indicated. The statistical package EZR (Saitama Medical Center, Tokyo, Japan) was used for R. The level of significance was set at p<0.05. All graphs were generated using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA). The sequence data obtained in this study have been deposited in the National Center for Biotechnology Information (NCBI) database (accession numbers for each strain are listed in Supplementary Table 2).

**Infections and competitive infection experiments.** Infections with individual strains, or co-infection experiments with the indicated strain mixtures, were carried out in 8- to 12-week-old mice (age- and sex-matched). Mice were kept on MD before switching to WD 24 h after the oral infection with 5×10\(^6\) c.f.u. per kg body weight. Additional experimental groups were treated with PBS or 200, 100, 50 or 10 \(\mu\)g of oleic acid, as indicated (100 \(\mu\)g was used as standard protocol; Sigma-Aldrich) or 4, 6 or 8 \(\mu\)g of chloramphenicol and 200 \(\mu\)g of ampicillin. The competitive index was calculated by dividing the output ratios (gcfu of the mutant/cfu of the WT of the inoculum). For analysis of bacterial loads in the organs, mice were euthanized by cervical dislocation at the indicated time point (untreated, day 1 p.i., day 4 p.i.), and caecum content, mesenteric lymph nodes, spleen and liver were recovered and homogenized in PBS supplemented with 0.5% tergitol and 0.5% bovine serum albumin. Minimal detectable values were 10 c.f.u. to 10\(^5\) c.f.u. in faeces and caecum content and 10 c.f.u. per organ in the systemic organs. Each experiment was performed in at least two independent biological replicates including the indicated number of mice and the data were pooled for statistical analysis. Typically, we used at least 5 to 6 mice per group in order to allow analysis by non-parametric statistical tests.

**Histological procedures.** Pathological scores were determined in a blinded fashion, as described in an earlier study\(^8\). In brief, samples from liver, spleen, ileum, caecum and colon were embedded in O.C.T. (Sakura), snap-frozen in liquid nitrogen, and stored at −80 °C. Sections (5 μm) were mounted on glass slides, air-dried at room temperature for 2 h, and stained with haematoxylin and eosin (H&E). Pathological score was determined by analysing four markers of inflammation: (1) submucosal oedema, (2) polymorphonuclear granulocyte infiltration into the lamina propria, (3) number of goblet cells harbouring mucin-filled vacuoles and (4) epithelial integrity. Pathological scores range from 0 to 13 arbitrary units (0–3, no or minimal inflammation; 4–6, slight inflammation; 7–9, moderate inflammation; 10–13, profound inflammation).

H&E pictures were taken with an Axioskop 2 plus (Carl Zeiss AG) microscope with a mounted AxioCam HRC (Carl Zeiss AG) camera. AxiosVision 4.8 (Carl Zeiss AG) software was used to capture images. Automatic best fit was applied for the best contrast.

**WT isogenic tagged strain analysis.** Mice were infected with a uniform mixture of the mutant and the WT of S. Typhimurium. A WTS-tag is a unique 40 bp DNA sequence tag within a non-coding region of the chromosome\(^9\). The faeces and caecum content of infected mice were collected at indicated time points (12 h p.i. and 4 days) and samples were homogenized in 500 μl PBS (50 μg ml\(^{-1}\) tetracycline and 0.5% bovine serum albumine) using a tissue lyser device (Qiagen; 2 min, 25 Hz). A 250 μl volume of the homogenate was inoculated into 3 ml LB broth culture containing 50 mg ml\(^{-1}\) streptomycin to enrich for the tagged strains for 4 h, then 50 μl of a serial dilution was plated on MacConkey agar plates (Oxoid) containing the appropriate antibiotics, as needed (50 μg ml\(^{-1}\) streptomycin, 50 μg ml\(^{-1}\) kanamycin, 15 μg ml\(^{-1}\) chloramphenicol and 200 μg ml\(^{-1}\) ampicillin). The competitive index was calculated by dividing the output ratio (gcfu of the mutant/cfu of the WT of the inoculum). For analysis of bacterial loads in the organs, mice were euthanized by cervical dislocation at the indicated time point (untreated, day 1 p.i., day 4 p.i.), and caecum content, mesenteric lymph nodes, spleen and liver were recovered and homogenized in PBS supplemented with 0.5% tergitol and 0.5% bovine serum albumin. Minimal detectable values were 10 c.f.u. to 10\(^5\) c.f.u. in faeces and caecum content and 10 c.f.u. per organ in the systemic organs. Each experiment was performed in at least two independent biological replicates including the indicated number of mice and the data were pooled for statistical analysis. Typically, we used at least 5 to 6 mice per group in order to allow analysis by non-parametric statistical tests.

**DNA extraction for 16S rRNA gene sequencing.** Caecum content was collected from CON mice kept on the MD, switched to WD or treated with 200 μg of oleic acid and analysed 6, 12 and 24 h after the switch or the treatment. Isolated caecum content samples were immediately shock-frozen in liquid nitrogen and stored at −80 °C. DNA was extracted using the AllPrep DNA/RNA/Protein Kit (Qiagen) with the following changes in the disruption and homogenization steps: 600 ml of RLT buffer and 3 × 4 mm metal beads were added to the tube containing the caecum content and bead-beaten at 10 Hz for 2 min using the Retsch M400. To separate fibres from bacteria, samples were centrifuged at 700 g for 2 min at 4 °C. Supernatants containing the bacteria were transferred to a tube containing 0.9 ml of 20% (v/v) sodium dodecyl sulphate (SDS) and samples were incubated at 25 Hz for 5 min. Before the transfer of the lysates to the QIAshredder column (Qiagen), lysates were pre-heated to 25 °C. The QIAshredder columns were centrifuged at 13,000 g for 3 min at room temperature for further homogenization. The flowthrough was centrifuged for 3 min at full speed to pellet the cell debris. The supernatants were loaded on the DNA columns and DNA was extracted...
according the manufacturer’s instructions with the modification that DNA was eluted in 100 µl EB.

Library preparation and 16S rRNA gene sequencing. The library was produced using the NextFlex 16S V4 Amplicon-Seq Kit 2.0 (barcodes 1–96; Bioo Scientific). The input concentration of genomic DNA was adjusted to 30 ng µl−1 for each PCR reaction. The library preparation was performed following the manufacturer’s instructions with the differences that the reaction volume was reduced to 25 µl per reaction and a modified primer pair was used for the first PCR reaction. Instead of the primer pair 515f-806r that is supplied with the kit, the degenerate primers 515f (5′-GTGCGACGCMGCCGCGGTAA-3′) and 806r (5′-GGACTACNVGGGTWTCTAAAT-3′), as described in refs. 45, 46, were applied. The first PCR reaction was performed using Q5 High-Fidelity DNA polymerase (BioConcept (NEB)) under the following cycle conditions: (1) initial denaturation, 95 °C for 1 min; (2) 20 cycles of denaturation, 95 °C for 30 s; (3) annealing, 56 °C for 30 s; (4) extension, 72 °C for 90 s; (5) final extension, 72 °C for 4 min. Cycles 2 to 4 were repeated eight times. After each PCR reaction, the PCR products were cleaned from oligos and nucleotides by AMPure XP magnetic beads (Beckman Coulter). The cleaned PCR product was eluted in 20 µl of resuspension buffer and used in a second PCR reaction using the modified primers. The quantity of amplicons was measured using a Qubit fluorometer. The quality of the amplicons was verified using a fragment analyzer (Advanced Analytical). The length of the amplicons after barcoding was ~450 bp. PCR products were adjusted to a final concentration of 60 ng per µl of multiplexed sample. Paired end read sequencing was performed on the Illumina MiSeq platform at the Functional Genomics Center, Zurich.

Analysis of microbiota composition. For CON mice, raw sequencing data were processed using custom scripts to execute several commands of the USEARCH software (version 9.1.13): paired reads were merged and quality-filtered using the fastx_mergepairs command with default settings. Merged reads were filtered using the fastq_filter command (fastq_maxee 0.1) and only merged reads with perfect primer matches and a minimum length of 100 bp were selected. Sequences were de-replicated using the fastx_uniques command and clustered into operational taxonomic units (OTUs) at 97% using the cluster_otsus command (mminsize 2), which also removed chimeric sequences. OTU abundances for each sample were quantified using the usearch_global command (strand both; id 0.97). Taxonomic annotation was performed by querying OTU sequences against the SILVA database (version 128) using the usearch_global command (-id 0.90; -maxaccepts 20; -maxrejects 500; -strand both; -top_hits_only). For Oligo mice, strain-specific qPCR amplification was performed using 1 mDa mass tolerance against a list of compounds derived from the KEGG database6 assuming single charged ions after deprotonation. Further analyses were performed in RStudio (version 1.0.143 based on R version 3.3.3) using the libraries vegan and ggplot2. Read counts were rarefied to 20,000 reads per sample. For performing a principal component analysis, Euclidean distances after Hellinger transformation were computed between samples using the vegdist function.

Determination of S.1m growth rates in the gut. S.1m growth rates in the gut were assessed using replication-incompetent plasmid pAM34, which has been described previously45,46. Briefly, pAM34 is a ColE1-like vector in which the replication of the plasmid is under the control of the LacI repressor, whereby plasmid replication only occurs in the presence of Suppressor β-galactoside (IPTG). S.1m carrying the pAM34 plasmid was therefore cultured for 12 h in the presence of 1 mM IPTG in LB broth and in the absence of antibiotics. Cultures were diluted 1:20 into fresh LB broth without IPTG and sub-cultured for 3 h at 37 °C. Inocula for in vivo infections were prepared as described previously47. Oligo mice were kept on the MD, switched to WD 24 h before infection or treated with 100 µg ampicillin and 1 mM IPTG. To quantify the total population size, samples were collected, rinsed with RPMI (15% FCS) and suspended in PBS (1% FCS). For intracellular staining, we first performed surface marker staining. Then, cells were fixed and permeabilized for 40 min at room temperature in the dark with Fix/Perm buffer (eBioscience/Thermo Fisher). Subsequently, cells were washed with permeabilization/wash buffer (eBioscience/Thermo Fisher) and stained for 40 min at room temperature in the dark. Antibodies/dyes used in this study: CD45 (30-F11, Biolegend), CD4 (RM4-5, Thermo Fischer), Foxp3 (FJK-16s, Thermo Fischer), CD69 (H12–2.1, Biolegend), CD95 (55-B5-6, Biolegend), CD11b (M1/70, Biolegend), CD11c (N418, Biolegend), SYTOX Green (Invitrogen) and Zombie-NIR (Biolegend).

Flow cytometric analysis of splenic immune cells of the caecal mucosa. Mice were euthanized and the caecum was excised and cut open longitudinally. To dislodge epithelial cells, the caecal tissue was cut into small pieces and incubated twice in 14 ml PBS (5 mM EDTA, 15 mM HEPES, 10% FCS; 20 min, 37 °C, mildly shaking). Subsequently, samples were washed in RPMI (30% FCS) and transferred to RPMI (1 mM γ-glutamyl transpeptidase (Sigma), 0.2 mM γ-glutamyltranspeptidase (Roche)). The cell suspension was then filtered through a 100 µm cell strainer and washed with RPMI. The cells were resuspended in RPMI and carefully pipetted onto a Nuocprep (Progen) 1.077 matrix. After centrifugation (30 min, 400g), cells from the interface were collected, rinsed with RPMI (15% FCS) and suspended in PBS (1% FCS). For intracellular staining, we first performed surface marker staining. Then, cells were fixed and permeabilized for 40 min at room temperature in the dark with Fix/Perm Fix/Perm buffer (eBioscience/Thermo Fisher). Subsequently, cells were washed with permeabilization/wash buffer (eBioscience/Thermo Fisher) and stained for 40 min at room temperature in the dark. Antibodies/dyes used in this study: CD45 (30-F11, Biolegend), CD4 (RM4-5, Thermo Fisher), Foxp3 (FJK-16s, Thermo Fisher), CD69 (H12–2.1, Biolegend), CD95 (55-B5-6, Biolegend), CD11b (M1/70, Biolegend), CD11c (N418, Biolegend), SYTOX Green (Invitrogen) and Zombie-NIR (Biolegend).
broth containing 50 mg/ml ampicillin, and inocula were prepared as previously described. CON mice were pretreated with ampicillin 24 h before infection, switched to WD 24 h before infection or treated with 100 μl oleic acid 1 h before and 4 h after infection with S. Typhimurium carrying pM972. Faecal samples were collected 12, 18 and 24 h p.i. and the caecum content was collected 24 h p.i. after elimination of the mice. All collected samples were diluted 1:20 (w/v) in sterile PBS. A 1 ml sample of caecum content was stained in 50 μl of PBS containing anti-O5 rabbit polyclonal antisemur (1:200 vol/vol) and incubated for 10 min. PBS (200 μl) was added to wash the cells. The samples were then centrifuged (4,000 rpm., 20 min) and supernatant was removed. Bacterial cells were suspended in 200 μl PBS. Samples were measured using an LSRII flow cytometer (Becton Dickinson) with both forward- and side-scatter parameters at a low, non-zero threshold value and acquired on a logarithmic scale. Data were processed using the Flowjo software (Treestar).

Flow cytometric assay for measuring cholate-dependent killing. S. Typhimurium WT strain and the mutant strains ΔacrB and ΔacrBΔtcr were grown overnight at 37 °C while shaking (160 rpm.) in LB broth containing the appropriate antibiotics. Ileum, caecum and colon content of untreated CON mice was collected and diluted 1:20 (w/v) in sterile M9 base salts. Different concentrations (0.125, 0.5, 1, 2 and 8%) of cholate were dissolved in M9 base salts. M9 base salts alone served as a control for potential killing of the gut microbiota on contact with oxygen. A 100 μl volume of M9 base salts only or the respective cholate solutions was pipetted into a 96-well plate, mixed with 1 μl of the different gut contents (harbouring microbiota) or the three S. Typhimurium strains and incubated for 20 min at room temperature. After this, samples were centrifuged (4,000 rpm., 20 min), supernatant was removed and bacterial cells were suspended in 200 μl PBS containing SYTOX green (1:5,000 vol/vol; Invitrogen, ThermoFisher) to detect bacteria with loss of membrane integrity. This dye can only penetrate and stain the DNA of microorganisms when the cell membrane is compromised. The SYTOX-positive (SYTOX+) fraction was quantified using a flow cytometer (Becton Dickinson) and side-scatter parameters at a low, non-zero threshold value and acquired on a logarithmic scale. Data were processed using the Flowjo software (Treestar).

Minimal inhibitory concentration assay. The sensitivity of S. Typhimurium WT, the mutants deficient in the AcrAB-ToLB and AcrAB-ToLB efflux pump against certain antimicrobials and primary bile salts, was assessed using the minimal inhibitory concentration (MIC) assay, which has been described previously. Briefly, overnight cultures of the different strains were adjusted to an optical density at 600 nm (OD600) of 1 and further diluted 1:2,000 in LB broth to obtain 1 × 106 c.f.u. per well (assay was performed in sterile 96-well plates). Stock solutions of the different antimicrobial agents were generated in LB broth as the following: 0.0625 μg/ml ciprofloxacin, 100 μg/ml rifampicin, 100 μg/ml ethyromycin, 6.25 μg/ml tetracycline, 10 μg/ml polymixin B, 8% cholate and 8% taurocholate. Each stock solution was either 10× or 100× concentrated. For the inocula, each of the different antimicrobial agent solutions was mixed with 50 μl of the respective inocula. The plates were incubated at 37°C and growth was measured at 0, 4, 6, 8 and 24 h by measuring the OD600 using a Spectramax plus system (Bucher Biotec). During the incubation between 8 and 24 h of growth, plates were sealed with Breathe-easier tape (Sigma-Aldrich) and covered with the lid of the 96-well plates. The function

\[
\frac{dS}{dt} = \frac{\beta}{\tau} S - \frac{r_S}{\Delta Tm} S - \frac{r_{Smax}}{\Delta Tm} S \quad (\text{measured growth rates 1.05 h}^{-1})
\]

for the microbiota population size was set to be 1, while for the microbiota without bile salt, it was set to zero. Maximum growth rates were set to 1.1 h^{-1} for both the microbiota and S. Typhimurium WT strain. This is based on measured data for both S. Typhimurium WT strain (measured growth rate is 1.10 h^{-1}) and Bacteroides thetaiotaomicron (measured growth rate 1.05 h^{-1} (ref. 11)).

Estimation of initial population sizes. To estimate the initial S. Typhimurium WT and S. Typhimurium ΔacrBΔtcr (mutant without functional AcrAB-ToLB efflux pump) population size S, we used the experimental inoculum size used for the infections, S = 5 × 10^6. The microbiota population size without bile salt was set to be 1 × 10^10 cells, and the results shown in Fig. 3a,b were generated using M = 10^11 consistently.

It is of interest that this number reflects a steady state of microbiota growth and loss through inflow. Inhibition of growth by bile salt, assuming outflow to be unchanged, could therefore lead to a decrease in microbiota population size that is directly proportional to microbiota growth, and can thus be modelled using the same kinetics (see previous paragraph for the estimation of parameters).

\[
M = 10^{11} \times e^{-kD t} \quad (\text{measured growth rate is 1.05 h}^{-1} (\text{ref. 11})).
\]

We tested how adding this dependence of the initial microbiota population size on bile concentrations to the model changes the outcome, and show the results below (see Methods, ‘Model results’).

Population dynamics. To describe the population dynamics of S. Typhimurium WT strain and the microbiota, we now formulate the following differential equations, where S denotes S. Typhimurium WT strain population size and M the microbiota population size:

\[
\frac{dS}{dt} = S \times (r_S - (S + M) \times d) \\
\frac{dM}{dt} = M \times (r_M - (M + S) \times d)
\]

where d is a description of clearance and death, introducing an effective carrying capacity for the total population size of (S + M), which dynamically relates the two populations.
Model results. The results for the modelled competition of S. Typhimurium and S. Typhimurium strains against the murine microbiota after 24h with constant initial microbiota population sizes are depicted in Fig. 3a,b. In Supplementary Fig. 15, we show in addition how changing the initial microbiota population size in response to bile (see Methods, Estimation of initial population sizes) affects the results of this competition. This modification of the model leads to increasingly smaller M with increasing bile salt concentrations. In the case of competition of S. Typhimurium against the microbiota, this only leads to small quantitative differences in realistic bile salt concentrations (that is, below 1%, Supplementary Fig. 15a) when compared to the model with constant initial microbiota population size (Fig. 3a,b). However, in the case of competition of the S. Typhimurium strain against the microbiota (Supplementary Fig. 15b), we observe a qualitative difference in the observed curves when comparing to the model with constant initial microbiota population size: at high bile salt concentrations, the S. Typhimurium population size increases (Supplementary Fig. 15b, cyan line). There is a simple explanation for this: very little growth happens in either population at these bile salt concentrations, and the observed population sizes are therefore largely determined by the initial population size, and, because the initial microbiota population size becomes smaller with increasing bile salt concentration, but the initial S. Typhimurium population size remains constant, we observe the behaviour shown in Supplementary Fig. 15b.

Numerical simulations. To solve the system numerically, we used the function ode in the package deSolve in the R language of statistical computing. The time resolution of the simulations was 12 min.

Sequence analysis of arcaB- and tolC-like genes. The Salmonella protein sequences were queried against the full proteome sequences of the other 27 sequences were queried against the full proteome sequences of the other 27

Statistical analysis. Sample size was determined from the data of previous studies. Scientists were not blinded for the assignement of the experiments and the data analysis. The exact Mann-Whitney U-test was performed when two groups were compared. One-way ANOVA was carried out when two or more treatment groups were compared. When time courses/different sampling sides were compared with two or more groups, two-way ANOVA was used to determine significance. Statistical analysis was performed by the software GraphPad Prism Version 7.02 for Windows (GraphPad Software). P values of less than 0.05 were considered to indicate statistical significance.

Ethical statement. All animal experiments were reviewed and approved by the Kantonales Veterinäramt, Zürich (licence 222/2013 193/2016) and are subject to the Swiss animal protection law (TschG).

Received: 22 August 2018; Accepted: 23 August 2019; Published online: 7 October 2019

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Acknowledgements

We thank members of the Hardt laboratory and R. Stocker for helpful scientific discussions and the RCHCI staff (especially K. Holzinger, D. Möllenhuener and S. Nowok) for excellent support of our animal work. E.S. is supported by the Swiss National Science Foundation (SNF, Marie Hein-Vogolin award PMDPP3_158364 and Ambizione award PZ00P3_136742) and the Gebert Rüf ‘Microbiota’ programme (GRS-073/17).

W.-D.H. is supported by the SNF (310030_153074 and 310030B_173338/1; Sinergia CRSII_154414/1; NRP 72 7074–7080 (2011).)

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41564-019-0568-5.

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| Sample preparation | See Materials and Methods for detailed sample preparation protocols. |
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| Instrument         | LSRll Flow cytometer (Becton Dickenson, NJ, USA) |
| Software          | FlowJo software 10.4.2 (Treestar, Ashland, OR, USA), FACSDiva (BD Biosciences) |
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