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PERSPECTIVE

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Sieving through gut models of colonization resistance

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The development of innovative high-throughput genomics and metabolomics technologies has considerably expanded our understanding of the commensal microorganisms residing within the human body, collectively termed the microbiota. In recent years, the microbiota has been reported to have important roles in multiple aspects of human health, pathology and host–pathogen interactions. One function of commensals that has attracted particular interest is their role in protection against pathogens and pathobionts, a concept known as colonization resistance. However, pathogens are also able to sense and exploit the microbiota during infection. Therefore, obtaining a holistic understanding of colonization resistance mechanisms is essential for the development of microbiome-based and microbiome-targeting therapies for humans and animals. Achieving this is dependent on utilizing physiologically relevant animal models. In this Perspective, we discuss the colonization resistance functions of the gut microbiota and sieve through the advantages and limitations of murine models commonly used to study such mechanisms within the context of enteric bacterial infection.

Two important roles have historically been associated with the microbiota. One is de novo synthesis of essential non-dietary vitamins and the production of other metabolites, such as short-chain fatty acids (SCFAs) via fermentation of carbohydrates[1,2]; the other is protection from invading pathogens by conferring what is known as 'colonization resistance'. Back in 1954, Bohnoff and Miller reported that pre-treatment with the antibiotic streptomycin significantly increases murine susceptibility to infection with *Salmonella*[3], and suggested that this was mediated by disrupting the integrity of the microbiota. In the following years, similar reports were made with different antibiotic formulations and pathogens[4]. The term colonization resistance was first coined in 1971, to quantify the pathogen load required to persistently colonize 50% of antibiotic-treated animals[5]. Nowadays, the term is used more loosely to qualitatively describe mechanisms by which the resident microbiota prevents colonization of exogenous microorganisms.

Deciphering the protective capabilities of the microbiota in a colonized niche may be achieved by characterizing microbiome composition, host-related physical conditions, and how the microbial, metabolite and immune landscape are shaped by host–microorganism interactions. While work has shown that commensals at many sites, including the skin[6] and nasal cavities[7], can confer direct and indirect protection against pathogens, this Perspective focuses on the gut, where much of our current knowledge of the microbiota stems from studies performed on the luminal content of the large intestine (and caecum in mice), due to the relative simplicity of sampling from the colon or collecting faecal samples.

The gastrointestinal (GI) tract is not a uniform niche for the microbiota. Bacterial concentrations reach 10^2–10^4 bacteria ml^{-1} in the stomach and upper two-thirds of the small intestine, whereas the ileum has a concentration of 10^7–10^8 bacteria ml^{-1} and the colon harbours 10^{10}–10^{11} bacteria ml^{-1} (refs [8,9]). Additionally, microbial community composition differs between the different regions of the large intestine in humans, and between luminal and mucosal-associated communities at the same site[10,11]. Various regions of the small intestine also display distinct compositions compared to the large intestine and among themselves[11–13], and complementary data from rhesus macaques suggest similar differences between luminal and mucosal small intestine communities[14]. Beyond bio-geographical differences, the microbiota also varies significantly between individuals, due to factors such as age[15], diet[16,17], antibiotic usage, food supplements[18,19], underlying medical conditions[20] and disturbances to circadian activity[21].

As enteric pathogens encounter this complex landscape during natural infection, it is important to understand how factors such as diet[22] and diurnal variation[21] can impact susceptibility or resistance to pathogen colonization. For example, host immune responses (including Toll-like receptor (TLR) and pro-inflammatory cytokine expression) and microbiota composition (specifically of mucosa-associated bacteria[23]) display diurnal variation[24,25]. These observations are probably linked, as the microbiota regulates the development and function of both the innate and adaptive immune systems (reviewed in ref. [26]) and antibiotic treatment downregulates immune responses in the gut, including pro-inflammatory cytokines and anti-microbial peptides[27], along with multiple additional host functions that naturally display circadian oscillations[23]. Consequently, the outcome of infection with pathogens such as *Salmonella*[24], *Listeria monocytogenes*[28] and *Streptococcus pneumoniae*[29] also displays diurnal variation and depends on functional clock activity, suggesting that antibiotic-mediated effects on circadian rhythm can play a role in the colonization potential of a host niche. In another example, both diet[22,30] and feeding status[31,32] can impact an infection outcome.

In this Perspective, we highlight the colonization resistance role of the gut microbiota and its interactions with pathogenic bacteria. We also evaluate current in vivo models used to study colonization resistance, with particular emphasis on physiological differences in the gut between conventional mice and mice depleted of microbiota (either through antibiotic treatment or through sterile breeding) and how these changes affect enteric infection.

Evidence supporting colonization resistance

Antibiotics are some of the most commonly prescribed drugs; in 2011, in the United States alone, more than 262.5 million courses of

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antibiotics were prescribed, equating to more than 0.8 prescriptions per person[33]. While antibiotics cause a rapid reduction in the burden of pathogenic bacteria and associated morbidity and mortality, an almost ubiquitous side effect of orally administered antibiotics is non-specific killing of commensal bacteria, leading to a significant reduction in the diversity of the gut microbiome and changes to its taxonomic composition (termed 'dysbiosis')[34]. The majority of evidence for colonization resistance stems from treatment of humans and mice with oral antibiotics, which is strongly associated with the overgrowth of gut pathogens and pathobionts, such as *Enterococcus faecalis*[35], *Escherichia coli*[36] and *Salmonella* Typhimurium[37], as well as enhanced toxin production by *Clostridium difficile*[38,39].

Colonization susceptibility post antibiotic treatment. In humans, *C. difficile* exists asymptomatically in the gut of both adults and children, and treatment with a variety of antibiotics, including carbapenems, fluoroquinolones and cephalosporins, are associated with increased risk of developing pseudomembranous colitis[40,41]. Antibiotic treatment is thought to increase the abundance of sialic acid and succinate, which are utilized by *C. difficile* as energy sources, and thus aid overgrowth post antibiotic treatment[42,43]. Further supporting a role for the microbiota, *C. difficile* infection can be effectively combatted through faecal microbiota transplantation from a healthy donor[44]. Differences in the taxonomic composition of the microbiota in humans have also been correlated with susceptibility to other enteric pathogens. For example, susceptibility to *Campylobacter jejuni* infection is associated with reduced bacterial diversity and lower abundance of members of the Lachnospiraceae family[45].

Antibiotic-treated mice demonstrate increased infection susceptibility, most commonly studied in the context of enteric infection. Historically, the investigated pathogens are largely restricted to genera of the Proteobacteria phylum (for example, *Salmonella, Escherichia* and *Campylobacter*) and the Firmicutes phylum (for example, *Enterococcus* and *Clostridium*). Despite mice usually being refractory to colonization by these pathogens, all are able to replicate to high levels in the mouse GI tract after antibiotic treatment. As such, many of the proposed mechanisms of colonization resistance are based on these murine models. A limited number of antibiotics are commonly used, typically either a single dose of 20 mg per mouse of streptomycin orogastrically or the addition of an antibiotic cocktail, such as metronidazole, neomycin and vancomycin, to drinking water[35,46]; both cause a significant reduction in gut microbiota load[47] and diversity[35,48]. *Citrobacter rodentium* is a natural murine-restricted pathogen, which adheres intimately to the enterocytes of the colon while forming attaching and effacing (A/E) lesions, and is used to model human enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) infection[49]. Strains of mice vary in their susceptibility to *C. rodentium*, and this is partially determined by the composition of their microbiota, as transplantation of microbiota from Swiss NIH mice (which develop mild, self-resolving *C. rodentium* infections) to C3H/HeJ mice (which are lethally susceptible) can partially confer resistance and delay mortality[50]. In general, murine susceptibility to enteric infections is associated with reduced diversity; however, individual species or simple consortia, which are capable of re-conferring colonization resistance to antibiotic-treated animals, have been identified[48,51,52].

Support from germ-free mouse studies. Studies with germ-free animals also lend credence to the colonization resistance function of the microbiota as, similar to antibiotic-treated mice, many reports show that germ-free mice are more susceptible to infection than their conventional counterparts, both in the gut and at other sites[53–56]. As with reconstitution in antibiotic-treated mice, commensal supplementation in germ-free models can often reverse the susceptibility phenotype. Using rational design, a low-complexity defined microbiota was recently developed that when introduced to germ-free mice conferred colonization resistance to *S.* Typhimurium, similar to that afforded in conventional mice[57]. Further, colonization of germ-free mice with a single murine isolate of the family Lachnospiraceae conferred partial resistance to *C. difficile*[58], and *Clostridium scindens* alone can confer resistance to *C. difficile* infection in antibiotic-treated mice by restoring the production of secondary bile acids from host-derived bile salts[51], which inhibit the growth of vegetative *C. difficile*. In one report, even re-colonization with a single eukaryotic virus partially restored intestinal morphology and lymphocyte function to germ-free mice[59].

Mechanisms of colonization resistance. Colonization resistance is mediated by a range of mechanisms, which generally include direct targeting of pathogens by the microbiota, modulation of the gut niche to exclude pathogens, and induction of immune responses to kill pathogens. In many instances, commensals are thought to directly kill or inhibit pathogens. One such mechanism is the production of anti-bacterial bacteriocins. Isolates of commensal *E. coli* can inhibit EHEC via the secretion of bacteriocins[60,61] and recently Enterobacteriaceae-produced microcidins were shown to mediate interspecies competition in the inflamed gut[62]. *Bacillus thuringiensis* produces the bacteriocin thuricin, which inhibits *C. difficile* and *L. monocytogenes*[63], and lactic acid bacteria produce a family of bacteroicins called lantibiotics, which can target both pathogens and other commensal bacteria[64]. However, in some cases bacteriocins can be narrow spectrum and they are less diverse in the gut than in other mucosal niches[65]. In addition to bacteroicins, commensals can antagonize pathogens by the production of SCFAs[66] or secondary bile acids[51]. Gram-negative pathogens including *Vibrio cholerae*[67] and *S.* Typhimurium[68], as well as commensals from the *Bacteroides* genus[69], are also capable of contact-dependent killing of other bacteria through effectors translocated via a type VI secretion system (T6SS). A recent study has shown that in conventional mice, a T6SS mutant of *Shigella sonnei* had reduced colonization, which was associated with a significant increase in the level of tissue-associated *E. coli*[70]. However, the effectiveness of T6SS killing might be affected by the fact that the interactions between pathogens and commensals are very specific, and that some require physical proximity in a densely populated niche and the broader utilization of T6SS by commensals to target enteric pathogens remains to be determined[71].

Commensals may alter the gut microenvironment to prohibit pathogen colonization. This can include competition for nutrients, which would otherwise be used by pathogens. For example, commensal strains of *E. coli* can compete with pathogenic *E. coli* for amino acids and sugars[72,73]; the microbiota also competes with pathogens for trace metals such as iron[74,75] and zinc[76], and pathogens without high-affinity transporters are outcompeted. Commensal facultative anaerobes can also sequester residual oxygen from the niche, thus reducing the virulence of pathogens that rely on oxygen for expression of virulence genes, such as *Shigella flexneri*[77], or for aerobic metabolism, such as *C. rodentium*[78]. Alternatively, commensals may also produce metabolites, such as butyrate, that can downregulate expression of virulence genes in both *S.* Typhimurium and *Salmonella* Enteritidis[79].

Host inflammation can provide favourable conditions for pathogen replication (see section 'Pathogen subversion of colonization resistance'), and several commensals have acquired the ability to suppress the host inflammatory response. For example, members of *Bacteroides, Clostridium, Lactobacillus, Streptococcus* and *Bifidobacterium* genera suppress inflammatory responses by promoting differentiation of FoxP3$^+$ regulatory T cells[80–82]. Immunosuppression can also be achieved through blocking of NF-κB activation and anti-inflammatory cytokine production by *Faecalibacterium prausnitzii*[83]. Germ-free mice have been found to be deficient in multiple components of the innate immune system,

Box 1 | Anatomy of the microbiota-depleted mouse GI tract

The mouse is a widely used model for human genetic disorders and infection. While mice partially (antibiotic-treated) or completely (germ-free) ablated of their microbiota are more susceptible to colonization with human pathobionts and enteric pathogens to which conventional mice are otherwise refractory, both of these interventions result in anatomical changes to the murine GI tract, which may complicate data interpretation.

Germ-free and antibiotic-treated animals have reduced gut motility, prolonged intestinal transit times, and display grossly enlarged caeca (an expanded fermentation vessel for dietary fibres in mice[130] that is relatively small in humans) compared to their untreated counterparts[131–134] (see figure). The production of metabolites by commensals, as well as the direct sensing of bacteria by the host, regulates GI motility. In humans, SCFAs have been reported to stimulate intestinal motility[135] and levels of serotonin (5-hydroxytryptamine), a neuroendocrine that stimulates gut motility, are depleted in antibiotic-treated mice[136]. Microbial products such as lipopolysaccharide (LPS) are directly sensed by the host and this too regulates motility. For example, murine enteric neurons are stimulated by LPS in a TLR4- and Myd88-dependant manner[137]. Additionally, a subset of macrophages present in the muscle surrounding the colon can be stimulated by LPS, regulate the enteric sympathetic nervous system and maintain gut motility[138].

The specific dysbiosis induced by antibiotics can vary depending on the class of antibiotics. Vancomycin, a glycopeptide that targets cell wall synthesis in Gram-positive bacteria, unsurprisingly leads to a bloom of Gram-negative Proteobacteria[35,139], whereas treatment with aminoglycosides (for example, streptomycin) has been associated with an overgrowth of members of the Bacteroidetes phylum[139]. Members of the aminoglycoside and glycopeptide classes of antibiotics are among those known to cause enlarged caeca when given orally to mice[144]. It has been reported that colonization of germ-free mice with a mixture of *Clostridia*, but not *Bacteroides uniformis*, is sufficient to correct caecal enlargement[140], but we are still lacking a comprehensive understanding of the relationship between a sterile gut, dysbiosis and caecal enlargement.

The figure shows a representative caecum and colon of C57BL/6 mice following three mock (–) or streptomycin (+) treatments. The enlargement of the caecum that is widely reported to occur in mice following antibiotic treatment can be seen.

which aid in protection against pathogens. Expression of pathogen recognition receptors such as Nod2 (ref. [84]), inflammasome activation[85] and production of antimicrobial peptides (AMPs)[86] are all reduced in germ-free mice compared to conventional mice. Furthermore, both germ-free and antibiotic-treated mice show reduced levels of myelopoiesis, rendering the host more susceptible to systemic *Listeria* infection[87]. These deficiencies can be partially restored by re-colonization of the sterile animals. Indeed, mono-colonization of germ-free mice with *Bifidobacterium breve* leads to upregulation of the production of Reg3γ[88]. The adaptive immune system is also modulated by commensals. Segmented filamentous bacteria, for example, are sufficient to induce T-helper-17 cells in germ-free mice[89], which prevent chronic inflammation and protect against *C. rodentium* infection.

Pathogen subversion of colonization resistance. Pathogens have co-evolved to topple colonization resistance. Mechanisms include the promotion of host inflammation[90], oxygenation of gut mucosa[91], alteration of metabolism in intestinal epithelial cells[91], and use of distinct energy sources to gain a growth advantage via metabolic pathways not present in commensal organisms. EHEC can overcome competition for nutrients by utilizing alternative sugar sources that are not utilized by commensal *E. coli*[92], and a number of enteric pathogens, including EHEC[93,94], *Salmonella*[95] and *Enterococcus*[96], are able to catabolize ethanolamine. Indeed, the ability to metabolize sugars elevated in the inflamed gut has been shown to be an important mechanism supporting pathogen growth. For example, *S.* Typhimurium and other Enterobacteriaceae are able to exploit an increase in the host-derived monosaccharides galactarate and glucarate, which gives them an advantage over the resident microbiota during inflammation[97]. Moreover, infection with *C. rodentium* results in increased cholesterol biogenesis and efflux, leading to elevated levels of faecal cholesterol and a bloom of commensal Proteobacteria that can catabolize cholesterol[91], which could potentially provide an energy source for the pathogen. Further, depletion of butyrate-producing commensal *Clostridia* alters host intestinal cell metabolism and results in increased luminal oxygen[98] and this can be exploited by *S.* Typhimurium, aiding luminal growth post antibiotic treatment[99].

During *Salmonella*-induced inflammation, reactive oxygen species (ROS) produced by recruited neutrophils react with byproducts of hydrogen sulfide produced by the microbiota to produce

Box 2 | Tackling colonization resistance to improve microbiota-based therapies

Gut dysbiosis can render the host more susceptible to infections with pathogens, and is associated with multiple additional pathologies. To date, there are no efficient therapies indicated for the prevention of this effect. While health practitioners often recommend consumption of live probiotic bacteria and the mechanisms by which probiotic strains can exert a beneficial effect have been suggested in preclinical studies[141–146], human clinical trials have yielded inconclusive results[147]. This may stem from the variation in the extent to which probiotic strains can colonize the human GI tract[148], and suggests that exogenous probiotic bacteria also face colonization resistance.

Engraftment with a full microbiota by means of faecal microbiota transplantation (FMT) is an efficient treatment of recurrent *C. difficile* infection, and animal models suggest that it may be useful for additional antibiotic-resistant infections, such as vancomycin-resistant *Enterococcus*[48]. Nevertheless, transplantation of a complete microbiota, especially to immunocompromised patients, bears the risk of transferring pathobionts, and clinical trials of FMT for inflammatory bowel disease suggest that some microbiota compositions are less efficient in colonizing the new host[149]. In order to circumvent the potential colonization resistance to probiotics and FMT and maintain efficacy while avoiding the risks, several novel microbiome-based therapeutics approaches have been suggested. The first is rational selection of a minimal number of strains that confer protection against a specific pathogen[48,51,150] rather than a generalised probiotics mixture. Another approach is based on utilization of beneficial microbiome-associate metabolites, either by filtering an entire FMT sample[151] or more precise therapy based on the biological function of the metabolite, both in a disease context (for example, diabetes[152], obesity[153]) or to circumvent colonization resistance[85]. Finally, therapies based on exogenous bacteria may be improved by additional modulation of the niche by diet. While this is in line with the classical 'prebiotics' point of view, recent advances suggest that in order to alter the bacterial composition in the gut, these dietary interventions should be personally tailored[154].

tetrathionate; unlike the commensals, *Salmonella* can utilize tetrathionate as an electron receptor, thus gaining a competitive advantage[100]. Moreover, via their cytokine (for example, IL-17 and IL-22) receptors, intestinal epithelial cells respond to inflammatory signals through production of AMPs (for example, Reg3β and Reg3γ) and iron scavengers such as Lipocalin-2 (Lcn2). While Reg3β and Reg3γ mainly target Gram-positive bacteria (commensal and pathogens alike), Lcn2 binds the siderophore enterochelin, which is utilized by many members of the Enterobacteriaceae to acquire iron. As *S.* Typhimurium encodes a glycosylated derivative of enterochelin that is resistant to Lcn2, it has a competitive advantage over commensal bacteria in the inflamed gut[101].

Evaluation of current in vivo colonization resistance models

Many human enteric pathogens exhibit a limited host range compared to their generalist ancestors, including *Salmonella* Typhi and Paratyphi A[102], pathogenic *E. coli*[103], *Shigella*[104] and *Yersinia pestis*[105]. This is generally mediated by a process of genome degradation and loss of multiple functions, including host-specific colonization factors such as fimbriae and adhesins, metabolic capabilities and virulence genes that enable colonization and survival in multiple hosts. This may result in complete host restriction, or disease courses of the same strain differing between humans and mice. To overcome this experimental limitation, antibiotic pre-treated and germ-free mice have been used to model human enteric infection.

Antibiotic pre-treated mice. In humans, EHEC and *S.* Typhimurium induce colitis and cause diarrhoea, but for reasons that are not fully understood, mice are not permissive to EHEC colonization[106,107]. Similarly, despite inoculation with high titres of *S.* Typhimurium (10^8 colony-forming units; c.f.u.), conventional mice do not shed detectable levels of *S.* Typhimurium in faeces two days post infection (d.p.i.) and do not develop diarrhoea or pathologies typically associated with human colitis[46]; instead, infection leads to systemic disease resembling human *S.* Typhi infection. Thus, in order to provide a genetically tractable small animal model of pathogen-induced colitis, antibiotic treatment of mice prior to inoculation with enteric pathogens of interest has been used to remove the species-specificity and colonization resistance barriers and enable colonization with fastidious human pathogens[46,106,108].

While evaluating the utilization of antibiotic-treated mice as a model to study the colonization resistance role of the enteric microbiota, it is important to keep in mind that the anatomy of the gut of these mice is significantly different from untreated conventional mice. In particular, antibiotic-treated mice have reduced gut motility and a significantly enlarged caecum (discussed in Box 1). Concomitantly, inoculation of antibiotic-treated mice with *S.* Typhimurium, EHEC or *C. rodentium* results in high levels of replication in the lumen of the GI tract, primarily in the lumen of the enlarged caecum. For example, in a streptomycin pre-treated murine model for *S.* Typhimurium-mediated colitis, the pathogen replicates to high titres (10^8 c.f.u. g^{-1} at 2 d.p.i.) in the caecal contents[46]. However, in line with high levels of luminal replication irrespective of virulence, an *S.* Typhimurium mutant lacking functional *Salmonella* pathogenicity islands 1 and 2 (SPI1 and SPI2)—which encode critical virulence factors needed for efficient cell invasion and intracellular replication—replicates in the caecal lumen comparable to the wild type[46,109].

Similarly, early studies using streptomycin-pre-treated mice as a model for EHEC infection relied on enumeration of bacteria in faeces as a readout for increased colonization of the GI tract, observing high titres of EHEC shed in the faeces of antibiotic-treated animals[108,110]. More recently, studies using bioluminescent EHEC showed that at 3 d.p.i., the vast majority of the bacteria are confined to the caecal lumen, with little bioluminescent signal observed on the colonic mucosa (and where this is seen, it is localized solely to the proximal colon)[106]. Importantly, when administered to streptomycin-treated mice, auxotrophic avirulent laboratory-adapted *E. coli* K12 strains are also shed at high titres (10^8 c.f.u. g^{-1} faeces)[111], suggesting that the caecum in antibiotic-treated mice may be permissive for bacterial growth in general. It is currently unclear why depletion of the microbiota facilitates high levels of replication in the gut lumen irrespective of virulence gene expression.

It has been suggested that commensal bacteria offer colonization resistance against EHEC in mice by competing for sugars[72,112]. However, in humans, EHEC transits the lumen of the GI tract and subsequently intimately adheres to the colonic mucosa (through A/E lesion formation), a niche distinct to that occupied by commensal *E. coli*. Therefore, competition assays that occur in the caecal lumen of antibiotic-treated mice do not necessarily reflect the challenges that EHEC faces under physiological conditions in humans and farm animals. Further, *C. rodentium* is able to intimately attach to the colonic mucosa and form A/E lesions in the murine colon without antibiotic pre-treatment[49] and this requires genes of the locus of enterocyte effacement (LEE) pathogenicity island, expression

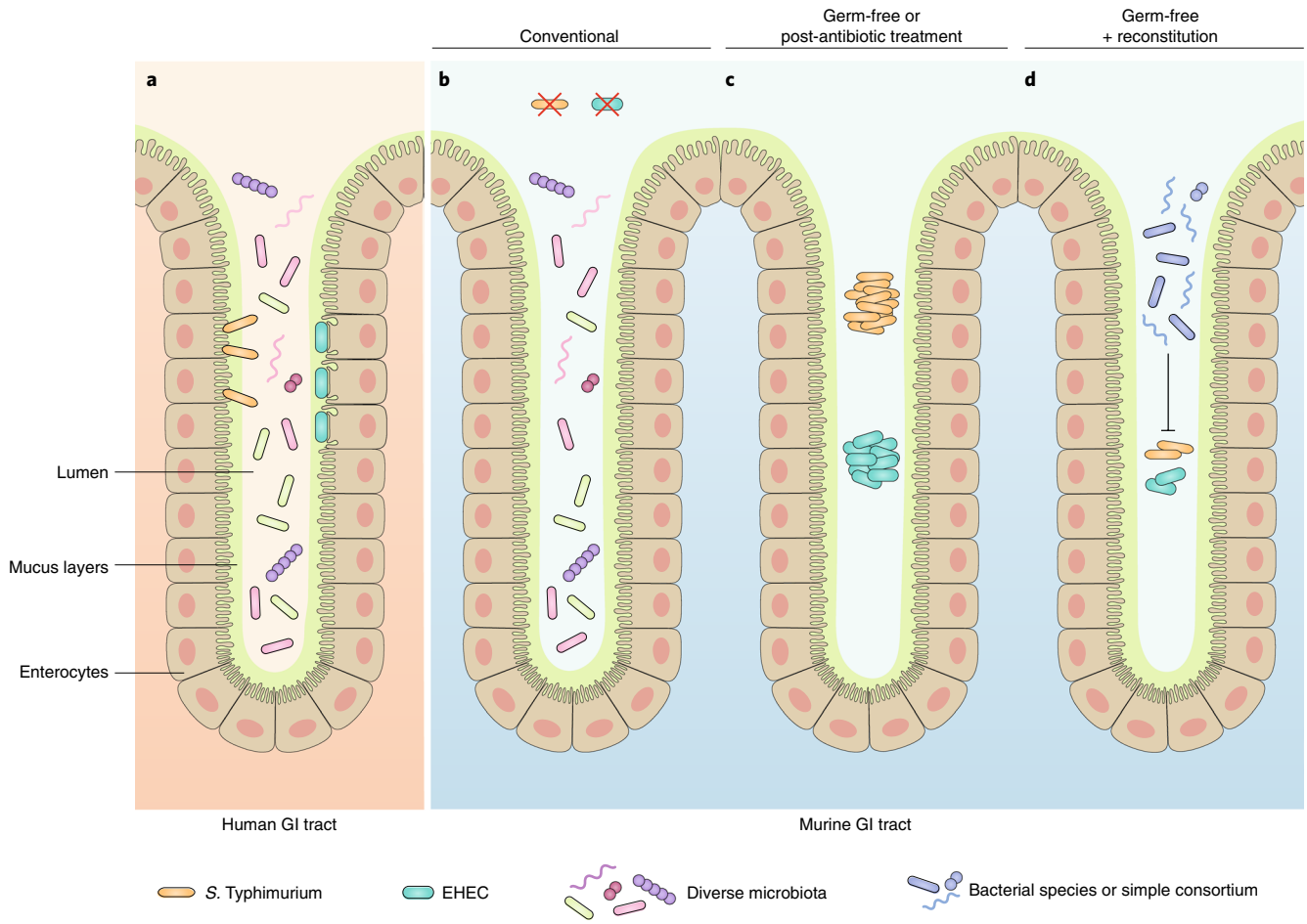

Fig. 1 | Colonization resistance in humans and mouse models. a, In humans, enteric pathogens such as EHEC and *S.* Typhimurium colonize the GI tract mucosa. EHEC intimately attach to colonocytes while forming A/E lesions, in the presence of the endogenous microbiota. **b**, Conventionally raised mice are not permissive to EHEC- and *S.* Typhimurium-induced colitis. **c**, In the presence of a disrupted microbiota (in antibiotic-treated or germ-free mice), for reasons which are not fully understood, pathogens and other bacteria can replicate to high titres in the lumen of the GI tract, primarily within the caecal contents, independent of virulence gene expression. **d**, Re-colonization of animals with commensals has been shown to reduce pathogen burdens; whilst suggestive, the protective role conferred by the re-introduced microbiota is not necessarily indicative of an interaction relevant within the niche occupied by the pathogen during physiological infection.

of which is controlled by the master transcriptional regulator Ler[113]. However, a single treatment of mice with kanamycin following colonic colonization with kanamycin-resistant *C. rodentium* leads to significant displacement of colonic *C. rodentium* and drastic expansion of the luminal caecal population, even with a strain constitutively expressing Ler[114]. This suggests that *C. rodentium* is dependent on commensals for survival in the colonic mucosa and, therefore, that antibiotic treatment can mediate a non-physiological infection course.

Antibiotics, including some that are commonly used in animal research, can also affect multiple non-bacterial host processes, such as immunomodulation[27], hypoglycaemia[115,116], altered gastrointestinal motility[117], mitochondrial damage[118], platelet inhibition[119], neuromuscular blockade[119], inhibition of cytochrome P450-mediated drug metabolism[120], inhibition of carbonic anhydrase leading to metabolic acidosis[116], and inhibition of monoamine oxidase potentially leading to serotonin syndrome[121]. Some of these changes may be a result of dysbiosis or decreased bacterial load, and can thus be useful in understanding indirect mechanisms of colonization resistance (for example, alterations in the immune landscape), but others may not be mediated by interactions with microorganisms and might thus be confounders worth noting in antibiotic-treated models. Further work on a greater array of enteric pathogens and antibiotic combinations may yield models that will allow alteration of the microbiota whilst minimizing changes in gut physiology. Nonetheless, until this question has been answered, we should be open minded to alternative cause and effect scenarios while utilizing the antibiotic pre-treatment models.

Germ-free mice. Localization of pathogens in the gut lumen is also seen in germ-free animals. Similar to antibiotic-treated models, this has mainly been investigated in the context of enteric infection with species belonging to the Proteobacteria and Firmicutes. Although direct comparisons between germ-free and antibiotic-treated models are infrequent, both models do report similar pathogen infection dynamics for *S.* Typhimurium infection[122].

In germ-free mice, the natural mouse pathogen *C. rodentium* persists in the caecal lumen at high titres, independent of *ler* expression and in the absence of A/E lesion formation. This is in contrast to conventional mice, where the *C. rodentium ler* mutant is unable to colonize[123]. An important question raised by these results and those from antibiotic-treated mice, is whether the ability of avirulent *C. rodentium*, and other bacteria, to colonize the caecal lumen reflects removal of commensals that promote colonization resistance or whether these changes reflect altered physiology of the enlarged caecum, which appears to impose little selective pressure.

Mechanisms of microbiota-enhanced pathogenesis

In addition to the protective role associated with the gut microbiota, multiple reports suggest that the microbiota can also in some instances facilitate pathogen colonization and expansion through diverse mechanisms, which are not represented in antibiotic pretreated and germ-free mice.

Pathogens can utilize signals derived both from the microbiome and the host to sense the niche and modulate the expression of virulence genes accordingly. While SCFAs can inhibit *S.* Typhimurium growth and virulence gene expression in the colon, the main infectious niche for *S.* Typhimurium is the distal ileum, where changes in the levels and composition of SCFAs upregulates expression of the invasion-associated SPI-1-encoded type III secretion system (T3SS)[124]. Similarly, the infectious site of EHEC is the colon, where high butyrate concentrations promote the expression of the T3SS[125]. Mucin O-glycans, derived from degradation of the GI protective mucus layer by commensals such as *Bacteroides thetaiotaomicron* and *Akkermansia muciniphila*, also serve as a virulence switch in EHEC; high levels in the lumen that can be utilized as energy source repress LEE genes through the FusKR two-component system to promote efficient replication, while lower levels near the epithelium signal the bacterium to upregulate LEE genes to promote T3SS-mediated infection[126]. Succinate, produced by several commensals, including *Bacteroides* spp., also activates virulence genes of EHEC and *C. rodentium*[127].

Commensals also provide nutrients for pathogens within the gut. Degradation of the mucus by *B. thetaiotaomicron* releases sialic acid, which is utilized as energy source by *S.* Typhimurium and *C. difficile*, and fucose, which is utilized by *S.* Typhimurium[42]. Succinate can be utilized by *C. difficile* as an electron receptor[43] and *S.* Typhimurium can also utilize hydrogen produced by commensals as energy source[128]. Further, on a low-fibre diet, several gut members switch from degrading dietary glycans to the degradation of the mucus, resulting in erosion of the barrier and promotion of aggressive *C. rodentium* colitis[22].

Similarly for non-bacterial pathogens, higher duodenal levels of the commensal *Lactobacillus taiwanensis* are positively associated with levels of regulatory T cells, which increase the susceptibility and enable colonization of resistant mice with the pathogenic nematode *Heligmosomoides polygyrus*[129].

Conclusions and perspectives

Bacteria and other microorganisms colonize every mucosal surface in the human body and play a crucial role in human health. The last few decades have seen a dramatic expansion in our knowledge about the composition and function of these commensal organisms within their respective niches, none more so than in the gut. Antibiotic-induced dysbiosis is in many cases undoubtedly a predisposing factor to the development of enteric infections and ongoing studies will certainly shed further light on the mechanisms by which commensals confer protection to their host, and this remains an important area of research.

However, antibiotic treatment of mice, routinely used to model enteric infections with pathogens to which the murine GI tract is usually refractory, induces pronounced changes to the mouse physiology, which alters the progression of infection. These changes, which at times have been overlooked, should be taken into consideration during data interpretation. For example, increased bacterial burden has been considered synonymous with increased virulence despite the alteration in anatomical localization and the ability of pathogens to replicate in the absence of virulence gene expression—*C. rodentium*, which is a natural mouse pathogen that can colonize in the presence of the endogenous microbiota, provides a prime example. Thus, a reduction in luminal replicating pathogens following re-colonization of antibiotic-treated or germ-free animals with commensal organisms, although suggestive, cannot be taken as a definitive indication of a colonization resistance role in the context of pathogens expressing virulence genes in a relevant and crowded niche (Fig. 1). Therefore, when studying pathogen–commensal–host interactions, the precise niche occupied by the pathogen following antibiotic treatment should be defined and related to conventional infections.

Reports from germ-free and antibiotic-treated mice may suggest a more passive form of colonization resistance in which antibiotics, rather than diminishing the presence of actively protective commensals, could be disrupting the microbial ecosystem in a manner that pathogens are able to exploit (for example, see ref. [99]). Importantly, rather that impacting only on the microbiota, antibiotics can also directly impact intestinal cells, which could potentially alter metabolism, peristalsis and inflammation. Therefore, there is a need to take a more holistic approach to host–microbiota–pathogen interactions to build a comprehensive understanding of colonization resistance. This will be especially critical when attempting to translate pre-clinical studies into microbiota-based treatments (Box 2) for enhancing human health and combating human and animal enteric infections.

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Competing interests

The authors declare no competing financial interests.

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