'Blooming' in the gut

How dysbiosis might contribute to pathogen evolution

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"Blooming" in the gut: how dysbiosis may contribute to pathogen evolution

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Preface:

Hundreds of bacterial species make up the mammalian intestinal microbiota. Upon perturbations by antibiotics, diet, immune deficiency or infection, this ecosystem can shift to a state of dysbiosis. This may involve overgrowth ('blooming') of otherwise underrepresented or potentially harmful bacteria (for example, pathobionts). Here we present evidence suggesting that dysbiosis may fuel horizontal gene transfer between members of this ecosystem, facilitating the transfer of virulence and antibiotic resistance genes, thereby promoting pathogen evolution.

Introduction

In 1996, Rodney T. Berg coined the following calculation: "In summary, there are … 10^{14} total gastrointestinal tract (GI) bacteria .... If we assume that one mutation in every 10^8 bacterial divisions is a viable mutation, 10^{14} total bacteria in the GI tract theoretically will produce 10^6 newly mutated viable bacteria at every division cycle. It is estimated that the bacteria in the GI tract divide every 20 minutes. This generation of large numbers of newly mutated bacteria at every division cycle allows the indigenous GI microflora to adapt rapidly to GI environmental changes"[1].

Given the fact that we are looking back on millions of years of microbiota-host co-evolution, consistently starting at birth with a first encounter of the microbiota, the combination of high bacterial numbers and their relatively short generation times implies that any contemporary microbiota can be regarded as the "snapshot" of a vast and continuing evolutionary process. Seminal contributions by large-scale sequencing consortia in the past years have offered unprecedented insights into the human microbiota´s assembly, individuality and stability over time [2-6]. Functional changes in the microbiota can derive from variations in the microbial transcriptome, proteome or metabolome. Altered microbiota functionality can also be introduced by diversification of the collective microbial gene pool (the microbiome), which can occur at three different levels: by intrusion or disappearance of individual members (for example, invading bacterial strains during maturation of an infant gut microbiota) [7]; by shifts in relative bacterial abundances such as those caused by dietary changes,

immune deficiency, antibiotic use or infections [8, 9], potentially leading to dysbiosis (**Box 1**) ; and finally by mutation or horizontal gene transfer (HGT). These alterations can significantly impact the overall functionally of the microbiota, by enhancing the individual fitness of certain keystone pathogens or keystone stabilizers **(Figure 1).**

HGT in particular enables bacterial evolution in quantum leaps rather than by step-wise adaptation through mutations, drive shifts in community composition and can potentially shift the system into dysbiosis. In the mammalian gut, HGT is thought to occur at a higher frequency than in other microbial ecosystems. This has essentially been attributed to its enormous local bacterial density[10, 11]. To date, the actual frequency of genetic exchange, its hotspots and limitations in terms of species boundaries and their contribution to overall ecosystem functionality can only be estimated from anecdotal evidence. Recent studies have established that environmental changes and microbiota perturbations can have profound and long-lasting effects on microbiota community structure and foster 'blooms' of otherwise low-abundant bacteria (**Table 1**). In particular, infections with enteric pathogens can give rise to enterobacterial blooms[12-14] , which can boost HGT between pathogenic and commensal bacterial species[15]. It seems reasonable to assume that bloom-driven HGT may promote bacterial evolution in the aftermath of such perturbations.

In this Opinion article, we argue that intestinal dysbiosis may act as a driver of HGT in the gut ecosystem, promoting pathogen evolution and the spread of antibiotic resistances.

HGT in the gut ecosystem

HGT-mediated acquisition of 'ready-made' genes, entire metabolic pathways, fitness and virulence factors as well as antibiotic resistance genes enables a swift adaptation of microbial communities to changing environmental conditions. Below we discuss mechanisms of HGT and why this process might be favoured in the conditions present in the intestine. *Mechanisms of HGT.* HGT is most efficient among closely related species, but can also occur between distantly related bacteria: *Enterobacteriaceae* can undergo HGT with Gram-positive species from the *Firmicutes* division [16-18]. Even inter-kingdom HGT has been described [19]. In general, HGT in bacteria can take place by three different mechanisms: transformation, transduction via bacteriophages and conjugation-mediated plasmid-exchange (**Figure 2A**).

Transformation involves the uptake, incorporation and expression of free DNA from the environment. Genetic elements such as prophages, transposons or plasmids are not required and natural competence, a developmental stage at which the acceptor strain can take up DNA from the environment and recombine it into its chromosome, is a trait encoded by the acceptor strain. So far, we do not know if transformation may occur frequently in the mammalian intestine. As the gut contains numerous DNA degrading enzymes, it seems likely that the concentration of high-molecular free DNA is low in the gut lumen and thus that transformation is relatively rare in this biosphere. However, stress conditions (as induced by perturbations) have been shown to promote natural competence in bacteria [20-22].

Bacteriophages can ferry genes between bacteria and contribute to HGT in two different ways. They can package and horizontally transfer bacterial genomic DNA between different bacterial species or

strains in a process known as transduction. Alternatively, many prophages have incorporated bacterial fitness factors ('morons') within their own genomes. These additional genes are not directly required for the phage lytic cycle (induced, for example, by stress conditions such as host DNA damage [23, 24]) but alter the bacterial phenotype upon integration into the host genome in the form of a prophage [25]. In contrast to conjugation, this mechanism of HGT does not require direct contact between the donor and the recipient. In fact, as phages are very stable in the environment over extended periods of time, the donor does not even have to reside in the same mammalian host as the recipient. Interestingly, many virulence factors of enteropathogenic bacteria are encoded by prophages[23, 26], and prophage integration was found to boost the competitive fitness of the commensal bacterium *Enterococcus faecalis* [27, 28]. In fact, lysogenic prophages integrated in the bacterial chromosome represent the dominant phage form in the intestine [29].

Intestinal ecosystems harbor a high diversity of conjugative plasmids [30]. Conjugative plasmids encode the genes required for formation of the conjugation machinery (i.e. the conjugative pilus) which is required for their own transfer. In contrast to transformation and phage-mediated HGT, conjugation requires physical contact of the donor and recipient bacteria [31]. Thus, conjugation is most efficient, when donors and recipients are present at extremely high densities [32], as occurs in the intestine (see below).

The intestinal ecosystem: an exquisite playground for HGT.

HGT between the gut microbiota and pathogens can have important consequences for human health, as intestinal bacteria act as reservoirs for fitness factors, virulence genes and antibiotic resistance genes[33-35]. Why are conditions in the gut especially favorable for HGT? First, the high nutrient inflow and constant temperature allows the maintenance of a continuously active bacterial metabolism. Second, microbial diversity and "amplifier donor strains" (i.e. extremely efficient plasmid donors) may have an "amplification effect" for plasmid transfer [36] , and host components such as catecholamines can further induce conjugative transfer. For example, norepinephrine at physiological concentrations was shown to enhance conjugative plasmid transfer from a clinical strain of *Salmonella enterica* subspecies enterica serovar Typhimurium to an *Escherichia coli* recipient *in vitro* [37] (see also ref [38]).**[AU: do we know why?]**

Last, the bacterial population densities are, in general, very high [39] and thus conducive for conjugal transfer. Ciliates within the intestine have been shown to increase conjugative plasmid transfer by several orders of magnitude by forming sites of very high bacterial density in their food vacuoles [40]. Likewise, the high bacterial density in the intestine of insects forms a favorable environment for plasmid exchange [41, 42]. A history of HGT leaves characteristic marks in bacterial genomes as for example shown in the case of the *Bacteroidetes*, which is the most prominent phylum of obligate anaerobic Gram-negative bacteria in mammalian gut ecosystems. A high number of integrative and conjugative elements provide evidence for past HGT events in *Bacteroides* spp.[43]. One remarkable example for horizontally transferred genes in this genus is the *Bacteriodes fragilis* toxin, which is encoded on a conjugative transposon [44]. Moreover, a recent study identified transposon-associated genes for degradation of algae-derived polysaccharides (for example, from nori, which is used in the Japanese cuisine) encoded by *Bacteroides* spp. that have been potentially obtained from marine

Bacteroidetes and are uniquely present in the microbiome of Japanese individuals [45, 46]. Moreover, it has been shown that intestinal inflammation elicits transient blooms featuring extremely high densities of commensal *Enterobacteriaceae*, in which conjugation-mediated HGT occurs at very high frequency [15]. This may explain why conjugative plasmid transfer is particularly pertinent between donor and recipient species that are able to locally bloom under the same environmental conditions. Thus, HGT does occur among the intestinal microbiota and it is further enhanced by blooms.

Blooms, HGT and pathogen evolution

Blooms are formed by bacterial species that are otherwise present in mammalian gut ecosystems at relatively low densities (that is, below 10^8 cfu/ml), for example *Enterococcaceae* and *Enterobacteriaceae* (**Table 1**). Conditions that affect the composition of the microbiota and can thus foster blooms include pathogen infection [12, 47, 48], genetic predisposition of the host (for example, in IL010- or TLR5-knockout mice [49, 50]) and inflammation triggered by colitogenic compounds (for example, dextran sulphate sodium)[47]. Such conditions may foster blooms by increasing high-energy nutrient availability (e.g. Nitrate as a new electron acceptor for anaerobic respiration)[51] or the elimination of competitors that keep colonization levels of bloom-associated species in check in a complex ecosystem (for example, by inducing colonization resistance[52]).

Enterobacterial blooms: a hotspot for HGT?

The best-known example of an *Enterobacteriaceae* that can form blooms is *Escherichia coli*, which is usually commensal but under certain conditions can become pathogenic **[AU: from below, ok?]**. Why are these bacteria blooming in the wake of perturbations? *E. coli* is the most abundant facultative anaerobic component of the mammalian gut microbiota but, under homeostatic conditions, represents only a minor fraction of the ecosystem vastly outnumbered by obligate anaerobic bacteria (10^5-10^8 cfu/g [52, 53]). Importantly, *E. coli* (like many other *Enterobacteriaceae*) has a very short doubling time and a highly flexible metabolic capacity, including anaerobic respiration of nitrate [51]and a multitude of catabolic pathways which makes it highly adaptable and allows it to bloom in the presence of perturbations [54]. This seems to explain why *E. coli* can exploit situations of reduced colonization resistance and blooms upon disruption of intestinal homeostasis. Such adaptation of *E. coli* to growth in a perturbed gut ecosystem may have opened the door for the acquisition of genetic material by other strains and by pathogens colonizing the gut. Indeed, the high variability in genomic content of *E. coli*, which reflects the high phenotypic diversity between strains, is indicative of constant evolution and diversification. The *E. coli* genome has an open pangenome structure, implying that the species constantly evolves by horizontal gene acquisition and diversification[55]. On average, strain-specific genes that are indicative of horizontal transfer (such as genomic islands, prophages, transposons and plasmids) make up more than 20% of an *E. coli* genome. Furthermore, *E. coli* contains a particularly high diversity of plasmids, which are thought to further enhance mobilization of genetic elements between different strains.

Evidence for conjugative transfer to *E. coli* in the context of blooms was recently obtained in the context of infection with the enteropathogen *Salmonella enterica* serovar Typhimurium (*S.* Tm). Infection facilitates not only *S.* Tm growth, but also elicits parallel blooms of commensal

Enterobacteriacae, such as *E. coli*. This can fuel the rapid transfer of a conjugative plasmid encoding the bacteriocin colicin from *S.* Tm to intrinsic *E. coli* strains of the normal mouse microbiota [15]. Thus, enterobacterial blooms enhance conjugative HGT among pathogenic and commensal members of this family.

In addition, phage mediated HGT might also be fuelled, as prophage DNA can represent up to 16% of *E. coli* genome - *Escherichia coli* O157 strain Sakai harbours 18 prophages[56]. As dysbiosis and antibacterial agents can increase phage titers in the gut [57-59], it is likely that these increased titers may boost lysogenic conversion of new host bacteria. Thus, in addition to conjugation, perturbation-induced blooms may also boost phage-mediated HGT, on the one hand by lytic induction and by elevating the overall abundance of donors and recipients

Inflammation-induced blooms fostering HGT.

Similarly to *E. coli*, closely related pathogens such as *Salmonella* spp. exhibit a clear history of HGT. During their evolution as pathogens, *Salmonella* spp. have acquired not only their characteristic virulence factors, but also an array of genes favouring growth in the inflamed gut. It has been hypothesized that the evolution of pathogenic *Salmonella* spp. may have happened in two phases (**Figure 2B**; [60]**)**. In the initial phase, the common ancestor of contemporary *Salmonella* spp. may have acquired genes enhancing growth in the inflamed gut. In this way, this ancestor might have resembled contemporary *E. coli* lineages of the ECOR B2 family, which can bloom in inflamed intestines [15, 52, 61]. If this were the case, some of these 'inflammation fitness factors' should display a history of HGT. An example of this in *E. coli* is the chromosomal *iroBCDEN* gene cluster, which allows bypassing iron-uptake interference by the host protein lipocalin 2 [62]. This cluster has been found on transmissible plasmids in uropathogenic *E. coli* strains [63], although little is known about the nature of this initial step of pathogen evolution, whether it occurred before or after the divergence from all *E. coli* lineages and which fitness factors had been acquired at this stage. In the second step of pathogenic *Salmonella* spp. evolution, the bacteria would have needed to acquire the capacity to elicit gut inflammation by themselves. Active triggering of gut inflammation has been attributed to a subsequent HGT event, namely the acquisition of the SPI-1 type III secretion system [60, 64, 65]. Notably, this key virulence factor is encoded by a pathogenicity island with clear evidence of HGT [66-68]. This enabled *Salmonella* spp. to trigger and sustain mucosal inflammation even when infecting healthy hosts.

Most likely, this had a dramatic effect and favoured the acquisition not only of further virulence factors, increasing the efficiency of inflammation, but also of additional fitness factors enhancing growth, survival and transmission in inflamed hosts (**Figure 2B**). In line with this hypothesis, *Salmonella enterica* genomes encode a significant number of prophages, pro-phage remnants, genetic islets and islands as well as plasmids coding for a large array of virulence and fitness factors such as fimbriae (such as *pef*), superoxide-dismutases (such as SodCI and SodIII), LPS (which mediates resistance to bile acid and antimicrobial peptides) [69, 70] and type III effector proteins[25, 71]. One example is the SPI-1 TTSS effector protein *sopE*. It is found in just some *S. enterica* strains, including *S. enterica* serovar Typhimurium SL1344 and *S. enterica* subspecies *enterica* serovar Typhi, and is encoded on prophages [23, 72, 73]. Functionally, it enhances host cell invasion [74] and contributes to gut inflammation [75, 76]. Furthermore, SopE triggers induction of the host nitric oxide synthetase, which

boosts pathogen growth in the intestinal lumen by promoting the conversion of nitric oxide to nitrate (which can be used for nitrate respiration) [77]. Another example is the ability of *S. enterica* subspecies *enterica* serovar Typhimurium to utilize tetrathionate, an electron acceptor generated by oxidative burst of the host inflammatory response [78]. Utilization of this unusual electron acceptor upon anaerobic growth on ethanolamine as carbon source provides a competitive growth advantage of the pathogen over the microbiota in the inflamed gut [79]. Genes required for tetrathionate-respiration are located on the border of *Salmonella* spp. SPI-2. In summary, these examples support the concept of continuing adaptive evolution of *S. enterica* subspecies *enterica* serovar Typhimurium by HGT to efficiently trigger and exploit gut inflammation.

In analogy to the continuing evolution of genuine enteric pathogens such as *Salmonella* spp., one may speculate that bacterial adaptation to growth in inflammation-inflicted blooms might foster the emergence of pathobionts (strains adapted to growth in disease-associated blooms by HGT). One might suppose that long-term growth of the microbiota in disease-associated blooms (for example, in inflammatory bowel disease (IBD)) could select for bacteria that are well adapted to withstand the selective pressures imposed by the host´s immune response. Resistance to host defences (for example to secretion of defensins, phagocyte killing and iron sequestration) may be lead to superior fitness under this condition and therefore become positively selected upon horizontal gene acquisition. As a result, a microbial community enriched in pathobionts may have enhanced disease-promoting potential. Indeed, a microbiota enriched in *Enterobacteriaceae* (*Klebsiella* spp. and *Proteus* spp.) isolated from genetically susceptible colitic mice was capable of driving colitis in genetically intact hosts [80, 81]. Thus, bacterial blooms occurring as a consequence of intestinal ecosystem perturbations may enhance selection for strains with higher pathogenic potential, e.g. improved capacities to utilize nutrients available in such a disturbed system. The subsequent increase in its population density may foster disease perpetuation. This may feed into a vicious circle of inflammation-induced pathobiont blooms and bloom-induced inflammation (**Figure 3A).** Most likely, additional mechanisms driving such processes will be identified, as we are learning more about the complex microbe-host interactions in the gut ecosystem.

Bloom-inflicted HGT of antibiotic-resistance ? **[Au: Please shorten to 45 characters including spaces]**

The human microbiome harbours a high diversity of antibiotic resistance genes [33]. Treatment with antibiotics induces pervasive changes in the composition of the human microbiota and its encoded resistance genes[8, 82, 83]. In the short-term, the antibiotic transiently decimates the microbiota, compromises colonization resistance and alters homeostasis of the gut-associated immune system [52, 84]. Interestingly, after a second course of antibiotic therapy, the disturbance of the intestinal ecosystem is less pronounced than what is observed after the first treatment [8]. This suggests that the ecosystem may be able to adapt to the perturbation - for example, by disseminating antibiotic resistances.

How could this adaptation be achieved? It is possible that antibiotic-resistance genes encoded on mobile genetic elements (such as plasmids and transposons) could have spread throughout the community, thus conferring resistance to a higher proportion of local strains [34, 85]. In this case, antibiotic-induced perturbation and concomitant horizontal spread of antibiotic resistance genes

would confer increased ecosystem stability. Indeed, it has been reported that the prevalence of antibiotic-resistant strains increased after antibiotic therapy [86] (**Figure 3B**). However, antibiotic therapy can damage the microbial ecosystem and severely compromise the resilience of the gut microbiota [87, 88] and increase the risk of infectious disease [89, 90]. [14, 48, 89].

Pathobionts may also acquire antibiotic resistance genes from commensal bacteria within blooms and may then become positively selected under long-term antibiotic therapy. This condition is of grave concern for human health and of major relevance in clinical settings. A growing number of outbreaks on intensive care units are caused by *Enterobacteriaceae* and Gram-negative non-fermenters like *Pseudomonas* and *Acinetobacter* spp. which are resistant to multiple antibiotics, including all clinically relevant β-lactams. This is mediated by the fast horizontal spread of CTX-M extended-spectrum β-lactamases (ESBLs), plasmid-encoded AmpC β-lactamases and KPC carbapenemases among the *Enterobacteriaceae* [91]. However, it remains unclear, if these gene cassettes have originated from commensals, pathobionts or pathogens.

Perspective

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The intestinal microbiota is exposed to a number of host- and environmental-derived stressors which affect its functionality by inducing changes in composition and gene expression. Clearly, those factors may also drive evolution of the microbial community over time by positive or negative selection of certain genetic traits. Our current view implies that evolution of certain low-abundance subgroups of the microbiota (e.g. *Enterobacteriaceae*) may not come about at constant rates and much rather happen in 'hot-spots' upon microbiota perturbation and dysbiosis: The ability of *Enterobacteriaceae* to grow in 'blooms' may provide the basis for efficient genetic exchange by HGT and further adaptation to pathobiont or pathogenic lifestyles.

The availability of novel sequencing technologies has opened up unprecedented possibilities to address functional evolution of the microbiota and thus gain a deeper understanding of their flexibility and stability. Now, we have the tools in hand to experimentally address the impact of host and environment-derived factors on the microbiota and its individual members and we can start investigating how these conditions drive short- and long-term microbial genome evolution. So far, metagenomic sequencing approaches have focused on bulk microbiome analysis. Only very recently have studies begun to assemble genomes of individual bacteria from such data[92]. However, this is still quite challenging owing to the short sequence reads and insufficient genome assembly, unless conditions with extremely low diversity are concerned[7, 93]. Therefore, at the genomic level, it has remained largely unclear to what extent the commensal bacteria in the intestine evolve over time, i.e. by accumulating mutations and by exchanging genetic material via horizontal gene transfer (HGT). The long read lengths of some third-generation sequencing platforms (for example, PacBio and Oxford Nanopore) may greatly enhance the detection of HGT events in microbial genomes even in metagenomic data sets[92].

Most of our current knowledge is derived from studies of enteropathogens and a few cultivable members of the microbiota[94, 95]. However, an increasing number of reference genome sequences of culturable human and mouse bacterial isolates are being generated (Human Microbiome project; GEBA project). This source of fully assembled genomes from metagenomic data is extremely valuable for analysing genome evolution of commensal bacteria over time, in particular of those species that cannot be cultured *in vitro* [96, 97]. In addition, genome sequencing of the same strains re-isolated after long-term colonization of the same ecosystem will be important to assess longitudinal genome stability of different bacterial types over time and to identify the confounding environmental variables. Recently, several studies shed light on a new aspect of bacterial genome diversification in the intestine: upon colonization of the gut by a single *E. coli* clone, different *E. coli* mutants that stably co-colonized the gut over long term were positively selected for according to increased stress resistance and nutritional competence [98, 99]. These data indicate a "sympatric diversification" into functionally diverse mutants that can exploit different nutritional or stress-imposed niches within the same gut ecosystem (known as the 'restaurant hypothesis' [99]). This possibility will further complicate the analysis: processes such as sympatric diversification among the gut microbiota pose extreme challenges to experimental and analytical systems which may be eventually overcome by genome sequencing strategies targeting individual bacterial cells retrieved from the microbiota and thereby allow detecting single nucleotide polymorphisms which may underlie such diversification.**[** Clearly, key discoveries on microbiota function in health and disease will rely on additional technological advances in DNA sequencing, and single cell analysis [100].

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Box 1: Dysbiosis

Early in the 20th century, the Russian Nobel prize laureate Elie Metchnikoff introduced the term dysbiosis (also known as dysbacteriosis) **[AU: ok?]** to describe the opposite of symbiosis - a state of co-existence in mutual harmony [101]. A dysbiotic microbiota therefore is an imbalanced intestinal microbial community (including bacteria, yeast, viruses and parasites) characterized by quantitative and qualitative changes in the **[Au: composition of?]** microbiota itself, its modified metabolic activities or changes in local distribution of its members. **[AU: moved from below]** During a state of dysbiosis, the microbiota is prone to invasion and blooms of pathogens exploiting niches left open after disturbances. Dysbiosis is a potential trigger of disease and is commonly associated with different diseases ranging from diarrhea and constipation to IBD [102], obesity [103], cancer [104], diabetes [105], allergy and asthma [106]. Most of these diseases involve inflammation, which itself is often caused by altered immune responses to intestinal bacteria. As such, immune dysregulation and gut dysbiosis frequently coincide and can occur as a result of one another.

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Figure legends

Figure 1: Functional classification of the members of the gut ecosystem.. Symbiosis describes a prolonged and close association between two species resulting in a benefit for at least one of these organisms. Symbiosis can be further subdivided into mutualism (right; each member benefits), commensalism (middle; only one member benefits, the other is unaffected) and parasitism (left; one organism benefits at the expense of the other). The intestinal microbiota is characterized by both commensal and mutualistic properties. Enteric pathogens including obligate and opportunistic pathogens lead a parasitic lifestyle. Colonization with an obligate pathogen leads to disease outcome (i.e. *Vibrio cholerae*, *Shigella* spp., *Salmonella* spp.). Opportunists colonize their host only under favorable conditions (i.e. immunosuppression; *Legionella pneumophila*, *Pseudomonas aeruginosa*) or show their pathogenic potential only under specific circumstances, such as during dysbiosis (=pathobionts; *Clostridium difficile*, vancomycin-resistant *Enterococcus* (VRE)). Low-abundance symbionts with the ability to destabilize a homeostatic microbiota towards a dysbiotic state are referred to as keystone pathogens (*Porphyromonas gingivalis*). Here, in contrast to pathobionts, an already imbalanced ecosystem is not a prerequisite. Symbionts with the opposite effect are referred to as keystone stabilizers (i.e. *Bacteroides thetaiotaomicron*) [107]. The blue gradient indicates the risk for bacterial blooms during a given condition.

Figure 2. Mechanisms of HGT and enteric pathogen evolution. A. Mechanisms of HGT. For each type of HGT, it is indicated whether it is dependent on bacterial blooms and whether it has been shown to occur in the gut. **B.** Two-stage model for the evolution of the enteric pathogen *Salmonella* spp.. In a first step, the common ancestor of commensal *E. coli* strains (i.e. ECOR B2) and contemporary *Salmonella* spp. may have acquired genes enabling growth in the inflamed intestine (e.g. genes for iron acquisition). While commensal *E. coli* remained at this level, *Salmonella* spp. acquired virulence factors (i.e. pathogenicity islands 1, 2 and 4) in the next stage to trigger gut inflammation itself. From stage two, horizontal acquisition of fitness- and virulence factors has been promoted by growth within inflammation-induced blooms. **[Au: Please add complete legend, describing each part of the figure sequentially]**

Figure 3. Perturbation-induced destabilization and stabilization of intestinal ecosystems. A. Perturbation-induced destabilization of the gut ecosystem. Perturbation-induced blooms can lead to dysbiosis and positive selection of pathobionts. Bacteria may adapt to growth in dysbiotic conditions and acquire even higher pathogenic potential by horizontal spread of virulence-factors. This process may end up in a vicious cycle in which perturbation-induced blooms increase inflammation in turn promoting pathobiont blooms. **B**. Perturbation-induced gut ecosystem stability. Perturbations may imply the spread of genes among the intestinal microbiota which confer resistance to the perturbation itself. This may be the case upon antibiotic treatment and thereby positive selection for horizontally

transferred antibiotic-resistance genes. After resilience, the evolved ecosystem will be more resistant to a second exposure to the same antibiotic.

Tables

Table 1: Studies reporting blooms of particular bacterial species in response to environmental changes or host-imposed perturbations **[AU: The text within the table is a little to long, so shortened to ensure that the table fits on one page. Also, we have added a new column to clarify the relevance of the table to HGT and the rest of the article]**

Ref.	***Species***	***Bloom characteristic***	***Function relevant for blooming***	***Evidence of HGT***[§]
Nutrient-induced blooms				
[45]	*Bacteroides plebeius*	Enrichment of *Bacteroides* spp. with metabolic capacity to degrade algae-derived polysaccharides	Porphyranases, etc. derived from marine bacteria	Genes are transposon-associated
[108]	*Bilophila wadsworthia*	Consumption of a diet high in saturated fat promotes the expansion of a low-abundance, sulphite-reducing pathobiont	Sulfite reduction	No
Antibiotic-induced blooms				
[90]	*E. coli* pathobiont	Multidrug-resistant blooms noted in antibiotic-treated mice and caused a sepsis-like disease via Naip-5-Nlrc4 inflammasome activation.	AB resistance genes (i.e. against ampicillin, neomycin)	Unknown
[88]	*Enterobacteriaceae*	One dose of clindamycin promotes enterobacterial blooms and contractions of other bacterial taxa in mice, enhancing susceptibility to *Clostridium difficile* induced colitis	Unknown	Unknown
[89]	*Enterococcus* spp., *Streptococcus* spp., γ-*Proteobacteria*	Intestinal blooms noted during allogeneic HSC transplantation **[AU: are these relevant to antibiotics? Or immunosuppresants?]**	Unknown	Unknwon
Inflammation-induced blooms				
[109]	Symbiotic *E. coli* NC101	Mucosa-associated strains increase in IBD and colorectal carcinoma (CRC) patients; monocolonization in CRC *Il10*-/- mouse model promotes invasive carcinoma	polyketide synthase (*pks*) genotoxic island	Gene encoded on genotoxic island

[15]	Symbiotic *E. coli* and *Salmonella* Typhimurium	Co-blooms in a mouse colitis model boosted conjugative horizontal gene transfer, which, under non-inflammatory conditions, was inhibited by the microbiota	Conjugative colicin plasmid	Gene encoded in plasmid
[35, 62, 78, 23, 77]	*Salmonella* Typhimurium *Citrobacter rodentium* *Campylobacter jejuni* *Enterobacteriaceae*	Inflammation shifts the balance between protective microbiota and the pathogen, promoting pathogen bloom	• *iroBCDEN* cluster • *ttrs*-cluster • *sopE*-phi	Gene encoded in genomic islands
[110]	Symbiotic *E. coli* [AU: is the bloom of E. coli or C. jejuni?]	Blooms in infant mice susceptible to *C. jejuni* and colitic mice. Artificial modification of the microbiota by feeding live commensal *E. coli* ; increased susceptibility to *C. jejuni* in healthy mice	unknown	unknown
mmune deficiency or host genetics factors resulting in blooms				
[50]	*Proteobacteria*	TLR-5 deficient mice show transient microbiota instability characterized by high levels of proteobacteria, encouraging inflammation.	unknown	unknown
Disease-dependent blooms				
[111]	Adherent and invasive E. coli (AIEC)	Higher prevalence within the ileal mucosa in Crohn's disease patients	unknown	unknown
[112]	*Helicobacter hepaticus*	T6SS mutants elicit increased inflammation in an experimental model of colitis (T cell transfer in $Rag1^{-/-}$ mice)	Absence of T6SS	unknown
Neonatal colonization				

113-115	Facultative anaerobes	Infants harbour increased levels of facultative anaerobic microorganisms and reduced levels of strict anaerobes. Such communities are intrinsically unstable and highly susceptible to interference by stressors and infections with opportunistic pathogens	unknown

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