

PERSPECTIVES

ESSAY

What are the consequences of the disappearing human microbiota?

Martin J. Blaser and Stanley Falkow

Abstract | Humans and our ancestors have evolved since the most ancient times with a commensal microbiota. The conservation of indicator species in a niche-specific manner across all of the studied human population groups suggests that the microbiota confer conserved benefits on humans. Nevertheless, certain of these organisms have pathogenic properties and, through medical practices and lifestyle changes, their prevalence in human populations is changing, often to an extreme degree. In this Essay, we propose that the disappearance of these ancestral indigenous organisms, which are intimately involved in human physiology, is not entirely beneficial and has consequences that might include post-modern conditions such as obesity and asthma.

"We have met the enemy, and he is us."
Walt Kelly, 1953.

"The enemy of my enemy is my friend."
Arabic Proverb.

This is an era of great change. We are exploring the outer reaches of the universe and can travel across continents in hours, but we are also warming the Earth and depleting its oceans. These major changes in our macroecology represent the price for the past century's technological progress. We have also begun to explore the microscopic universe within us. Is this smaller-scale biosphere affected by similar changes? In particular, has the progress that has lowered infant mortality and prolonged lifespan also caused unanticipated alterations in our microecology and, consequently, our health?

In this Essay, we focus on our intimate interface with microorganisms: residents and transients, and symbionts and pathogens. We will explore whether and how our microecology is changing, in analogy to our altered macroecology.

What are our microbiota?

We live in a microbial world¹. When animals emerged, microorganisms had long (>3 billion years) been part of a vast community of life that included complex endosymbiotic

relationships, and it has been calculated that animals have carried resident microorganisms since at least the emergence of sponges^{2,3}. Humans have become host to a myriad of microorganisms that assemble into complex, largely beneficial communities that outnumber human cells by tenfold. The dominant forms of human-microorganism interactions are those in which microorganisms benefit the host without causing harm (commensal relationships) and those in which both host and microorganism benefit

(symbiotic or mutualistic relationships). Co-evolution, co-adaptation and codependency are all features of our relationships with our indigenous microbiota^{4,5}.

The vertebrate microbiota can be characterized as: ancient, with deep ancestries; conserved in their host species; often present for defined life cycle events or persisting for life; and host niche specific. These properties imply that the microbiota have been selected, but it is becoming increasingly clear that although they are bounded by these rules they are also highly individual.

The human microbiome is the subject of intensive studies, including the international Human Microbiome Project⁶ (BOX 1). Although possibly germ-free (gnotobiotic) before birth, humans develop a resident microbiota shortly after birth. In the neonatal period, the community assembly process is dynamic and is influenced by early environmental (in particular, maternal) exposure and stochastic effects⁷. The composition of the indigenous microbiota evolves in a generally orderly manner in response to diet and other environmental factors and is also influenced by diverse human genetic backgrounds. The bacterial diversity in the human body is striking in its richness of distinct species and strains, but it is noteworthy that a limited number of phyla are commonly found in indigenous microbial communities. Only 4 of the more than 50 bacterial phyla that have been identified in the environment

Box 1 | The Human Microbiome Project

In recent years, there has been a growing appreciation of the extent of the commensal microbial populations in humans. Collectively, these populations have been termed 'the human microbiome', originally by Joshua Lederberg¹⁰⁷, and also the 'human microbiota'. Owing to advances in DNA sequencing technologies and improvements in bioinformatics, it has become possible to characterize the great diversity in the human microbiota. In 2007, the US National Institutes of Health (NIH) launched the [Human Microbiome Project](#) (see Further Information) as one of its major roadmap initiatives, earmarking ~US\$140 million for its completion¹⁰⁸. This major scientific endeavour has the following aims:

- to determine whether individuals share a core human microbiome;
- to understand whether changes in the human microbiome can be correlated with changes in human health;
- to develop the technological tools to support these goals;
- to address the ethical, legal and social implications of human microbiome research.

One value of the Human Microbiome Project is that it can help us to ascertain how much the microbiota have been changing. Work is now ongoing, and the European Union and other countries are committed to similar projects, all working under the umbrella of the [International Human Microbiome Consortium](#) (see Further Information).

Box 2 | What is a pathogen?

- Microbial pathogens can range from bacteria to metazoa to viruses or prions. All pathogens are excellent cell biologists, taking advantage of chinks in the armour of their hosts to propagate. There is no intrinsic difference between a pathogen and a symbiont, only context. However, there are fingerprints that are sufficiently characteristic of pathogens or symbionts to be used to help distinguish between them:
- Pathogens are generally clonal^{109–112}.
- Pathogens induce host responses. They usually cannot do their deeds without a trace.
- Infection with a pathogen can sometimes cause disease, which is a function of the interaction between the pathogen and the specific host, as determined by a matrix of host, microbial and environment circumstances.
- The gene pool of the host includes the genes of its resident microorganisms, which may be why many members of the microbiota are selected for by the host: to prevent colonization by pathogens instead.

(Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria) dominate human mucosal and cutaneous habitats, which suggests that strong selective forces have limited diversity over at least hundreds of thousands of years of co-evolution^{8–16}. Despite this stereotypical assembly process, each individual in a single mammalian species, including *Homo sapiens*, has a virtually unique microbiota^{10,11}. The composition of the microbiota and the phenotypes that are expressed affect, as well as reflect, the overall biological diversity of humans.

Most research has focused on defining the core elements of the microbiota that are shared by all, or at least most, humans⁸. To date, the rules that govern life on and in humans are poorly understood, and we are still in the earliest stages of discerning the functions of the microbiota in health and disease⁴. The human microbiota facilitate the extraction of energy from food, provide accessory growth factors, promote post-natal terminal differentiation of mucosal structure and function, stimulate both the innate and adaptive immune systems, and provide ‘colonization resistance’ against pathogen invasion^{17–20}. However, studies of gnotobiotic animals have shown that microorganisms

(or at least bacteria) are not necessary for life or for the completion of an animal life cycle^{19–21}. Paradoxically, therefore, there seems to be no absolute requirement for a functional resident microbiota, but nor does gnotobiosis occur in nature.

Although the relationship between animals and their resident microbiota is thought to be largely beneficial, it can also become detrimental^{19,22}. In humans, commensal α -haemolytic streptococci colonize the mouth and intestinal tract and, in part, protect us against incursions by pathogenic streptococci^{17,23}. However, these commensal organisms are often transiently present in the bloodstream after we chew food, brush our teeth or defecate^{24,25}. If there is damaged tissue present, such as an ageing heart valve, these long-term residents can cause a fatal infection. Thus, our generally beneficial microbiota have the capacity to cause our demise. They thin the herd, particularly if the normal defences that hold microorganisms in check are compromised in some manner.

It is clear that we share a complex biological relationship with our microscopic companions. Are these organisms symbionts or parasites? (BOX 2). The ecologist Theodor Rosebury created the term ‘amphibiosis’ to

describe relationships that can be symbiotic or parasitic, depending on the biological context¹⁸. This concept is the guiding principle for the remainder of this Essay.

When influenza sweeps across the globe, infecting millions and causing disease and sometimes death, we agree that the virus is a pathogen, as is *Yersinia pestis*, the cause of bubonic plague. However, the distinction between a commensal resident microorganism and a pathogen can be blurred at times, in part because some commensals cause disease, albeit often in immunocompromised hosts, whereas some of the most feared pathogens can persist in humans for a lifetime without causing any symptoms²⁶. For example, several members of the nasopharyngeal microbiota, including *Streptococcus pneumoniae* and *Neisseria meningitidis*, regularly cause disease. Such ‘commensal pathogens’ colonize a substantial proportion of the human population, most of whom are asymptomatic carriers²⁷. Are these organisms pathogens, or should they be considered to be members of the indigenous microbiota that have evolved to live in perilous locations²⁸, such as in respiratory tract-associated lymphatic tissue? From this viewpoint, the specific indigenous microbiota in such niches would regularly come into contact with elements of an immune system that hold them at bay most of the time²² but that occasionally fail to do so, resulting in disease. Immunization against these microorganisms not only protects against disease but also prevents host colonization, in an antigen-specific manner (reviewed in REF. 29). Serotype replacement is an acknowledged deleterious effect of such immunization programmes, but may there be other deleterious effects that we have yet to uncover? It is crucial to improve our understanding of the relationships between humans and our microbiota, so that we can assess the risks, in addition to the potential benefits, of modifying the

Glossary

Commensal relationship

An intimate, although generally benign, relationship between a resident microorganism and its host. Probably the product of a long evolutionary interplay between the microorganism and the host. The relationship need not be symbiotic.

Gene transfer

The transfer of individual genes or their components, islands of genes, entire organisms, or communities of organisms from parent to offspring (vertical transfer) or between individuals not in a direct lineage (horizontal transfer).

Microbiome

The collective genomes of the microorganisms that reside in an environmental niche.

Nash equilibrium

In game theory, a solution concept in which players in a game are aware of the strategies of the other players but do not deviate from their own, because they do not have anything to gain; it will be disadvantageous to deviate (to ‘cheat’).

Pan-genome

The set of all of the genes that are found in members of a single species.

Parasite

An organism that diminishes the reproductive fitness of another organism or benefits from another organism without reciprocity.

Pathogen

In medicine, any organism that causes disease. In biological terms, a pathogen is a microorganism that has the inherent capacity to cross anatomical barriers and resist host defences that ordinarily restrict most other microorganisms.

Symbiont

An organism that has a biological relationship with one or more organisms that leads to mutual benefit.

Syntrophic interactions

Interactions in which organisms do more together than alone.

Virulence

A quantitative estimate of the ability of one organism to harm another.

Table 1 | Changes in human ecology that might affect microbiota composition

Change	Consequence
Clean water	Reduced faecal transmission
Increase in Caesarean sections	Reduced vaginal transmission
Increased use of pre-term antibiotics	Reduced vaginal transmission
Reduced breastfeeding	Reduced cutaneous transmission and a changed immunological environment
Smaller family size	Reduced early life transmission
Widespread antibiotic use	Selection for a changing composition
Increased bathing, showering and use of antibacterial soaps	Selection for a changing composition
Increased use of mercury-amalgam dental fillings	Selection for a changing composition

composition of the microbiota deliberately or inadvertently. The use of molecular Koch's postulates³⁰ may assist in this risk assessment.

How are the microbiota stabilized?

Is there any underlying biological regulation that stabilizes the diverse microbial populations that we carry, even in the face of host immunity, competing organisms and the daily continuous flux of newly introduced microorganisms? Cooperation between competing life forms is a challenging concept and, although much of the literature on microorganisms focuses on the evolution of virulence^{31,32}, there is an increasing focus on the rules that govern the evolution of cooperation^{33,34}.

In an earlier report, a hypothesis was presented that Nash equilibria enable pathogens such as *Mycobacterium tuberculosis* and *Salmonella enterica* subsp. *enterica* serovar *Typhi* to persist³⁵. Such a view has also been applied to *Helicobacter pylori*, the dominant member of the gastric microbiota¹⁶. In game theory, a Nash equilibrium represents a particular circumstance in which any player who deviates from the rules of the game is in an inferior position compared to those who have played by the rules^{36,37}. Such conditions can exist in a limited number of situations in nature, and biological co-evolution is a prime example.

For any equilibrium to function, there must be boundary conditions or limits and strong penalties against transgressors that, in net, remove any advantage from 'cheating'³⁸. This is characteristic of a Nash equilibrium, but the complex evolved biology of metazoans such as humans requires solutions that operate on multiple scales. A series of nested dynamic equilibria was envisioned, in which each stratum contributes to creating the boundary conditions for the others³⁵. This is made possible, in part, by the fractal nature of biological communities, in which ecosystems nest within one another, a structure that provides both stability and resilience in a world

of opportunists, invaders and cheaters. In steady state, the equilibria form a continuum, analogous to a Mobius Strip, with no beginning or end. This population structure permits the co-evolution of competing organisms (for example, host and microorganism) that would otherwise lead to an 'arms race'³⁹ and the destruction of one organism or the other. However, even in such a derived evolutionarily stable strategy⁴⁰, all is not fixed. The equilibrium has sufficient elasticity for invasions (by pathogens or 'cheaters') to be contained under normal conditions of flux. However, even with such capacitance, there must be boundaries beyond which the systems lose equilibria and descend towards the extinction of one partner or the other.

In the human biosphere, the boundaries for flux are unknown. Even a catastrophe like the AIDS epidemic has so far been contained within the overall population, in part because of the inefficiencies of HIV transmission⁴¹. However, scenarios involving more transmissible agents in a 'smaller world' in which population connectivity selects for virulence and the ecosystem has been perturbed are worrisome⁴². This is directly germane to considerations of the stability of the microbiota. On the basis of humanity's long association with our indigenous microorganisms and their conservation^{4,10}, the equilibria that maintain the composition of our microbiota should be considered to be major pillars of our biological stability. Seen from this view, pathogens that arise are 'cheaters' that can break the equilibria. In principle, 'cheaters' can cause the extinction of the microorganism, the host or even both, although in reality variants usually emerge and equilibria ultimately rule. For example, if the host-mediated penalties against 'cheating' (such as host immunity to the microorganism or the microorganism's loss of a niche in the host) are sufficiently high, then less virulent variants that can evade biological surveillance can become successful.

The 'disappearing microbiota' hypothesis

In general, it seems that the human microbiota and their hosts evolved their equilibria together, in an orderly way¹⁰, as diet, geography and occasional ecological disturbances had their effects on distinct, albeit diverse, human genetic backgrounds⁴³. Our complex protective commensal microbiota facilitate nutrient and vitamin acquisition, promote tissue development and integrity, and stimulate multiple aspects of immunity^{18–22}. However, in the face of modern global ecological changes, has there been stability for the descendants of the microbiota that colonized humans during the ~2 million years that they lived in small groups as hunter-gatherers?

As human health and longevity have improved in developed countries, new diseases have arisen without obvious explanation. The 'disappearing microbiota' hypothesis^{44,45} has been advanced to explain the rise and fall of several common diseases in developed countries. Beginning in the nineteenth century and accelerating in the twentieth century, there have been dramatic changes in human ecology (TABLE 1), including cleaner water, smaller families, an increase in the number of Caesarian sections, increased use of pre-term antibiotics, lower rates of breastfeeding and more than 60 years of widespread antibiotic use, particularly in young children. How have these changes affected the transmission and maintenance of the indigenous microbiota⁴⁴?

We postulate that the important factor in modern allergic and metabolic diseases might not be our decreased sampling of the microorganisms in food, air, water or soil, as has been postulated by the 'hygiene hypothesis' (REF. 46), but instead could reflect the loss of our ancestral microorganisms. As the representation of particular species diminishes in one generation, the potential for vertical transmission to the next generation⁴⁷ can decrease in a stepwise manner (FIG. 1). Diminished horizontal transmission resulting from changes in human ecology (TABLE 1) makes it more difficult to overcome losses in vertical transmission, and this then manifests as a birth cohort phenomenon. We believe that alterations in human macroecology have progressively affected the composition of our indigenous microbiota, which in turn has affected human physiology and, ultimately, disease risk. The increases that have occurred in recent years in the prevalence of conditions such as obesity and asthma, as well as oesophageal disorders that are a consequence of reflux, have been so rapid that an environmental cause must be present^{48–50}. Is

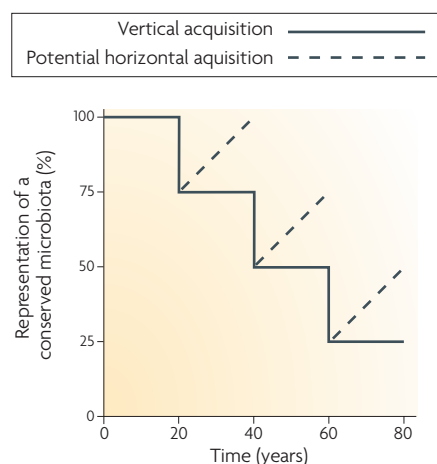


Figure 1 | The effect of maternal status on the resident microbiota of the next generation. We propose that, since the earliest days of the evolution of mammals, there has been major maternal transmission of microbiota to their offspring (vertical transmission). However, loss of the conserved microbiota in one generation leads to its loss in the next. For humans, until recently, horizontal microbial transmission also occurred and could compensate for the loss of vertical transmission. Members of the microbiota were horizontally transmitted through faecally contaminated drinking and bathing water, and high physical contact as a result of social crowding and large families; in many modern societies, these routes have diminished. The progressive loss of vertically transmitted microorganisms without horizontal replacement represents a cumulative birth cohort phenomenon.

one of these causes the loss of one or more constituents of the indigenous microbiota?

H. pylori, an ancient member of the human microbiota⁵¹, generally dominates the gastric niche (BOX 3). Accordingly, its presence can be used as a means to assess the status of our microbiota. Surprisingly, *H. pylori* has been progressively disappearing^{52–54} from individuals in developed countries (FIG. 2) during the twentieth century, with secondary alterations in gastric secretory, hormonal and immune physiology^{55,56}. These alterations have been associated with a progressively declining incidence of important illnesses with long latent periods, such as gastric cancer. We now have multiple tools to detect *H. pylori*⁵⁷. But if a lesser known ancestral indigenous microorganism disappeared in the colon, mouth, skin or vagina, could we identify that change and, crucially, could such a change (whether it was a loss or a replacement and subsequent ‘overgrowth’ by a different indigenous component) contribute to some of the diseases that are becoming more prevalent? The Human Microbiome Project⁶ should begin to address such questions (BOX 1).

The consequences of loss

Modelling of the specific host–microorganism relationships suggests that there are several potential physiological relationships that can occur (FIG. 3). Several specific examples illustrate the concept that the loss of an indigenous microorganism will have consequences for the host.

Loss of *H. pylori*. *H. pylori* colonization and the response that it induces in the host affects the regulation of gastric hormones, including gastrin and somatostatin⁵⁸. As such, *H. pylori* status affects gastric pH and its regulation⁵⁵. Over the decades of *H. pylori* colonization, the mass of gastric acid-secreting glands in the host progressively decreases, owing to the long-term effects of inflammation^{58,59}. Although the rates of glandular decrease reflect both host-specific and population-specific factors, overall there is a progressive decrease in acid production⁶⁰ that is greater than the decrease observed in *H. pylori*-negative hosts^{60,61}. The increasing gastric atrophy and hypochlorhydria that are associated with the presence of *H. pylori*^{60–62} contribute to the risk of gastric cancer^{63,64}, but the sustained acidity in *H. pylori*-negative hosts increases the risk of gastroesophageal reflux disease (GERD) and its consequences, including oesophageal and gastric cardia adenocarcinomas^{64,65}.

As *H. pylori* is disappearing from human populations, reflecting both diminishing transmission and increasing antibiotic treatment (TABLE 1), both ‘idiopathic’ peptic ulcer disease and gastric cancer rates are diminishing^{66,67}, which is clearly salutary. However, oesophageal reflux, Barrett’s oesophagus and adenocarcinoma are increasing, which is clearly deleterious^{50,66}. Are these reciprocal phenomena? The *cag* pathogenicity island⁵⁵ (or perhaps a better term would be the *cag* ecological fitness island⁶⁸) status of the *H. pylori* population has a strong influence on the development of Barrett’s oesophagus and adenocarcinoma, as predicted by the strong interaction between *cag*-positive strains and host cells^{60,63,69,70}. The stomach also produces the hormones ghrelin and leptin, both of which have multiple roles in energy homeostasis⁵⁵. Patients in whom antibiotic treatment eliminates *H. pylori* have increased circulating ghrelin levels⁷¹. Investigations are ongoing, but it is already clear that in recent generations of children growing up in developed countries there has been little gastric *H. pylori*-mediated⁵⁶ regulation of these adipokines at the developmental stage, when long-term adiposity is being programmed.

It is possible that the disappearance of *H. pylori* might be contributing to the current epidemics of early-life obesity, type 2 diabetes and related metabolic syndromes.

Gastric T cell and B cell populations also differ in *H. pylori*-positive and *H. pylori*-negative hosts^{72–74}. This might be expected, as the *H. pylori*-negative stomach has a diminished presence of the cellular elements of the immune system and reduced cytokine traffic, whereas the *H. pylori*-positive stomach has a rich population of immune cells (FIG. 3). The enhanced T cell populations in *H. pylori*-positive hosts include greater proportions of particular T cell subsets, including cells expressing forkhead box protein P3 (FOXP3), which regulate immune functions^{73,74}. The stomachs of *H. pylori*-negative hosts have much lower numbers of these cells, which have systemic, as well as local, activities. Several epidemiological studies (reviewed in REF. 75) show that *H. pylori*-positive individuals (especially individuals carrying *cag*-positive strains) have lower risks of childhood asthma, allergic rhinitis and skin allergies than those without *H. pylori*^{56,76,77}. The rise in childhood asthma and related disorders has occurred while *H. pylori* has been disappearing; the loss of gastric T cell populations and their systemic effects could provide a mechanism for these allergic diseases^{78,79}.

Subtherapeutic antibiotic treatment. In the early 1950s, not long after the discovery of antibiotics, scientists found that feeding low doses of antibiotics (termed subtherapeutic antibiotic treatment (STAT)) to farm animals increased their rate of growth and ability to convert food into body mass (otherwise known as their ‘feed efficiency’; reviewed in REF. 80). The younger the animals are when STAT is started, the stronger the effect⁸⁰. The fact that several antibiotics can produce this effect in poultry, cattle and swine^{80–82} has led to the widespread usage of antibiotics as animal feed supplements in the United States and other developed countries (this practice has now been banned by the European Union because of the spread of antibiotic-resistant bacteria).

Why does giving STAT change energy homeostasis in this broad group of vertebrates? As growth promotion can be induced by many different antibacterial (but not antifungal) agents, this activity is not a side effect but a consequence of the antibacterial activities of these agents, which presumably affect the microbiota of the exposed animals⁸². However, the affected microbial populations and metabolic pathways are

Box 3 | The gastric microbiome

The human body is colonized by a highly complex microbiota, but the stomach is an exceptional niche. When *Helicobacter pylori* can be detected by conventional means, including culture or biochemical assays, tissue histology and host serological responses, it is the numerically dominant organism, representing >50% of all of the bacterial cells in the niche^{16,103}. This phenomenon is unlike other human niches that house a large range of bacterial species but no single predominant organism (in the vagina, a single *Lactobacillus* species often predominates, but its identity differs in different women¹¹³). The dominance of the gastric niche by *H. pylori* is consistent with its adaptations that enable gastric persistence⁵⁵. In persons in whom *H. pylori* cannot be detected by conventional means, the organism may still be detected by PCR-based techniques, usually as more minor populations¹⁶. These observations suggest that there may be a 'stealth' phenomenon in which *H. pylori* exists below the radar of histological, biochemical and immunological fingerprints, but its characteristics are not yet understood.

In addition to *H. pylori*, numerous other bacterial species may be present, but whether they are resident in the stomach or transient from the upper gastrointestinal tract has not been determined¹⁶. The phyla that are observed (chiefly Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes) seem to be similar in *H. pylori*-positive and *H. pylori*-negative hosts¹⁶.

unknown. Given that this manipulation of the microbiota has such effects on early life energy homeostasis and body mass development in farm animals, what might the effects be of the widespread exposure of children to antibiotics early in life? Instead of the continuous, low-dose antibiotics that are administered on the farm, we are giving our children short, high-dose pulses. How is this affecting their microbiota, energy homeostasis and 'feed efficiency'? Even single courses of widely used antibiotics can change the stable structure of microbial communities, with the presence of selected organisms continuing for years after the antibiotic exposure has ceased^{83,84}. We speculate that the widespread treatment of young children with antibiotics has caused alterations in the compositions of their intestinal microbiota, and the luminal signals to the host (FIG. 3), that are directly contributing to the epidemic of obesity in developed countries.

The pneumococcus and Staphylococcus aureus. *S. pneumoniae* (known as the pneumococcus) is an important human pathogen, causing pneumococcal pneumonia, infections of the upper respiratory tract and its appendages, and occasionally lethal diseases such as meningitis and endocarditis⁸⁵. Consequently, the development of a pneumococcal vaccine has been a priority for >100 years. However, pneumococci are carried by healthy persons in the nasopharynx, often for months, and are part of the consortia of microorganisms inhabiting this niche. Pneumococci are naturally transformable and vary extensively, with a major source of variation being the presence and type of polysaccharide capsule⁸⁶. Most human disease is caused by encapsulated pneumococci, and particular serotypes predominate^{87,88}. Consequently, preventative strategies have focused on the most virulent

capsular subtypes, and polyvalent vaccines have been developed⁸⁹. These vaccines are effective and have reduced the incidence of serious pneumococcal infections in high-risk populations⁹⁰. Immunization not only protects against disease but also prevents colonization by those pneumococci with the capsule types that are present in the vaccine⁸⁹. One of the earliest concerns of researchers was that increasing the immunity to certain capsular types would lead to serotype replacement^{88–90}, which might result in new pneumococcal infections, but presumably at a lower rate than rates of infection by the more virulent pneumococci that are targeted by the vaccine. Although serotype replacement and the subsequent pneumococcal disease would lower the net 'effectiveness' of vaccination, there would still be residual utility.

All of these predicted consequences have come to pass^{89–92}. However, in addition to replacement with non-vaccine serotypes of *S. pneumoniae*, replacement with an unantigenated organism, *Staphylococcus aureus*, has occurred^{92–95}. A growing body of evidence indicates that the encapsulated pneumococci and *S. aureus* are ecological competitors^{93,94}, and that the loss of the former is leading to the expansion of the latter⁹⁵. This can take place through direct competition or through microbial manipulation of host immunity^{96–98}. This is occurring just as we are witnessing an unprecedented epidemic of methicillin-resistant *S. aureus* (MRSA) infections in the community among persons who have not been recently hospitalized or received antibiotics. In the absence of obvious exposure or selection pressure, these community-acquired MRSA (CA-MRSA) strains are being widely transmitted from person to person and with occasional, but often serious, clinical phenotypes^{99,100}. The ecology of *S. aureus* has clearly changed³⁴. Whether or not this reflects the

consequences of pneumococcal vaccination, antibiotic pressure selecting for particularly virulent clones, ecosystem degradation due to effects on the nasopharyngeal and cutaneous microbiota of widespread antibiotic use, or a combination of these factors remains to be determined.

Recolonizing a vacated niche

If the indigenous microbiota are disappearing, have there been replacements? Are there model systems in which to explore this question? As discussed above, *H. pylori*, the ancient and dominant microbial inhabitant of the human stomach, is disappearing with remarkable speed. In two or three generations, human societies have moved from near-ubiquitous (>80%) *H. pylori* prevalence rates to rates in single digits among contemporary native-born children in the United States (FIG. 2) and western Europe^{56,101,102}. This is an unprecedented change in human microecology.

What about the gastric niche itself: with *H. pylori* disappearing, will there be new tenants and, if so, how will they interact with the host? The stomach is bounded proximally by the oropharynx and oesophagus and distally by the intestine, all of which possess resident microbiota^{8–11}. The gastrointestinal tract is also a portal to the outside world through ingested food and drink. Although *H. pylori* is the dominant gastric microorganism, there is DNA evidence for the presence of other transient or residential microorganisms^{16,103} and indications that a more diverse microbial population is present in the *H. pylori*-vacated stomach¹⁰³.

For occupancy of a vacated niche to occur, the transmission potential to other hosts is crucial¹⁰⁴. Several possible scenarios should be considered. The first possibility is that each individual will become colonized by their own host-specific microorganisms that can change over time but that are essentially unsuccessful at transmitting to new hosts. This would lead to billions of ongoing long-term experiments (one per human birth) in microbial transmission fitness. The second possibility is that the overall level of resident microorganisms will remain low, as other microorganisms cannot use the resources of the *H. pylori*-free stomach as efficiently as *H. pylori*. The third possibility is that, without *H. pylori* competition and inhibition, other microorganisms will better use the niche's resources. Such organisms could be a microbial consortium involving a few or many species, or a single species with a varied and ultimately robust pan-genome. Alternatively, they may be exogenous

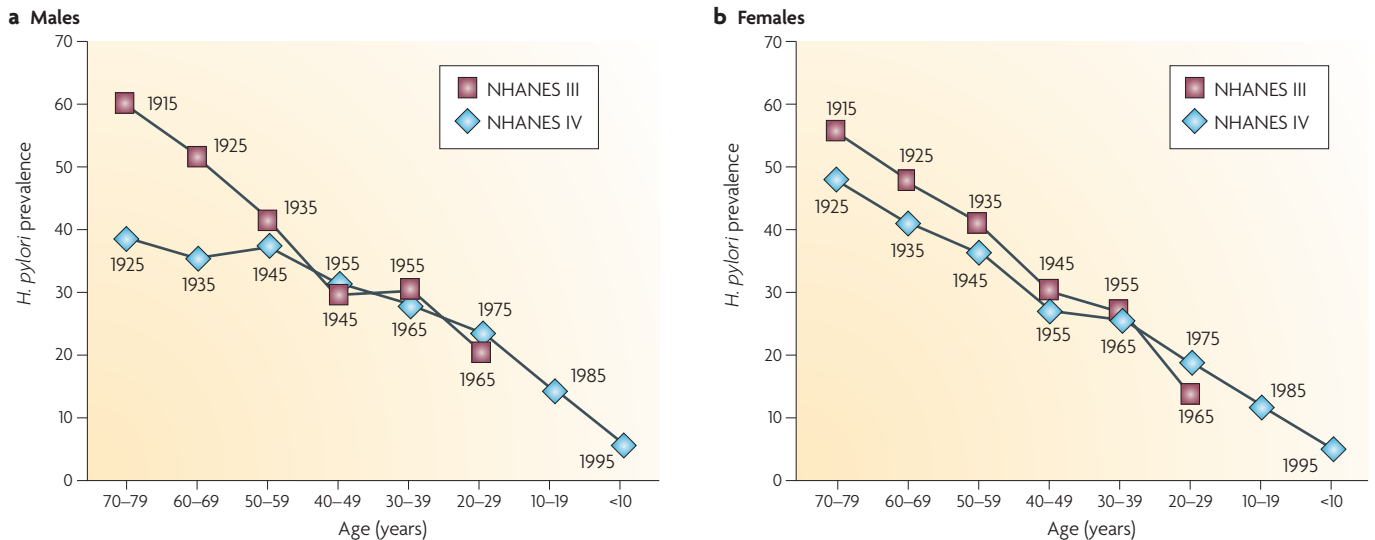


Figure 2 | *Helicobacter pylori* prevalence in the United States by age and year of birth. *Helicobacter pylori* prevalence in males (a) and females (b) was determined in two large national studies (the National Health and Nutrition Survey (NHANES) III and NHANES IV), which were conducted in 1988–1991 and 1999–2000, respectively. These data, involving 15,000 subjects, are consistent with a now well-recognized birth cohort

effect^{52–54} in which *H. pylori* acquisition, and thus prevalence, has been declining in industrialized countries for >100 years. The numbers next to each point represent the midpoint year of birth for each group of survey subjects (with each group covering one decade of births). Image is modified, with permission, from REF. 56 © (2008) University of Chicago Press.

organisms that are acquired during ongoing environmental exposure. The transmission potential will determine whether the replacement organisms will be host-specific populations or emergent, highly selected organisms that are common between hosts. A potential consequence of the third scenario is the emergence of a highly transmissible microorganism that is well-adapted for the now sparsely populated niche. Because in this situation selection is based on the ability to transmit to new hosts, a range of virulence is possible, with the potential for positive selection for virulence¹⁰⁴. This could be dangerous, because the next main inhabitant of the gastric niche might not be as benign as *H. pylori*, which has co-evolved with humans at a moderate level of virulence. A newly acquired organism that evolved to colonize and efficiently use the resources would probably be more virulent¹⁰⁵ and could selectively sweep through the population.

These possibilities are not gastric specific but represent the general consequences that might accompany the extinction of species from the microbiota of any niche. The more dominant the species that are lost, the larger the void and the greater the potential risk of replacement with a highly virulent organism.

Future therapeutics?

Will there be cases in which human health is improved by the replacement of some of the lost microbiota? Certainly, a trip

through the dairy section of any supermarket in the Western world would lead one to believe so. Nonetheless, translating this marketing concept into scientifically based beneficial activities is limited by several outstanding questions. These include: which particular microorganisms should be replaced; what will their sources be; and what will the timing of their replacement be? We should begin to think about these options and their expected outcomes. Replacement will necessarily lead to the development of new classes of probiotics; we speculate that one day we will see microbial additives that are specific for particular hosts, for specific durations of exposure and eradication. This is one vision for the future, but it will require a much deeper understanding of the metabolic, hormonal and immunological interactions with our microbiota (FIG. 3).

One emerging concept in microbial pathogenesis is the notion of the 'community as a pathogen', in which a conserved broad swathe of the microbial community, rather than any one specific member, contributes to disease. This concept might be relevant to a range of inflammatory processes of the skin and mucosa, including inflammatory bowel disease and chronic periodontitis. It suggests that studies of pathogenesis should consider the general properties of microbial communities, such as resilience, or conserved functional interactions, such as syntrophic interactions and the importance of gene transfer, rather

than the role of single microorganisms, especially for the development of new approaches for maintaining or restoring health. Thus, under some circumstances, restoration of communities might be more appropriate than replacing single microorganisms.

Conclusions

It is predictable that social and medical progress that affects the composition of the microbiota will also have consequences for our physiology and health. However, the specific outcomes will only be learned empirically, as the human ecosystem is too complex for anything more than the most superficial predictions. Improving our prognostication is an important technical challenge.

Is the selective disappearance of the microbiota contributing to oesophageal diseases, obesity and its consequences, asthma and related disorders, and the epidemic spread of high-grade pathogens? Further investigation will clarify these points, but the theoretical basis exists: ecological changes involving our ancient microbiota have the power to affect physiology and, ultimately, health. Individuals who are 'normal' in modern societies might be representative of the population at large but not of our historical heritage¹⁰⁶. Studies of persons who are indigenous to those developing countries that have had little impact from modern health practices (including exposure to antibiotics) could be ideal to define our historic 'norms'.

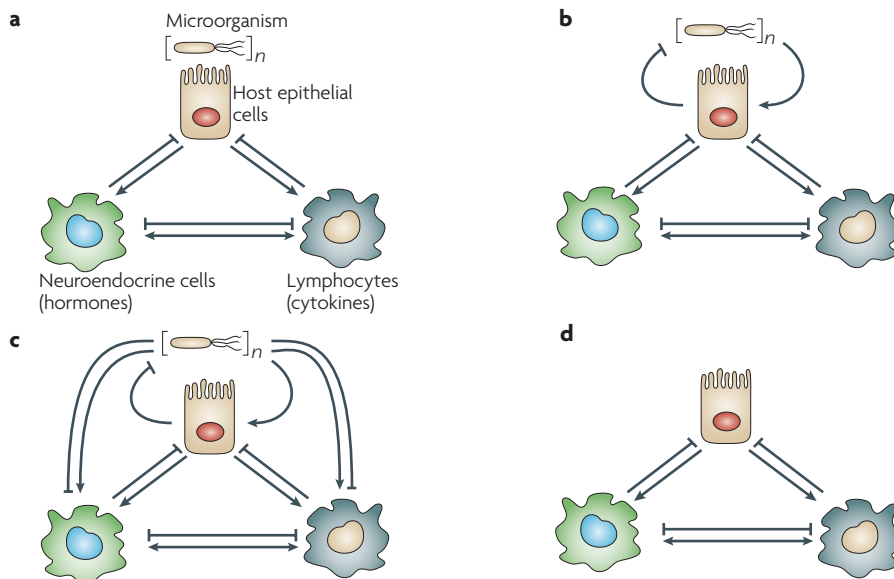


Figure 3 | Interactions between host and microbiota. **a** | For luminal microbiota that do not interact with host cells, the host-specific regulatory networks are essentially unaffected by transient microbiota or by luminal microbiota that are distant from the epithelium. This could represent a large class of organisms in the microbiota. **b** | For microbiota that interact with epithelial cells, there is an equilibrium relationship involving signalling between the microbial populations and the host³⁵. **c** | For co-evolved microbiota that interact with multiple cell types, the cross-signalling in the most fully co-evolved states involves multiple host cell types, including epithelial, immunological and neuroendocrine cells. The interaction of *Helicobacter pylori* with the gastric mucosa is representative of this model. The introduction of drugs, such as the commonly used proton pump inhibitors that reduce gastric acidity, affects equilibrium values and selects for a differing microbiota^{59,114}. **d** | In the absence of a microbiota, the indigenous host regulatory mechanisms predominate and reach different homeostasis values than are reached when interacting microbiota are present. In the situation of a disappearing microbiome, **a** and **d** predominate.

Moving forward, we must learn to better distinguish between pathogens and amphibions and to better assess in whom to eliminate, leave alone or restore the microorganism (or metabolic pathway) in question. Or is it even wise to contemplate any action until we understand more about our most intimate residents? Public health deals with trends in populations, but medical care inevitably involves individuals. A greater understanding of the characteristics of a host's genome and microbiota, and their interactions, will lead to individualized approaches to the prevention and treatment of specific diseases. We are at a scientific frontier.

Martin J. Blaser is at the Department of Medicine and the Department of Microbiology, New York University Langone Medical Center, New York, New York 10017, USA.

Stanley Falkow is at the Department of Microbiology and Immunology and the Department of Medicine, Stanford University School of Medicine, Stanford, California 94307, USA.

Correspondence to M.J.B.
e-mail: Martin.Blaser@med.nyu.edu

doi:10.1038/nrmicro2245

Published online 9 November 2009

- Whitman, W. B., Coleman, D. C. & Wiebe, W. J. Prokaryotes: the unseen majority. *Proc. Natl Acad. Sci. USA* **95**, 6578–6583 (1998).
- Iyer, L. M., Aravind, L., Coon, S. L., Klein, D. C. & Koonin, E. V. Evolution of cell-cell signaling in animals: did late horizontal gene transfer from bacteria have a role? *Trends Genet.* **20**, 292–299 (2004).
- Hoffmeister, M. & Martin, W. Interspecific evolution: microbial symbiosis, endosymbiosis and gene transfer. *Environ. Microbiol.* **5**, 641–649 (2003).
- Dethlefsen, L., McFall-Ngai, M. & Relman, D. A. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* **449**, 811–818 (2007).
- Hickman, C. S. in *Influence of Cooperative Bacteria on Animal Host Biology* (eds McFall-Ngai, M. J., Henderson, B. & Ruby, E. G.) 3–34 (Cambridge Univ. Press, Cambridge, UK, 2005).
- Turnbaugh, P. J. *et al.* The human microbiome project. *Nature* **449**, 804–810 (2007).
- Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A. & Brown, P. O. Development of the human infant intestinal microbiota. *PLoS Biol.* **5**, e177 (2007).
- Eckburg, P. B. *et al.* Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638 (2005).
- Gill, S. R. *et al.* Metagenomic analysis of the human distal gut microbiome. *Science* **312**, 1355–1359 (2006).
- Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R. & Gordon, J. I. Worlds within worlds: evolution of the vertebrate gut microbiota. *Nature Rev. Microbiol.* **6**, 776–788 (2008).
- Pei, Z. *et al.* Bacterial biota in the human distal esophagus. *Proc. Natl Acad. Sci. USA* **101**, 4250–4255 (2004).
- Lepp, P. W. *et al.* Methanogenic Archaea and human periodontal disease. *Proc. Natl Acad. Sci. USA* **101**, 6176–6181 (2004).
- Aas, J. A., Paster, B. J., Stokes, L. N., Olsen, I. & Dewhirst, F. E. Defining the normal bacterial flora of the oral cavity. *J. Clin. Microbiol.* **43**, 5721–5732 (2005).
- Gao, Z., Tseng, C. H., Pei, Z. & Blaser, M. J. Molecular analysis of human forearm superficial skin bacterial biota. *Proc. Natl Acad. Sci. USA* **104**, 2927–2932 (2007).
- Fierer, N., Hamady, M., Lauber, C. L. & Knight, R. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proc. Natl Acad. Sci. USA* **105**, 17994–17999 (2008).
- Bik, E. M. *et al.* Molecular analysis of the bacterial microbiota in the human stomach. *Proc. Natl Acad. Sci. USA* **103**, 732–737 (2006).
- Crowe, C. C., Sanders, W. E. Jr & Longley, S. Bacterial interference. II. Role of the normal throat flora in prevention of colonization by group A *Streptococcus*. *J. Infect. Dis.* **128**, 527–532 (1973).
- Rosebury, T. *Microorganisms Indigenous to Man* (McGraw Hill, New York, 1962).
- Mackowiak, P. A. The normal microbial flora. *N. Engl. J. Med.* **307**, 83–93 (1982).
- Smith, K., McCoy, K. D. & Macpherson, A. J. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin. Immunol.* **19**, 59–69 (2007).
- Gordon, H. A. & Pesti, L. The gnotobiotic animal as a tool in the study of host microbial relationships. *Bacteriol. Rev.* **35**, 390–429 (1971).
- Pamer, E. G. Immune responses to commensal and environmental microbes. *Nature Immunol.* **8**, 1173–1178 (2007).
- Roos, K., Holm, S. E., Grahn-Hakansson, E. & Lagergren, L. Recolonization with selected α -streptococci for prophylaxis of recurrent streptococcal pharyngotonsillitis — a randomized placebo-controlled multicentre study. *Scand. J. Infect. Dis.* **28**, 459–462 (1996).
- Durack, D. T. Prevention of infective endocarditis. *N. Engl. J. Med.* **332**, 38–44 (1995).
- Roberts, G. J. Dentists are innocent! “Everyday” bacteremia is the real culprit: a review and assessment of the evidence that dental surgical procedures are a principal cause of bacterial endocarditis in children. *Pediatr. Cardiol.* **20**, 317–325 (1999).
- Falkow, S. Is persistent bacterial infection good for your health? *Cell* **124**, 699–702 (2006).
- Murphy, T. V., Pastor, P., Medley, F., Osterholm, M. T. & Granoff, D. M. Decreased *Haemophilus* colonization in children vaccinated with *Haemophilus influenzae* type b conjugate vaccine. *J. Pediatr.* **122**, 517–523 (1993).
- Mazmanian, S. K., Round, J. L. & Kasper, D. L. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* **453**, 620–625 (2008).
- Segal, S. & Pollard, A. J. Vaccines against bacterial meningitis. *Br. Med. Bull.* **72**, 65–81 (2004).
- Falkow, S. Molecular Koch's postulates applied to bacterial pathogenicity — a personal recollection 15 years later. *Nature Rev. Microbiol.* **2**, 67–72 (2004).
- Maynard Smith, J. & Szathmari, E. *The Major Transitions in Evolution* (Oxford Univ. Press, 1995).
- Messenger, S. L., Molineux, I. J. & Bull, J. J. Virulence evolution in a virus obeys a trade-off. *Proc. R. Soc. Lond. B* **266**, 1480–1482 (1997).
- Michod, R. E. Evolution of the individual. *Am. Nat.* **150**, S5–S21 (1997).
- Sachs, J. L. & Bull, J. J. Experimental evolution of conflict mediation between genomes. *Proc. Natl Acad. Sci. USA* **102**, 390–395 (2005).
- Blaser, M. J. & Kirschner, D. The equilibria that allow bacterial persistence in human hosts. *Nature* **449**, 843–849 (2007).
- Nash, J. F. Equilibrium points in n -person games. *Proc. Natl Acad. Sci. USA* **36**, 48–49 (1950).
- Nash, J. Non-cooperative games. *Ann. Math.* **54**, 286–295 (1951).
- Ma, C.-T., Moore, J. & Turnbull, S. Cheating and equilibrium. Stopping agents from ‘cheating’. *J. Econ. Theory* **46**, 355–372 (1988).
- Obbard, D. J., Jiggins, F. M., Halligan, D. L. & Little, T. J. Natural selection drives extremely rapid evolution in antiviral RNAi genes. *Curr. Biol.* **16**, 580–585 (2006).
- Smith, J. M. in *Evolution and the Theory of Games* 10–27 (Cambridge Univ. Press, Cambridge, UK 1982).
- Gray, R. H. *et al.* Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet* **357**, 1149–1153 (2001).
- Galvani, A. P. Epidemiology meets evolutionary ecology. *Trends Ecol. Evol.* **18**, 132–139 (2003).
- Turnbaugh, P. J. *et al.* A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484 (2009).

44. Blaser, M. J. Who are we? Indigenous microbes and the ecology of human diseases. *EMBO Rep.* **7**, 956–960 (2006).
45. Blaser, M. J. Disappearing microbiota: *Helicobacter pylori* protection against esophageal adenocarcinoma. *Cancer Prev. Res. (Phila. Pa.)* **1**, 308–311 (2008).
46. Strachan, D. P. Hayfever, hygiene, and household size. *BMJ* **299**, 1259–1260 (1989).
47. Nahar, S. *et al.* Evidence of intra-familial transmission of *Helicobacter pylori* by PCR-based RAPD fingerprinting in Bangladesh. *Eur. J. Clin. Microbiol. Infect. Dis.* **28**, 767–773 (2009).
48. Flegal, K. M., Graubard, B. I., Williamson, D. F. & Gail, M. H. Cause-specific excess deaths associated with underweight, overweight, and obesity. *JAMA* **298**, 2028–2037 (2007).
49. Eder, W., Ege, M. J. & von Mutius, E. The asthma epidemic. *N. Engl. J. Med.* **355**, 2226–2235 (2006).
50. Pohl, H. & Welch, H. G. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J. Natl Cancer Inst.* **97**, 142–146 (2005).
51. Linz, B. *et al.* An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* **445**, 915–918 (2007).
52. Banatvala, N. *et al.* The cohort effect and *Helicobacter pylori*. *J. Infect. Dis.* **168**, 219–221 (1993).
53. Roosendaal, R. *et al.* *Helicobacter pylori* and the birth cohort effect. Evidence of a continuous decrease of infection rates in childhood. *Am. J. Gastroenterol.* **92**, 1480–1482 (1997).
54. Harvey, R. F. *et al.* Relationship between the birth cohort pattern of *Helicobacter pylori* infection and the epidemiology of duodenal ulcer. *QJM* **95**, 519–525 (2002).
55. Blaser, M. J. & Atherton, J. C. *Helicobacter pylori* persistence: biology and disease. *J. Clin. Invest.* **113**, 321–333 (2004).
56. Chen, Y. & Blaser, M. J. *Helicobacter pylori* colonization is inversely associated with childhood asthma. *J. Infect. Dis.* **198**, 553–560 (2008).
57. Suerbaum, S. & Michetti, P. *Helicobacter pylori* infection. *N. Engl. J. Med.* **347**, 1175–1186 (2002).
58. Odum, L., Petersen, H. D., Andersen, I. B., Hansen, B. F. & Rehfeld, J. F. Gastrin and somatostatin in *Helicobacter pylori* infected antral mucosa. *Gut* **35**, 615–618 (1994).
59. Kanno, T. *et al.* Gastric acid reduction leads to an alteration in lower intestinal microflora. *Biochem. Biophys. Res. Commun.* **381**, 666–670 (2009).
60. Kuipers, E. J. *et al.* Long-term sequelae of *Helicobacter pylori* gastritis. *Lancet* **345**, 1525–1528 (1995).
61. Kuipers, E. J., Perez-Perez, G. I., Meuwissen, S. G. & Blaser, M. J. *Helicobacter pylori* and atrophic gastritis: importance of the *cagA* status. *J. Natl Cancer Inst.* **87**, 1777–1780 (1995).
62. Argent, R. H. *et al.* Toxigenic *Helicobacter pylori* infection precedes gastric hypochlorhydria in cancer relatives, and *H. pylori* virulence evolves in these families. *Clin. Cancer Res.* **14**, 2227–2235 (2008).
63. Peek, R. M., Jr. & Blaser, M. J. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nature Rev. Cancer* **2**, 28–37 (2002).
64. Kamangar, F. *et al.* Opposing risks of gastric cardia and noncardia gastric adenocarcinomas associated with *Helicobacter pylori* seropositivity. *J. Natl Cancer Inst.* **98**, 1445–1452 (2006).
65. Islami, F. & Kamangar, F. *Helicobacter pylori* and esophageal cancer risk: a meta-analysis. *Cancer Prev. Res. (Phila. Pa.)* **1**, 329–338 (2008).
66. el-Serag, H. B. & Sonnenberg, A. Opposing time trends of peptic ulcer and reflux disease. *Gut* **43**, 327–333 (1998).
67. Howson, C. P., Hiyama, T. & Wynder, E. L. The decline in gastric cancer: epidemiology of an unplanned triumph. *Epidemiol. Rev.* **8**, 1–27 (1986).
68. Hacker, J. & Carniel, E. Ecological fitness, genomic islands and bacterial pathogenicity. A Darwinian view of the evolution of microbes. *EMBO Rep.* **2**, 376–381 (2001).
69. Backert, S. & Selbach, M. Role of type IV secretion in *Helicobacter pylori* pathogenesis. *Cell Microbiol.* **10**, 1573–1581 (2008).
70. Viala, J. *et al.* Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* *cag* pathogenicity island. *Nature Immunol.* **5**, 1166–1174 (2004).
71. Nwokolo, C. U., Freshwater, D. A., O'Hare, P. & Randevara, H. S. Plasma ghrelin following cure of *Helicobacter pylori*. *Gut* **52**, 637–640 (2003).
72. Mattsson, A., Lönroth, H., Quiding-Jarbrink, M. & Svennerholm, A. M. Induction of B cell responses in the stomach of *Helicobacter pylori*-infected subjects after oral cholera vaccination. *J. Clin. Invest.* **102**, 51–56 (1998).
73. Robinson, K. *et al.* *Helicobacter pylori*-induced peptic ulcer disease is associated with inadequate regulatory T cell responses. *Gut* **57**, 1375–1385 (2008).
74. Lundgren, A. *et al.* Mucosal FOXP3-expressing CD4⁺CD25^{high} regulatory T cells in *Helicobacter pylori*-infected patients. *Infect. Immun.* **73**, 523–531 (2005).
75. Blaser, M. J., Chen, Y. & Reibman, J. Does *Helicobacter pylori* protect against asthma and allergy? *Gut* **57**, 561–567 (2008).
76. Chen, Y. & Blaser, M. J. Inverse associations of *Helicobacter pylori* with asthma and allergies. *Arch. Intern. Med.* **167**, 821–827 (2007).
77. Reibman, J. *et al.* Asthma is inversely associated with *Helicobacter pylori* status in an urban population. *PLoS ONE* **3**, e4060 (2008).
78. Codolo, G. *et al.* The neutrophil-activating protein of *Helicobacter pylori* down-modulates Th2 inflammation in ovalbumin-induced allergic asthma. *Cell Microbiol.* **10**, 2355–2363 (2008).
79. Del Prete, G. *et al.* Immunosuppression of T_H2 responses in *Trichinella spiralis* infection by *Helicobacter pylori* neutrophil-activating protein. *J. Allergy Clin. Immunol.* **122**, 908–913.e5 (2008).
80. Jukes, T. Antibiotics in animal feeds and animal production. *Bioscience* **22**, 526–534 (1972).
81. Butaye, P., Devriese, L. A. & Haesebrouck, F. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on Gram-positive bacteria. *Clin. Microbiol. Rev.* **16**, 175–188 (2003).
82. Gaskins, H. R., Collier, C. T. & Anderson, D. B. Antibiotics as growth promotants: mode of action. *Anim. Biotechnol.* **13**, 29–42 (2002).
83. Sjölund, M. *et al.* Long-term persistence of resistant *Enterococcus* species after antibiotics to eradicate *Helicobacter pylori*. *Ann. Intern. Med.* **139**, 483–487 (2003).
84. Sjölund, M. *et al.* Persistence of resistant *Staphylococcus epidermidis* after single course of clarithromycin. *Emerg. Infect. Dis.* **11**, 1389–1393 (2005).
85. Musher, D. M. *et al.* Bacteremic and nonbacteremic pneumococcal pneumonia. A prospective study. *Medicine (Baltimore)* **79**, 210–221 (2000).
86. MacLeod, L. C. & Kraus, M. R. Relation of virulence of pneumococcal strains for mice to the quantity of capsular polysaccharide formed *in vitro*. *J. Exp. Med.* **92**, 1–9 (1950).
87. Bries, D. E., Novak, L., Hotomi, M., van Ginkel, F. W. & King, J. Nasal colonization with *Streptococcus pneumoniae* includes subpopulations of surface and invasive pneumococci. *Infect. Immun.* **73**, 6945–6951 (2005).
88. Weinberger, D. M. *et al.* Epidemiologic evidence for serotype-specific acquired immunity to pneumococcal carriage. *J. Infect. Dis.* **197**, 1511–1518 (2008).
89. Ghaffar, F. *et al.* Effect of the 7-valent pneumococcal conjugate vaccine on nasopharyngeal colonization by *Streptococcus pneumoniae* in the first 2 years of life. *Clin. Infect. Dis.* **39**, 930–938 (2004).
90. Albrich, W. C., Baughman, W., Schmotzer, B. & Farley, M. M. Changing characteristics of invasive pneumococcal disease in Metropolitan Atlanta, Georgia, after introduction of a 7-valent pneumococcal conjugate vaccine. *Clin. Infect. Dis.* **44**, 1569–1576 (2007).
91. Veenhoven, R. *et al.* Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media: a randomised study. *Lancet* **361**, 2189–2195 (2003).
92. Madhi, S. A. *et al.* Long-term effect of pneumococcal conjugate vaccine on nasopharyngeal colonization by *Streptococcus pneumoniae* and associated interactions with *Staphylococcus aureus* and *Haemophilus influenzae* colonization in HIV-infected and HIV-uninfected children. *J. Infect. Dis.* **196**, 1662–1666 (2007).
93. Bogaert, D. *et al.* Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. *Lancet* **363**, 1871–1872 (2004).
94. Regev-Yochay, G. *et al.* Association between carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in children. *JAMA* **292**, 716–720 (2004).
95. Regev-Yochay, G. *et al.* Does pneumococcal conjugate vaccine influence *Staphylococcus aureus* carriage in children? *Clin. Infect. Dis.* **47**, 289–291 (2008).
96. Selva, L. *et al.* Killing niche competitors by remote-control phage induction: the case of *Streptococcus pneumoniae*–*Staphylococcus aureus* interference. *Proc. Natl Acad. Sci. USA* **106**, 1234–1238 (2009).
97. Lysenko, E. S., Ratner, A. J., Nelson, A. L. & Weiser, J. N. The role of innate immune responses in the outcome of interspecies competition for colonization of mucosal surfaces. *PLoS Pathog.* **1**, e1 (2005).
98. Regev-Yochay, G. *et al.* The pneumococcal pilus predicts the absence of *Staphylococcus aureus* co-colonization in pneumococcal carriers. *Clin. Infect. Dis.* **48**, 760–763 (2009).
99. Herold, B. C. *et al.* Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* **279**, 593–598 (1998).
100. Dufour, P. *et al.* Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. *Clin. Infect. Dis.* **35**, 819–824 (2002).
101. Tindberg, Y. *et al.* *Helicobacter pylori* infection in Swedish school children: lack of evidence of child-to-child transmission outside the family. *Gastroenterology* **121**, 310–316 (2001).
102. Rothenbacher, D., Bode, G. & Brenner, H. History of breastfeeding and *Helicobacter pylori* infection in pre-school children: results of a population-based study from Germany. *Int. J. Epidemiol.* **31**, 632–637 (2002).
103. Andersson, A. F. *et al.* Comparative analysis of human gut microbiota by barcoded pyrosequencing. *PLoS ONE* **3**, e2836 (2008).
104. Brown, N. F., Wickham, M. E., Coombes, B. K. & Finlay, B. B. Crossing the line: selection and evolution of virulence traits. *PLoS Pathog.* **2**, e42 (2006).
105. Lenski, R. E. & May, R. M. The evolution of virulence in parasites and pathogens: reconciliation between two competing hypotheses. *J. Theor. Biol.* **169**, 253–265 (1994).
106. Marini, E. *et al.* *Helicobacter pylori* and intestinal parasites are not detrimental to the nutritional status of Amerindians. *Am. J. Trop. Med. Hyg.* **76**, 534–540 (2007).
107. Lederberg, J. & McCray, A. T. 'Ome sweet 'omics. A genealogical treasury of words. *The Scientist* **15**, 8 (2001).
108. Peterson, J. The NIH Human Microbiome Project. *Genome Res.* 9 October 2009 (doi:10.1101/gr.096651.109).
109. Tibayrenc, M. & Ayala, F. J. The clonal theory of parasitic protozoa: 12 years on. *Trends Parasitol.* **19**, 405–410 (2002).
110. Rich, S. M., Sawyer, S. A. & Barbour, A. G. Antigen polymorphism in *Borrelia hermsii*, a clonal pathogenic bacterium. *Proc. Natl Acad. Sci. USA* **98**, 15038–15043 (2001).
111. Sarkar, S. F. & Guttman, D. S. Evolution of the core genome of *Pseudomonas syringae*, a highly clonal, endemic plant pathogen. *Appl. Environ. Microbiol.* **70**, 1992–2012 (2004).
112. Kennedy, A. D. *et al.* Epidemic community-associated methicillin-resistant *Staphylococcus aureus*: recent clonal expansion and diversification. *Proc. Natl Acad. Sci. USA* **105**, 1327–1332 (2008).
113. Zhou, X. *et al.* Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. *Microbiology* **150**, 2565–2573 (2004).
114. Vesper, B. J. *et al.* The effect of proton pump inhibitors on the human microbiota. *Curr. Drug Metab.* **10**, 84–89 (2009).

Acknowledgements

M. Blaser gratefully acknowledges funding from the US National Institutes of Health (NIH; grants R01GM62370 and UH2AR057506) and from the Diane Belfer Program for Human Microbial Ecology. S. Falkow gratefully acknowledges funding from NIH (grants R01AI026195 and R01AI038459).

DATABASES

Entrez Genome Project: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomemap>
Helicobacter pylori | *Homo sapiens* | *Mycobacterium tuberculosis* | *Neisseria meningitidis* | *Streptococcus pneumoniae* | *Salmonella enterica* subsp. *enterica* serovar Typhi | *Staphylococcus aureus* | *Yersinia pestis*

FURTHER INFORMATION

Human Microbiome Project: <http://nihroadmap.nih.gov/hmp>
 International Human Microbiome Consortium: <http://www.human-microbiome.org>
 Martin J. Blaser's homepage: <http://www.med.nyu.edu/medicine/labs/blaserlab>

ALL LINKS ARE ACTIVE IN THE ONLINE PDF