Microbial evolutionary medicine: from theory to clinical practice

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Medicine and clinical microbiology have traditionally attempted to identify and eliminate the agents that cause disease. However, this traditional approach is becoming inadequate for dealing with a changing disease landscape. Major challenges to human health are non-communicable chronic diseases, often driven by altered immunity and inflammation, and communicable infections from agents which harbour antibiotic resistance. This Review focuses on the so-called evolutionary medicine framework, to study how microbial communities influence human health. The evolutionary medicine framework aims to predict and manipulate microbial effects on human health by integrating ecology, evolutionary biology, microbiology, bioinformatics, and clinical expertise. We focus on the potential of evolutionary medicine to address three key challenges: detecting microbial transmission, predicting antimicrobial resistance, and understanding microbe–microbe and human–microbe interactions in health and disease, in the context of the microbiome.

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Introduction

A diverse range of microbes play a crucial role in human health and disease. Opportunistic or specialist pathogens can colonise the urinary tract,[1] the gut,[2] or the lungs,[3] whereas the gut microbiome affects nutrient metabolism and absorption,[4] and resilience to infection.[5] Global antibiotic use is on the rise[6] and antibiotic-resistant bacteria are now so widespread that WHO warns that the world is running out of functional treatments[7] while bacteria continue to evolve resistance to new drugs. This problem is caused by the extensive use of antibiotics in the clinic as well as unregulated over-the-counter purchases, and therapeutic, prophylactic, and growth-promoting use in agriculture. Another major challenge is disease attributed to the perturbation of the healthy microbiome, in which changes can occur because of a diet of processed food, altered hygiene practices, and antibiotic use. These practices leave individuals susceptible to opportunistic infections and prone to develop metabolic syndrome.[8,9]

The emerging field of evolutionary (or Darwinian) medicine seeks to apply the approach of evolutionary biology to address challenges to human health, and to identify the ultimate causes of disease.[10] This supplements classic medicine by focusing on why we become ill, rather than how, by considering that disease might be the consequence of maladaptation to the environment, in particular because our living conditions have changed over a short period of time. Using an understanding of evolutionary processes to predict how a disease will develop or respond to treatment will help to design new therapies (figure 1, panel 1). This approach is particularly relevant for understanding the microbes present on human inner and outer surfaces. Many microbes have a long history of association with their host,[12] they frequently co-occur and interact with other microbes, and they evolve rapidly because of their large population sizes and short generation times.[13] Traditionally, evolution (ie, changes in allele frequencies within a population) is thought to be slow relative to ecology (changes in the relative abundance of different species or populations). However, given the potentially rapid evolution of microbes, the scales of ecology and evolution can overlap, leading to substantial feedback between ecological and evolutionary processes.[14,15] Evolutionary medicine is inseparable from ecological medicine in the context of microbes because of their specific ability to rapidly evolve and adapt, leading to substantial feedback between ecological and evolutionary processes. Theoretical and experimental biology have been instrumental in elucidating how microbial interactions shape the stability of bacterial consortia such as that in the gut,[16,17] and how clinical interventions affect bacterial evolution and coexistence within such consortia.[18] Ecoevolutionary approaches have not often been applied to clinical systems, despite some success stories (panel 2). In this Review, we focus primarily on bacteria, although progress has been made in the field for other infectious agents including viruses.[25,26]

We focus on three key challenges where an interdisciplinary ecoevolutionary approach to infectious diseases might be beneficial, feasible, and useful. First, we discuss the timely detection of pathogen transmission; second, the prediction of antimicrobial resistance; and finally, the understanding of microbial interactions in health and disease, in the context of the human microbiome. Given the increasing availability of large-scale genomic and metagenomic sequencing data from clinical samples, we will focus on how such data, in conjunction with experimental laboratory studies, could be used to infer evolutionary and epidemiological dynamics. We caution that a sequencing-based approach is not equivalent to an evolutionary medicine approach; however, it is an indispensable tool to generate and test evolutionary hypotheses. We also discuss challenges of the interdisciplinary approach in addressing the aforementioned issues, exemplified by the sharing and use of high-throughput sequencing data for both basic research and clinical applications (figure 2).

Inferring evolutionary patterns to detect microbial transmission

Many pathogens are measurably evolving over time, even within individual patients. This evolution enables the tracking and understanding of transmission chains via genome sequencing with the aid of phylodynamics. A mature field in its own right, phylodynamics can be thought of as the application of molecular evolution to epidemiology.[19,27–29] Globalisation, urbanisation, changing climate and land-use patterns, and large-scale farming all contribute to the risk of epidemics. An epidemic spread of specific bacterial clones can often be unnoticed for some time, in particular when the causative agent is considered a common pathogen, such as *Pseudomonas aeruginosa* clones, which can spread between patients with cystic fibrosis,[3,30] or the ST131 *Escherichia coli* clone, which was not detected until 2008 when it had already spread globally.[31,32]

How can early detection of epidemics be facilitated?

To identify and predict the transmission of bacteria, knowledge of which genetic elements to monitor is crucial. Many studies on clinical isolates focus on the core genome, which is the portion of alignable genomic regions common to a collection of isolates. This particular focus is primarily to ease comparisons across strains. Yet, as many virulence factors and antibiotic resistance genes are found on mobile elements such as plasmids, bacteriophages, and transposons, these elements could be the most important to monitor, as their transmission might go undetected when the focus is on strain transmission[33] (eg, the colistin resistance-conferring *mcr-1* gene that is widely spread on plasmids across *E coli* clones).[34,35] Increasingly, genomic approaches are used to track plasmid dissemination,[36] and a cohesive view of both core and accessory genomic components is needed to fully understand pathogen evolution, transmission, and virulence.[37] For instance, bacterial chromosomes can be thought to coevolve or coadapt with antibiotic resistance plasmids, which might incur fitness costs that must be compensated with mutations on the chromosome. Laboratory evolution experiments have shown that a combination of mutations on chromosomes and plasmids can lead to high antibiotic resistance with reduced fitness cost; however, the resulting codependence could limit the spread of coadapted plasmids to other lineages.[38] Such complex evolutionary interactions merit further study in the clinic and in the laboratory to better predict the risk of antibiotic resistance epidemics.

Looking beyond the core genome raises the question of whether the microbe is merely a vector of disease, in cases where virulence and antibiotic resistance stem from transferable mobile elements.[39,40] When extensive recombination occurs, mobile resistance genes are effectively decoupled from their host genomes—and genes rather than bacterial genomes or strains would become the relevant unit of evolution and epidemiology. Mobile genes are associated (at least for some measurable amount of time) with a particular genomic background (eg, a bacterial clone) and thus transmission of both bacterial clones and mobile genes is worth tracking. Could these transmission events be detected earlier, when

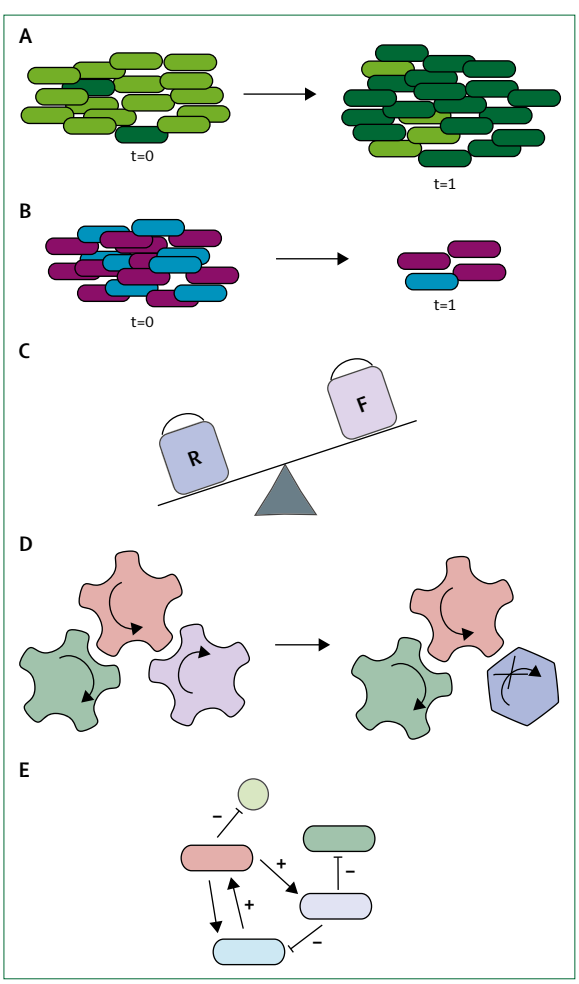

Figure 1: **Concepts from evolution and ecology that can be used to understand clinical challenges**
(A) Mutants that confer a reproductive advantage (dark), compared with ancestral genotypes (light) increase in frequency through positive natural selection while time elapses from t=0 to t=1. (B) Allele frequencies might change randomly by genetic drift in small populations (eg, following bottlenecks). Blue and purple shapes correspond to bacteria with two different alleles. (C) A fitness trade-off occurs when a mutation improves one trait (eg, antibiotic resistance; R) but leads to the deterioration of another trait (eg, lowered growth rate, fitness; F). (D) Pleiotropy occurs when a mutation affects more than one trait. Intracellular dependencies could then be detrimental to overall cell function. A mutation affecting one trait (purple wheel and corresponding mutated blue hexagon) affects fitness by disrupting communication to other cellular processes (curved arrows). (E) The positive and negative interactions between species in a community can be described as an ecological network. The type of interactions between the species (positive, arrows, or negative, Ts) between the different bacterial species (coloured spheres and rods) determine the stability of the community.

there is still time to prevent further transmission? Through retrospective evolutionary investigation of bacterial pathogen population dynamics, we are now beginning to understand how epidemics begin.[41] Faster sharing of genomic data and annotations (eg, pathogenicity islands, plasmids that easily spread, and virulence gene markers) through public databases can support the tracking of ongoing epidemics, allowing scientific consortia of local and global scientists to contribute to analysis and timely detection of transmission.[42]

What makes a pathogen?

A better understanding of the environmental niches and genetic variation of opportunistic pathogens is required for the detection of virulence determinants. Do disease-causing strains represent a random sample from an environmental or host reservoir, or do they harbour specific characteristics that facilitate human colonisation and infection? A comparison of *Vibrio cholerae* genomes from environmental and clinical sources revealed that a combination of specific core-genome single nucleotide polymorphisms already present in the environment, was a prerequisite for the acquisition of mobile elements encoding key virulence factors that affect colonisation and virulence in the human host.[43] Likewise, sampling from animal hosts can show linkage between animal and human reservoirs.[44–46] Various studies investigating bacteria residing in the urinary tract have shown that what are traditionally thought of as pathogens can actually be present in both patients and controls.[47,48] Sequencing of microbial pathogens in conjunction with laboratory studies could reveal the selective effect of the host environment. Such a combined approach could increase knowledge of which circumstances favour the evolution of pathogenicity, the selective enrichment and subsequent spread of pathogens, and whether the success of a pathogen occurs because of the genetics of the pathogens or the different niches provided by genetically or physiologically variable human hosts.[49]

Predicting and manipulating the evolution of antimicrobial resistance

Because of the widespread use of antibiotics in the community and in agriculture, and the proximity of humans and livestock, resistance spreads rampantly back and forth from livestock to waste water and to humans. This pattern of spread has been observed in small family farms in Vietnam[50,51] and in industrial-sized farms in China.[52] The spread of resistance back and forth between livestock and humans is probably dependent on the socioeconomic environment—eg, in the Netherlands such bidirectional spread appears to be uncommon.[53] Although antibiotic resistance is a widespread occurence, and also occurs in organisms never exposed to synthetic antibiotics,[54–56] resistance is not distributed equally across all environments.[57] Can an understanding of ecology and evolution help predict and avoid the emergence of antibiotic resistance?

Panel 1: **Concepts from evolution and ecology that can be used to understand clinical challenges**

Natural selection is the evolutionary force that drives a change in frequency of genotypes, leading to adaptation to the environment. Mutants that confer a reproductive advantage compared with ancestral genotypes could increase in frequency through positive selection (figure 1A). Treatment with high concentrations of antibiotics selects for resistant genotypes. By contrast, genotypes that confer a reproductive disadvantage could decrease in frequency through negative selection. The fitness cost of antibiotic resistance could select against the dissemination of resistant pathogens outside of the clinic. In small populations allele frequencies can change via genetic drift because of chance, as has been observed in population bottlenecks at transmission events in tuberculosis.[11] Observed changes might not necessarily reflect selection (figure 1B).

The concept that natural selection can act at different levels of organisation (the gene, genome, or even community), usually on different timescales, is termed levels of selection. A so-called selfish gene or organism could favour its own replication on short timescales (eg, within a patient), but might become extinct in the long term. Therefore, we might observe patterns of mutations that appear counterintuitive at first glance but can be explained by evolutionary theory.

Fitness trade-offs and genetic constraints are organism limitations for simultaneously optimising all traits. With a limited amount of resources available, adaptation is expected to come at a cost because investing resources in one trait often comes at the cost of another. When a strong selection gradient is applied (eg, antibiotic treatment) fitness trade-offs could result in increased resistance leading to slower growth, and consequently lower fitness, or increased sensitivity to other drugs (figure 1C). Furthermore, the benefit of a mutation might depend on the genetic background, in which a mutation or genetic element leading to antibiotic resistance in one organism might not confer resistance in another. Pleiotropy occurs when a mutation affects more than one trait. Intracellular dependencies could lead to detrimental functioning, and thus slower growth (figure 1D). In all cases, evolution can be constrained and the course of adaptation could be biased.

Ecological succession describes how community composition could change over time, as the inhabitants or outside forces change resource availability. For successful colonisation, some species rely on the presence of other so-called pioneers, or their modification of the habitat. Priority effects describe the positive or negative effects that primary colonisers have on the ability for later arrivals to thrive.

Ecological networks describe the positive and negative interactions between species in a community. The type of interactions between the species determine the stability of the community (figure 1E).

Panel 2: **Successes of microbial evolutionary medicine**

- The exploitation of bacterial evolution (molecular clock) to trace transmission events over time at the scale of hospitals and continents[19]
- The use of signatures of strong positive natural selection on antibiotic resistance mutations to identify potentially causal (or diagnostic) resistance mutations[20]
- The identification of evolutionary trade-offs that limit the acquisition of resistance genes[21,22]
- The identification of the role of intraspecies and interspecies microbial interactions in pathogen ecology and adaptation[17,23]
- The discovery that gut microbes with a long evolutionary history of cospeciation with mammals tend to be depleted in human disease states, suggesting that ancient associates can tend to be beneficial to health[12]
- The introduction of *Wolbachia* spp symbionts into mosquitoes to control malaria transmission by inhibiting *Plasmodium* spp development[24]

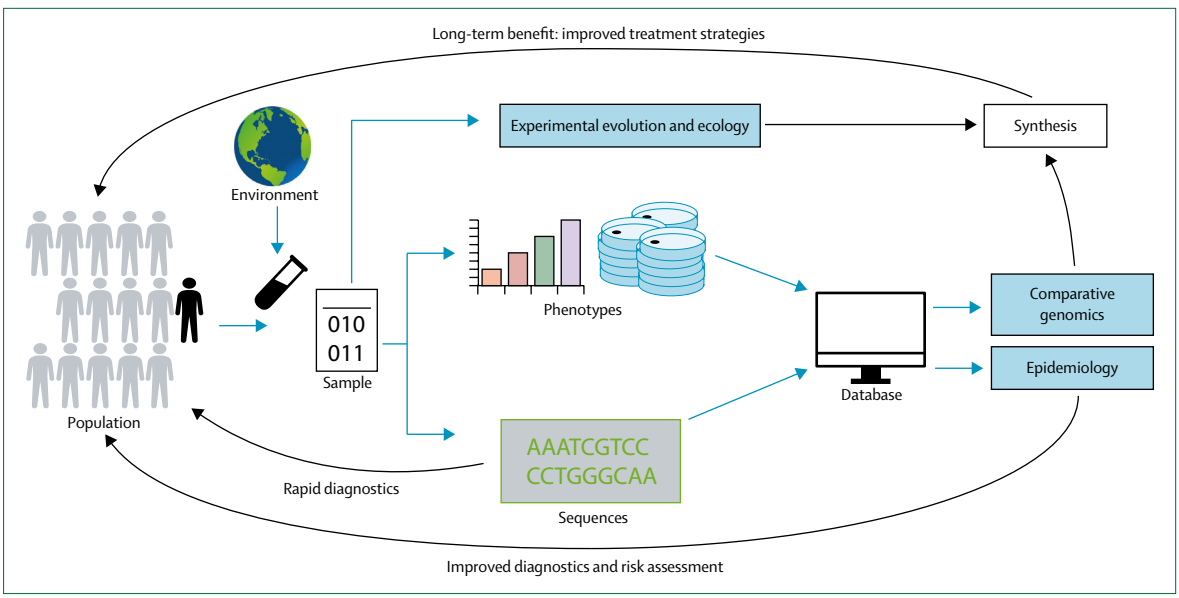

Figure 2: **How information flow in an interdisciplinary microbial evolutionary medicine approach could improve basic knowledge, public health, and patient care**

A microbial sample collected from a patient with a specific disease, a healthy volunteer, or the environment can be sequenced and phenotyped (eg, by assessing antibiotic susceptibility). Sequencing and annotation of isolates could serve as a rapid diagnostic to directly benefit the individual patient. Compiled sequences (potentially including genomes, metagenomes, and metatranscriptomes) and phenotypic data (eg, antibiotic resistance of microbes, and metabolomes) in an accessible database with appropriate metadata (eg clinical patient data), could be useful for subsequent analyses. Inferred epidemiological transmission patterns can be used for improved diagnostics and future risk assessments. Comparative genomics and experimental ecology and evolution can help in the formulation of hypotheses of why a given clinical outcome occurred. In turn, such hypotheses might be experimentally verified with collaborative, interdisciplinary efforts. This framework has the potential to lead to improved treatment strategies and precautions, which will benefit the population in the long term.

Can experimental evidence regarding the evolution of antibiotic resistance be used to inform treatment decisions?

In the laboratory, microbes are capable of quickly adapting to high concentrations of antibiotics.[58] However, for experimental work on resistance evolution to be clinically relevant, the question of whether resistance in vivo evolves under strong or weak selection pressures needs to be addressed (panel 1). Because of the unequal distribution across tissues and low penetration of biofilms, the antibiotic concentration that reaches microbes in situ at infectious sites might be different from that in experimental conditions, even if clinically relevant concentrations of antibiotics are used in experiments.[59,60] The strength of selection in the host is unknown in most cases, but might affect the type of resistance mutations that arise, and the rate at which these mutations increase in frequency. A weak selective pressure has been found to be more likely to select for a broader range of resistance mutations at little to no cost to the bacteria that carry them.[61]

Fitness trade-offs between growth rate and antibiotic resistance are expected to create a barrier to the spread of resistance (panel 1).[62] For example, clinical isolates of *Enterococcus faecium* have markedly larger genomes than non-clinical isolates, in part because they carry a large pathogenicity island and other mobile genetic elements[63,64] that often code for antibiotic resistance. DNA content, and coinciding rates of transcription and translation, are generally assumed to be negatively correlated with growth rate.[65] Additionally, in many species, resistance against fluoroquinolones and aminoglycosides can have a deleterious effect on either mobility or growth in the absence of the antibiotics.[66–69] The fitness burden, which leads to negative selection in the absence of antibiotics, can effectively select against the dissemination of resistant pathogens outside of the clinic.[70] These trade-offs can inform on how to select the optimal dose of antibiotic treatment. The general rationale has been that a so-called hit hard approach of using the highest possible dose, lowers the risk of resistance development because of a reduced pathogen population size and thus a smaller total number of resistant mutants in the population.[71] However, eco-evolutionary theory predicts that hitting hard actually selects for highly resistant bacteria, which have fewer moderately resistant competitors when population size is small (ie, clonal interference is reduced in small populations). Therefore, under some conditions, the use of a light-touch treatment at the lowest clinically effective dose will lead to a lower level of resistance development and a more effective treatment.[71] Conventions on the duration of treatment have also been challenged on the basis of studies showing that a shorter treatment period might be preferable for pneumonia.[72] Yet another

approach might be to exploit intraspecies competition by lowering the availability of essential nutrients for the pathogen, thereby intensifying the competition to a level where fitness trade-offs limit the spread of resistance.[73]

Crossresistance, which is when the same mutation confers resistance to multiple antibiotics, has been found to be more likely to evolve under strong selective pressures. However, under some conditions, collateral sensitivity can occur. In such a case, mutations that confer resistance to one drug can induce susceptibility to another drug.[74] Cycling of antibiotics in the clinic has been suggested to use collateral sensitivity to limit the development of resistance to each of the cycled drugs,[75,76] yet studies have found contradictory results regarding the effectiveness of cycling strategies.[77,78] A major challenge for evolutionary medicine is to understand which mechanisms and which environmental conditions can diminish the emergence and spread of antibiotic resistance. Understanding these mechanisms and conditions can inform the development of so-called evolution proof or more likely so-called evolution-proof antibiotics (panel 2).[79] Discrepancies between the experimental conditions in which drugs are developed, and the clinical settings where they are used, could cause the failure of drugs during preclinical trials, even if they might be effective for patients.[80] Therefore, the ecological environment is a key factor for investigating and predicting the emergence of drug resistance.

The genetic architecture of resistance

The role of the bacterial genetic background should be studied to help determine the likelihood of resistance evolution via de-novo mutations or uptake of mobile elements conferring resistance. The genetic background plays a key role in shaping the outcome of the evolution of resistance to antibiotics by point mutation,[81–83] and can also effect the evolution of resistance by horizontal gene transfer.[84] For example, clinical isolates of the nosocomial pathogens *Enterococcus faecalis* and *E faecium* lack CRISPR-cas systems, making them more prone to accept foreign DNA and thus more likely to acquire antibiotic resistance genes.[21,64] Similarly, the most recombinogenic strains of the human pathogen *Streptococcus pneumoniae* are also the most likely to become antibiotic resistant.[85] Even without barriers to recombination, genetic constraints could select against mobile elements once they arrive in a new host genome. Fitness costs can be in the form of regulatory inefficiency experienced when new elements are incorporated,[86] or when biochemical incompatibilities are present,[87] leading to negative selection. Therefore, the fitness effect of mutations or horizontal gene transfer events can depend on the genetic background wherein they occur and could constrain or facilitate the evolution of antibiotic resistance (panel 1). Approaches aimed at detecting resistance should not only focus on specific resistance genes, but also consider mutations in coding and non-coding sequences such as promoters and intergenic regions.[38] Assessing the level of resistance and corresponding fitness purely on the basis of the presence or absence of certain genetic elements in the genome is difficult. Developing tools for predicting which strains of a pathogen have a high risk of evolving resistance is a daunting task, but one that could help guide the use of antibiotics in clinical settings. This task will require richly annotated genome and mobilome data, in publicly accessible databases, which includes data for antimicrobial susceptibility and growth rates in different relevant environments, as well as metadata.

Microbial interactions and the eco-evolutionary dynamics of the human microbiome

Microbe–microbe and host–microbe interaction can take place in the context of diverse and complex host-associated communities called microbiomes. When considering the adaptive potential of pathogens, we need to consider both intraspecies and interspecies interactions—the social or ecological environment in which bacteria evolve and interact. The composition, structure, and stability of a healthy microbiome is affected by human genetics, diet, and other environmental factors.[88,89] Key concepts from ecological theory, and experiments that guide the study of our microbiome, include the importance of diversity, ecological networks, and succession (panel 1). Metagenome sequencing, transcriptome sequencing, and phenotypic assays can inform about which bacterial organisms are where, their specific actions, and how they evolve, whereas experimental work guided by evolutionary theory can help to determine why bacteria evolve. To facilitate the accessibility of data to study these objectives, the Human Microbiome Project, the American Gut project and the Global Microbiome Conservancy, among others, are providing data from different body sites and different human populations.

For more on the **Human Microbiome Project** see https://hmpdacc.org/

For more on the **American Gut project** see http://americangut.org/

For more on the **Global Microbiome Conservancy** see http://microbiomeconservancy.org/

Can virulence be lowered by manipulating bacterial interactions?

An example of an intraspecies interaction that can affect pathogen virulence is the production of extracellular molecules that represent a so-called public good in game theory terminology—for example, those that facilitate bacterial communication, nutrient acquisition, and the breakdown of antibiotics.[90] These compounds are produced and shared within a population (or community) in a cooperative manner but are, by definition, exploitable, since non-producers might reap the benefit of their use without paying the cost of production.[91] The fact that this process can drive the loss of public-good production is well understood from game theory and laboratory experiments.[92] Additionally, analyses of the order of mutations in genome sequences of the opportunistic pathogen *P aeruginosa* showed that intra-species bacterial interactions also can drive changes in production of virulence factors in patients.[23] Manipulation of such interactions could have clinical applications.[93] An example is the elimination of *Staphylococcus aureus* from

the human gut by probiotic bacillus strains that block the signalling of *S aureus* with public-good quorum sensing molecules, and thereby turn off its production of virulence factors.[94] The importance of bacterial interactions in infections is further shown by the ability of non-pathogenic *E coli* to outcompete pathogenic salmonella, by exploiting their cooperative iron uptake.[95]

Why is microbiome diversity important?

A diverse microbiome is often, but not universally, associated with health and protection from disease;[96,97] the strongest and most consistent association is found between low diversity and diarrhoeal diseases.[98] The primary interactions between the constituents of the microbiome are thought to be competitive,[99] and diversity can reduce the available niches for invading diarrhoeal pathogens. For example, *Clostridioides difficile* colonisation is facilitated by a low diversity of the gut microbiome,[100] and repopulating the system towards a healthy, diverse state could cure such infections.[101] Faecal microbial transplants (FMT) were found to be about 85% effective in the treatment of recurrent *C difficile* infections[102] and hold great promise for the future design of microbiome-based therapeutics. Exactly which components of the transplant led to success remain unclear,[103] but the effect of FMT can be partly predicted on the basis of microbiome composition of donor and recipient.[104] In patients with cancer undergoing stem-cell transplants, low gut microbiome diversity and presence of specific organisms can be used to predict the risk of bloodstream infection a week later. Microbiome profiling could inform the choice of antibiotics or suggest the use of FMT to increase diversity.[105]

Several studies have shown that humans have experienced depletion of gut bacterial biodiversity, particularly populations that have embraced modern lifestyles and diets.[106–108] Processed foods, the use of antibiotics, and overly hygienic environments could be responsible for the disappearance of human ancestral gut symbionts, which could drive the rise of non-communicable diseases worldwide.[9] The microbiome composition can affect the propensity for non-communicable diseases through the immune system, because a diverse and stable microbiome has been suggested to be a key contributor to maturation. Early life events are particularly important in shaping the microbiome ecosystem,[109] and treatment with antibiotics at a young age is found to be associated with an increased risk of developing asthma and obesity later in life.[110,111] A prospective cohort study showed that an adequate maturation of the gut microbiome in the first year of life was crucial in the protection of children against asthma at age 5 years, especially for children born to asthmatic mothers.[112]

Are the effects from bacterial interation with the host dependent on the ecology?

Gut microbiome research is particularly focused on identifying taxa that contribute to health and disease. For instance, the abundance of an *Akkermansia* species was observed to be reduced in hosts with metabolic disorders,[113,114] but an increased abundance was observed in patients with Alzheimer's disease and ulcerative colitis.[23,92] *Akkermansia* species have been found to interact with other gut bacteria in metabolic networks[115] and the differential effect on health could reflect the different ecological backgrounds in which this species occurs.[100] The stomach bacterium *Helicobacter pylori* was characterised as a major risk factor for the development of peptic ulcers and stomach cancer late in life, but has been shown to be implicated in the protection against asthma and allergies early in life.[116,117] The term pathobiome is used to describe the fact that the effect of bacteria on health or disease is dependent on the other species of the microbiome.[118]

Does pathogen diversity matter for colonisation?

In urinary tract infections *E faecalis* facilitates the invasion of otherwise avirulent *E coli* in an animal colonisation model and can even affect disease development after the infection has cleared.[119] The stochastic nature of which microorganisms arrive first could have a role in disease development, as some bacteria can only colonise after early so-called pioneers clear the way (panel 1).[120] Interactions between different bacterial species derived from polymicrobial urinary tract infections affect ecological stability and antibiotic tolerance in vitro.[17] In chronic urinary tract infections in older people with subacute symptoms, polymicrobial infections are common.[121] An additional layer of complexity in microbe–microbe dynamics can come from bacteriophages that affect bacterial growth and could mediate interactions.[122] Ecology and evolution both have an important role in the outcome of infections, as microbial interactions might affect pathogen colonisation and survival.

Who is in control?

The degree of coevolution between mammals and their microbiomes is a topic of debate, but phylogenetic studies show that several gut bacteria have been vertically inherited over millions of years of evolution and have cospeciated with mammals.[12] Some long-term members of the mammalian microbiome might confer specific benefits. For example, the absence of certain human-associated bacteria is linked with inflammatory bowel disease.[12] The host has evolved to control some aspects of the microbiome composition (eg, through oxygen regulation) and so-called dysbiosis can result if control is lost.[99,123] The term holobiont is at times used to describe the microbiome in the context of the host.[124] However, even though the microbiome might have a specific function in the host and be beneficial, it has not necessarily coevolved with the host.[99,125,126] Therefore, whether natural selection acts at the level of the holobiont, or mainly at the level of individual species or genes within the microbiome, remains unclear.

Challenges to the use of ecoevolutionary approaches in the clinic

Applying ecoevolutionary approaches, such as phylogenetics,[127] game theory,[128] and modelling parameterised with empirical data from clinical and experimental laboratory studies[17,129] might enable the scientific community to prioritise certain approaches in the clinic. Ideally, pathogen and the presence of virulence and resistance markers could be identified, the co-occurrence of interacting species observed, and the best course of treatment for a patient predicted on the basis of their metagenomic data. In principle, metagenomes can contain information about the microbiome, pathogens, and human genetics,[130] potentially allowing predictions on the basis of host-microbe genetic interactions. However, making these predictions is generally not feasible at present, partly because of a lack of understanding of the ecoevolutionary interactions, and partly because high-throughput sequence analysis of both microbe and host is not yet routine practice in the clinic.[26] A clear exception are slow-growing pathogens, such as *Mycobacterium tuberculosis*, for which a variety of resistance-conferring mutations can be identified more rapidly by whole-genome sequencing (WGS) than by culture-based drug sensitivity assays.[131–134] However, communication of genomic data to clinicians is still a challenge. We suggest that genomic literacy should be taught during medical training, teaching clinicians to interpret sequencing-based results and to gauge when metagenomics or culture-based approaches are the appropriate tools for solving a problem. To increase the accessibility of WGS data, incorporating clinically desirable user-interfaces in future clinical WGS analysis software and databases would be useful.[135] These databases could additionally be equipped with warnings on the detection of specific resistance mutations in submitted genomes, or alerts of possible transmission if a specific clone has been found elsewhere, thereby benefiting global detection and information exchange (figure 2).

The path to interdisciplinarity

To further the understanding of the causal explanations of disease, increased collaboration is needed between clinicians and basic researchers. A database for matching clinical strain collections and scientists, with questions, specific hypotheses to test experimentally, and funds, could facilitate such interdisciplinary investigations. To improve reproducibility across studies, funders and journals should encourage higher standards in data submissions, including standardised metadata to allow reuse of data for comparative studies.[136] The bundling of human-related metadata (eg, microbiome sequencing, comorbidities, and diet data) could raise issues related to privacy. For example, microbiome data can be used to identify individuals.[137] However, we believe that the benefits of open data sharing, with appropriate checks and balances, clearly outweigh the potential risks.

***Panel 3:* Open questions and challenges for microbial evolutionary medicine**

- How does evolution and natural selection of microbes in the environment (or non-human hosts) effect their ability to colonise and cause disease in humans?[43]
- Can treatment strategies be designed that minimise the evolution of resistance—eg, by exploiting fitness trade-offs to reduce the spread of antibiotic resistance?[73,138–140]
- Can treatment strategies be designed that favour beneficial microbes, and prophylactic treatments that disfavour invasion by pathogens?[94,95]

A hope for the future in the field of microbial evolutionary medicine is to establish a common ground between clinicians, epidemiologists, bioinformaticians, public health officials, and cell biologists, microbiologists, and ecoevolutionary biologists to tackle the extensive interdisciplinary challenges that lie ahead (panel 3). A major aim is to be able to detect antibiotic resistance and virulence on the basis of genomic signatures of both host and pathogen, to improve diagnostics,[26] and to predict the development and spread of antibiotic resistance. Large advances are being made in relatively simple systems toward this aim, exemplified by tuberculosis, which can serve as a test case for more complex systems (panel 2). Eventually, we aim to be able to predict the health of the host on the basis of the ecology of the personal microbiome, in conjunction with the genetics of the individual patient, and to assess the risk of invasion of pathogens in complex systems, such as the gut. All these challenges cannot be solved by single disciplines in isolation. The path to applying ecoevolutionary theory to improve patient care seems discouragingly long at times;[141,142] however, initiatives such as the incorporation of evolutionary medicine in biological and medical curricula in universities throughout the world can serve as encouragement to pursue this goal.

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All authors contributed to the writing of the manuscript.

Lorentz Center Workshop on Darwinian Microbial Medicine Consortium

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Declarations of interests

We declare no competing interests.

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