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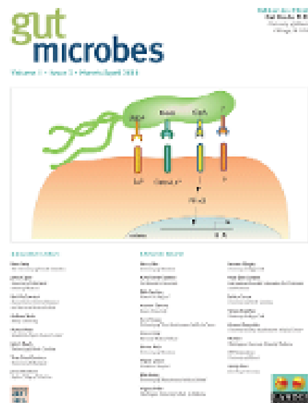

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Comparing the microbiota of the cystic fibrosis lung and human gut

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Comparing the microbiota of the cystic fibrosis lung and human gut

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In recent articles,[1,2] we discussed a fundamental shift in the way in which polymicrobial infections can be viewed. In these articles, we used chronic bacterial infections of the lower airways, and specifically those that occur in cystic fibrosis (CF) patients, as a model system. These infections are of course critical in clinical terms for these patients; respiratory failure due to a combination of these chronic infections with the host immune response that they elicit remains the leading cause of mortality in CF. Given the importance of maintaining lung health in these patients, the CF airways are the focus of a wide range of scientific and clinical studies. In particular, research momentum has built in relation to identifying the microbes present in the CF lung. Already, many important insights have been gained through the application of increasingly sophisticated culture-independent analytical methodologies to identify all microbial nucleic acids in the CF lung,[3-6] and those from viable or metabolically active bacteria.[7,8] In doing so, the data generated have revealed microbial assemblages of far greater diversity and in turn complexity than has previously been recognised in this context. These studies, which have served to highlight the inadequacy of traditional culture-based diagnostic microbiology to fully characterise such infections,[9] have also led to a significant shift in our view of what the word "infection" represents for these and other chronic diseases, with potentially important implications for their optimal treatment. In this article we contrast the information we and others have accrued from chronic lung infections with data generated from studies examining the microbial communities present in the gut. In doing so we highlight parallels between these two contexts and discuss how these commonalities can inform clinical thinking.

Common Beginnings

The process of isolating microbes through enrichment culture was developed in the 19th century, and culture-based strategies remain the basis of diagnostic bacteriology to this day. Whilst there was recognition that certain infections were in fact polymicrobial in nature at the beginning of the 20th century,[10,11] there existed neither the theoretical basis on which to construct models of polymicrobial activity, nor the tools to dissect these systems well.

In a diagnostic context, culture-based microbiology relies on the detection of aetiological agents by providing the conditions that a given species requires for growth in vitro. In many cases, even where the involvement of a specific pathogen is suspected, the provision of such suitable growth conditions can be extremely difficult. This problem is further compounded when diseased tissues contain multiple microbes, challenging the association of a single species with a particular clinical condition. In such circumstances, the size of the pool of uncharacterised species, and the relative distribution of those species within that pool, may not easily be determined using culture-based approaches. To illustrate, culture-independent surveys of phylogenetically-informative ribosomal RNA genes have indicated that more than 75% of the phylotypes detected in the human large intestine do not correspond

closely to cultured species.[12-14] In addition, any analysis based on obtaining species in axenic culture does not afford any insight into the interactions between the microbes present in a tissue, or consequently into the impact that these interactions may have. For these reasons, the rational characterisation of highly diverse microbial systems, and their relationship with disease pathogenesis, using traditional culture-based techniques is severely hampered.

Molecular Advances

The development of polymerase chain-reaction amplification (PCR) provided a basis for assays that exploit differences between species at the nucleic acid level. Here, variation in DNA sequence, particularly the presence of nonconserved genes, can be used to derive species-specific PCR-based assays. These techniques are applied directly to nucleic acids extracted from clinical samples to determine whether particular species are present, often quantitatively using real-time PCR.[15] In all such culture-independent analyses, the first step is the extraction of nucleic acids directly from specimens of interest. As such, these methods avoid many of the biases associated with in vitro culture. However, as with any process however, nucleic acid extraction can introduce other forms of analytical biases, such as through differential lysis of cells of different bacterial species.[16,17] Nevertheless, such species-specific assays enable direct bacterial detection in a wide range of samples, with the popularity of this approach reflected in the ever increasing number of such assays.[18] Nevertheless, whilst conferring advantages in terms of speed, cost and accuracy, these assays share some of the drawbacks associated with the use of selective media in culture-dependent approaches. Again, application of these methods requires a prediction to be made as to which agents are likely to be associated with a particular sample, so that the required reagents (e.g., the appropriate growth medium or species-specific primers) are used. They also share, even with automation, a practical upper limit to the number of species-specific assays that can be performed on one sample.

Alternatives to species-specific approaches are available. In one PCR-based method, primers are used that amplify regions of particular phylogenetically informative genes that are conserved across the Domain Bacteria, in theory producing PCR product for any bacterial species reasonably abundant within a sample. The key to this process is that, between the two conserved regions recognized by the primers, there is sufficient sequence variation among different bacteria to enable species discrimination and identification. As such, this method provides a basis for the characterisation of bacterial species present within a clinical sample without the need for prior prediction of which species may be present, addressing many of the drawbacks of both culture-based assays and species-specific PCR.

By far the most commonly used region in such 'broad-range PCR' strategies is the 16S ribosomal RNA (rRNA) gene.[19] PCR primers are targeted towards regions of the 16S rRNA gene that show conservation across the Domain Bacteria, meaning that a broad range of ribosomal gene products are generated from DNA templates extracted from a mixed bacterial community. To resolve this mixture of gene products and to identify the individual species present in the community, a number of strategies can be applied. These include Single Strand Conformation Polymorphism, Denaturing Gradient Gel Electrophoresis, Temperature Gradient Gel Electrophoresis, Terminal Restriction Fragment Length Polymorphism (T-RFLP) profiling and 16S rRNA gene sequencing (reviewed in refs. 20–22). Each technique exploits the variable internal regions directly or indirectly, with the strategy used selected on the basis of the clinical question and the type of data required.[20,22] Such broad-range strategies are finding increasing use, and in an expanding range of clinical scenarios.[23]

Concepts Emerging from the CF Airways

As highlighted in earlier publications, the application of molecular techniques to the analysis of CF airway infections has significantly changed the way in which such infections are viewed. Traditional culture-based diagnostic microbiology focused on a relatively small number of bacterial species that were both amenable to laboratory cultivation and considered to have important roles in CF lung disease.[24] Of these, the most notable were *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, *Staphylococcus aureus*, *Haemophilus influenzae, Alcaligenes xylosoxidans* and *Stenotrophomonas maltophilia*.[25,26] However, the use of molecular techniques has shown a far greater range of bacterial species to be present (and abundant) in CF respiratory samples than was previously appreciated,[3-6] and the use of careful culture techniques not commonly used in the clinical arena has corroborated these findings.[27]

Whilst it is difficult to succinctly describe the taxonomy of such a wide diversity of species, to give some insight, the traditionally recognised CF pathogens fall into two phyla; Proteobacteria (*H. influenzae, P. aeruginosa, S. maltophilia, B. cepacia* complex*, A. xylosoxidans*) and Firmicutes (*S. aureus*). Culture-independent studies have also shown CF secretions to be abundant in other members of these phyla, including species of the genera Veillonella, Streptococcus, Abiotrophia and Gemella (Firmicutes), and Neisseria and Acinetobacter (Proteobacteria). Culture-independent studies have further shown however that species belonging to four other phyla are also commonly resolved: Actinobacteria (including Actinomyces spp., Rothia spp.), Bacteroidetes (including Prevotella spp., Porphyromonas spp., Capnocytophaga spp.), Spirochaetes (Treponema spp.) and Fusobacteria (Fusobacterium spp.).

A common but unexpected finding in adult CF respiratory samples has been the high relative representation of species requiring anaerobic conditions for growth, such as those in the genera Veillonella and Prevotella.[9,27] The presence in CF secretions of bacteria with diverse growth requirements is however less surprising once the biology of the CF respiratory tract has been considered. The CF airways have been found to be both chemically and physically diverse, containing complex nutrients and carbon sources.[28] Levels of important factors required for growth by some species, such as iron,[29] and carbon sources such as mucin and alginate,[30,31] and amino acids,[32] vary in concentration across

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the airways, as do electron acceptors such as oxygen and nitrogen oxides.[33,34] As such, this heterogeneity provides conditions that are potentially suitable for colonisation by a wide range of microorganisms.

It is important to also recognise that the diversity seen in the CF lung is not only reflected in the number of bacterial species present, but also in their relative abundance. This distribution of bacterial cell numbers between species within the lung may be ecologically important in terms of interactions between different community members. There is also diversity at bacterial sub-species levels, as exemplified by the co-occurrence of multiple strains of *P. aeruginosa*.[35,36] Furthermore, the diversity observed among microbes is not limited to bacteria, but extends, for example, to viruses[37] and to fungi.[38] Whilst of an apparently lesser magnitude, the microbial diversity seen in the CF lung is reminiscent of the microbial communities normally found in other areas of the body, such as the oral cavity or gut. As such, in keeping with the terminology used to describe those polymicrobial communities, the diverse system seen in the CF lung may also be regarded as a "microbiota".

Microbial diversity in turn leads to the potential for "social interactions",[39-43] that may in turn dampen or exaggerate outcomes in relation to pathology and response to therapy. In work examining the bacterial communities that colonise the oral cavity, Jenkinson and Lamont[44] raised the possibility that, through social interactions, a microbial community may itself represent a pathogenic entity. There is mounting evidence that this concept may also be true as applied to the microbes in the CF lung. Sibley et al.[45] demonstrated that the virulence of known CF pathogens, such as *P. aeruginosa,* can be increased significantly by the presence of species previously dismissed as clinically insignificant, such as those derived from the oral cavity. We have therefore proposed that the aggregate microbial content of the CF lung might usefully be considered as a distinct pathogenic entity, whose impact on the host may be greater than the combined impacts of its individual component species alone.[2] In light of this concept, constructing a detailed and comprehensive model of the polymicrobial system in the CF lung is essential if the drive to understand and treat this disease is to be fully effective.

Insights from Studies of the Intestinal Microbiota

Infections in the CF airways were initially selected as a model system for applying molecular techniques to complex microbial communities based on a number of factors. These included the clinical importance of the condition and the assumption that the bacterial assemblages involved would be comparatively simple (as suggested by conventional culture-based analysis). However, as the true complexity of the CF airway microbiota started to emerge, parallels with highly complex microbial systems found in other areas of the body have become increasingly apparent.

The gut microbiota represents a significant difference in scale and complexity compared with that in the CF lung; 16S ribosomal RNA gene analysis has led to estimations that the gastrointestinal (GI) microbiota consists of hundreds of genera and thousands of bacterial species.[46-48] This complexity confounds the use of conventional culture-based analysis, and makes this context an ideal candidate for culture-independent analysis of the community.[48] Over the past decade these techniques have given a substantially broader and more accurate view of the gut microbiota.[49,50] The relatively high costs of large scale 16S rRNA dideoxynucleotide sequencing has limited its use in such systems and, as is the case with the CF airways, studies have utilised community profiling approaches such as T-RFLP[51,52] and denaturing gradient gel electrophoresis (DGGE)[53-55] to ascertain overall community structure and track its dynamics. More recently, the wider applicability of pyrosequencing, combined with the highly abundant data that it is able to provide, has led to an expansion of the use of this approach to the analysis of microbes that colonise the gut.[56-59]

Comparisons of the microbial systems in the gut and the CF lung may help to distinguish factors that underpin the typically mutually beneficial relationships usually found in the GI tract from the injurious relationships in the CF lung. As might be expected when comparing pathogenic and non-pathogenic systems, there are a number of clear differences. In CF, the presence of high densities of bacteria in an area of the body normally free from large-scale microbial colonisation is associated with consequences that can be assumed to be wholly negative. By contrast, the presence of a complex microbiota in the gut has a number of important functions ranging from supply of nutrients to the host, immune system development, angiogenesis and fat storage.[60-67] Rather than representing a pathogenic system, the relationship between the gut microbiota and the host is assumed to be typically symbiotic.

However, the relationship between the host and the microbial community in the CF lung, and that between the host and the normal gut microbiota, may represent opposite ends of a continuum of pathogenicity (**Fig. 1**). Further, this is a spectrum onto which all microbiota at all colonised sites of the body fall. In this scheme, the normally symbiotic relationship between the host and the gut microbiota can occasionally become unbalanced (a phenomenon referred to as dysbiosis), as with the introduction of new pathogens or antibiotics,[68] in some cases resulting in precipitation of disease. Whilst CF airway disease is characterised by acute periods of pulmonary exacerbation in which the relationship between the host and infecting microbial community is considered highly pathogenic, in much of the intervening periods the relationship is of relatively low pathogenicity, referred to as periods of stability, with exacerbations perhaps precipitated by new viral infections or other perturbing factors.[69]

These commonalities between the gut and the CF lung are reflected in a number of significant similarities in the behavior of their microbial systems. One example is the social interaction between microbial community members, exemplified among colonising bacteria by the expression of quorum sensing mediated behaviors such as biofilm formation, which is important for bacterial colonisation in both contexts.[70,71] Other quorum-sensing-mediated behaviors that are also key to infection, such as the expression of virulence factors, have also been shown to be exhibited

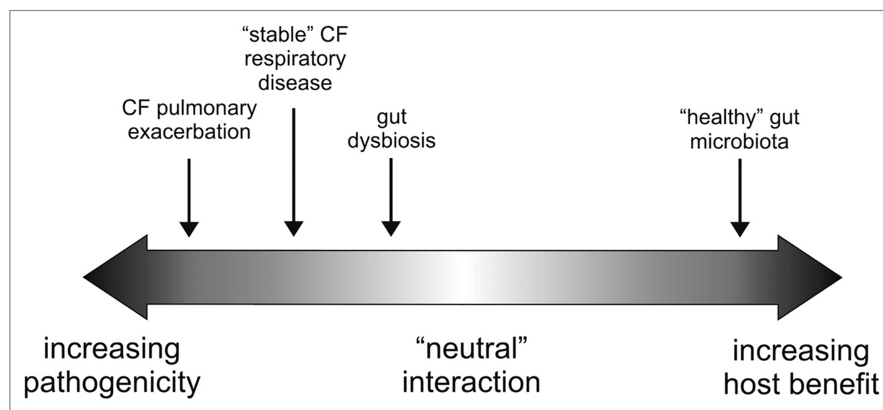

Figure 1. The relative positions of the interactions between the host and the microbial community in stable and exacerbating CF pulmonary disease, and in the healthy and dysbiotic gut, on a conceptual continuum of pathogenicity.

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by bacteria in both chronic respiratory infections,[72,73] and the gut.[74,75] Whilst the impact of such coordinated behavior on both virulence and drug impact is well recognised in the CF lung, it has been the focus of less intense study in the gut to date. However, in common with the CF lung, the virulence of clinically important pathogens in the gut has been shown to be modulated by other members of the bacterial community. For example, the synthesis of Shiga toxin by enterohaemorrhagic *Escherichia coli* O157:H7 has been shown to be inhibited by factors secreted by other members of the intestinal microbiota.[76]

The Developing Microbiota

Once established, the microbiota associated with a particular region of the body, such as the gut, is relatively stable in terms of its composition within individuals compared to the levels of variation in microbial constituency among different niches or among the same niches in different individuals.[77] Further, data derived from the study of murine models and humans suggest that, following challenge such as antibiotic treatment, these microbiota will largely revert to their pre-perturbation state,[68,78] provided that perturbation is not too severe.[79,80] Data derived from analysis of the CF lung suggest a comparable situation (data not shown). By comparison, differences that are seen when comparing the same microbiota from body sites in different individuals are likely to reflect a range of factors, including host genetics,[65] niche physiology,[81] as well as the range of environmental microbes that an individual is exposed to.[82,83] Determining the reasons for such differences will require further in depth study; however, some clues may help to elucidate the manner in which these microbiota develop.

The temporal progression of the gut microbiota in neonates has been investigated through metagenomic analysis, with evidence emerging of an ordered progression toward the microbial communities found in the guts of adults.[84,85] Whilst microbial communities varied widely from infant to infant in their composition, the distinct features of the microbial community of each infant were recognizable for intervals of weeks to months. Whilst still distinct, a convergence toward a profile characteristic of the adult gastrointestinal tract was observed by the end of the first year of life.[85] Further analysis of these data by Trosvik et al.[86] suggested the convergence observed is driven, in the main, by simple, phylum-level interactions between colonising species. Further, interactions between the gut microbiota and the host during this process of development may be key to the emergence of the adult intestinal immune system,[87,88] also reviewed by Guarner et al.[89]

While such detailed analysis of the temporal progression of airway colonisation in CF is yet to be performed, recent work has exposed the complexity of microbial communities in the airways of young children with CF, and there is evidence of species succession in this context. Culture-based studies indicate that *Staphylococcus aureus* and non-encapsulated *H. influenzae* are isolated early in life, whereas most CF patients become infected with *P. aeruginosa* over time.[90] In a previous article, we described the use of culture-independent, T-RFLP analysis of bronchoalveolar lavage fluid (BALF) samples from sputum-producing paediatric patients. The data derived showed that there were no significant differences in diversity between the bacterial communities detected among samples from young children with CF and those from CF adults.[91] Further, Harris et al.[92] applied 16S ribosomal RNA gene clone sequence analysis to the characterisation of bacteria in BALF from non-sputum and sputum producing children at single time points. This process identified a total of 65 different bacterial species (including the traditionally recognised CF pathogens) in BALF sampled from 28 children with a mean age of 8 years. These data suggest that, even in the paediatric CF airways, the bacterial diversity is significantly higher than previously recognised. As such, application of further metagenomic analysis will greatly enhance our understanding of the progression of microbial colonisation and infection of the CF airways, the processes that drive this progression, and why some species are able to persist in this niche whilst others are not.

The Influence of Microbiota on Known Pathogens

"Colonisation resistance" is a term used to describe the phenomenon whereby pathogenic species are excluded from a region of the body, such as the gut, by the presence of a microbiota.[93] This exclusion may be due to a range of factors, including changes in oxidation-reduction potential and pH,[94] elaboration of inhibitory substances (e.g., bacteriocins, fatty acids, hydrogen sulfide),[94,95] competition for nutrients,[95] and competition for adhesion sites.[96] Specific examples include the competition for proline with indigenous *Escherichia coli* that has been shown to limit growth of *E. coli* O157:H7 in the intestines of mice,[97] and the age-associated changes in gut microbiota that have been correlated with increased risk of *C. difficile* infection.[98] Interestingly,

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the interaction between the host and the gut microbiota can be exploited by certain enteropathogenic bacteria in order to overcome colonisation resistance. The presence of species such as *Citrobacter rodentium* and *Salmonella enterica* in the gut triggers a inflammatory host response. The resulting production of antimicrobial peptides by the host affects the composition of the gut microbial community and suppresses its growth, allowing the pathogenic species to become established.[99-101]

Disruption of gut microbiota through the use of antibiotics can also change its composition, potentially facilitating pathogenic behavior by some bacterial species and infection with new pathogens,[78] and also reviewed by Walk et al.[102] The concepts of clinically relevant interactions between microbial community members, and further complication by antibiotics, have important implications for the CF lung where, once established, clinically significant pathogens are rarely eradicated despite frequent antibiotic treatment. Whilst the CF lung has been the focus of less work in this area, there are some examples of interspecies interactions that influence colonisation by clinically significant pathogens. For instance, as patients acquire *P. aeruginosa, S. aureus* tends to be detected less frequently,[103,104] although both species are commonly co-isolated.[105] A mechanism for this relationship has been suggested involving the production by *P. aeruginosa* of anti-staphylococcal substances, including 4-hydroxy-2-heptylquinoline-N-oxide (HQNO), when co-infecting CF airways with *S. aureus*.[104] However, this compound also protects *S. aureus* during co-culture from commonly used aminoglycoside antibiotics.[105] Furthermore, it has been shown that prolonged growth of *S. aureus* with *P. aeruginosa* selects for typical *S. aureus* small colony variants, which have stable aminoglycoside resistance,[106] and are persistent in chronic infections.[107]

Importantly, however, the presence of a particular species in the polymicrobial community of the CF airways or the gut is not necessarily sufficient to cause clinically evident disease. For example, pathogenic bacteria are able to exist in a carrier state, where the host does not develop disease but harbours an infective organism that may cause disease in others. This phenomenon is exhibited in the GI tract by both *Neisseria meningitidis* and *Vibrio cholerae*, with great clinical significance.[108,109] Similarly, lung infection with nontuberculous mycobacteria can cause substantial decline in lung function in some, but not all, people with CF.[110] An example of a mechanism for such variation in pathogenicity within individual microbial species is the ability of some pathogenic bacteria to turn on or off virulence genes depending on circumstance, as with the production of exotoxins by *Clostridium difficile*.[111] Moreover, where pathogenicity is conferred by the expression of discrete traits, the potential influence of co-infecting species to affect this expression of pathogenesis is clear. In vitro evidence for the potential for clinically relevant polymicrobial interactions in the CF lung comes from Sibley et al.[45] who demonstrated that pathogenically important behaviors of *P. aeruginosa* can be impacted by the presence of bacterial species that are routinely disregarded by most treating clinicians.

Host-Microbiota Interactions

As discussed above, once established, the gut microbiota is typically characterised by a symbiotic relationship with the host. The impacts of this interaction can be far-reaching and profound. The gut microbiota can impact host metabolism and energy storage, with the breakdown of this symbiotic relationship implicated in the development of obesity and type 2 diabetes,[112] as well as conditions such as irritable bowel syndrome (IBS)[113] and inflammatory bowel disease.[114] Further, as discussed by Lyte,[115] microbes both produce and recognise neuroendocrine hormones, potentially allowing for direct signalling with the host. This capacity may be manifested in the suggested association between changes in the composition of gut microbes and changes in the normal functioning of the nervous system,[116] and further, between gut dysbiosis and changes in host behavior.[113,117]

The relationship between the gut microbiota and the host also appears to be bi-directional. Metaproteomic analysis has shown that a significant proportion of the proteins found in faeces is involved in innate immune defense, indicating an extensive effort by the host to regulate the microbial population.[118] The intestinal microbial balance may also be temporarily changed by an alteration in diet,[119] in turn linked to an impact on memory and anxiety-like behavior.[120]

Evidence of such a complex interrelationship between the gut microbiota and the host has implications for the fundamental ways in which we consider CF lung infections. They present the possibility that a microbial community may impact not only the host tissues with which it is in direct contact, but also physically and functionally distant areas of the body. This raises important considerations both for the way in which exacerbations of chronic infections may be triggered, and for the effects of the antimicrobial therapies commonly used in their treatment.

Disruption of the symbiotic relationship between the gut microbiota and the host has been implicated in serious diseases, such as ulcerative colitis, irritable bowel syndrome and *Clostridium difficile* colitis,[113,121,122] and as reviewed by Peterson et al.[114] If the gut model is translated to the CF respiratory tract, a context where any colonising bacterial community is likely abnormal and highly pathogenic, the question can be asked: can a balance between the host and the infective bacterial community in CF be achieved that results in a reduction in pathogenicity? For example, are there bacterial communities that may be associated with minimal pathogenesis? Are there treatments, such as probiotics, nutrients, or other chemicals, that would favor such "beneficial" communities? This concept may be controversial given the presumed highly deleterious impact of airway colonisation. However, in light of the current lack of microbiological explanation for the transition between periods of increased and decreased symptoms, and the common use but only limited efficacy of antimicrobial strategies designed to significantly disrupt this polymicrobial community, this is a possibility that warrants consideration. Further, there is evidence that, as airway disease progresses, primary colonising

species in the CF lung often behave less and less like traditional pathogens. This is illustrated by *P. aeruginosa,* where strains present in advanced CF infections differ substantially from "wild-type" *P. aeruginosa,* with the counterintuitive loss of virulence factors required for acute infection,[66,123] potentially resulting in a reduction in immune response.[66,124] Therefore, the behavior of the bacteria present in the community may be as important as the identification of the species present, rendering the clinical effects of antibiotics less predictable.

Importantly, the polymicrobial nature of the bacterial community infecting the CF lung or the gut microbiota may influence host immunity in a way that affects pathogenesis. For an example from the oral cavity, *Streptococcus cristatus* has been shown to be capable of dampening the IL-8 response induced by infection with *Fusobacterium nucleatum* in epithelial cell culture. Using oral epithelial cells as a model, Mans et al.[125] demonstrated that the degree of complexity of a mixed microbiota influenced the transcriptional response to infection of the epithelium. Thus, both the behavior of the microbiota and the host response can vary depending upon the complexity of the microbiota. In an example of potential relevance for the CF airway, species such as *Streptococcus salivarius* strain K12 have been shown to antagonise *Pseudomonas aeruginosa*-induced IL-8 secretion from human bronchial epithelial cells. This again suggests a role for commensal species in modulating human epithelial cell immune responses in the nasopharynx and, potentially, other parts of the airway.[126]

Elucidating the Relationships Between the Host and Microbiota

Culture-independent techniques are increasingly being used to explore the bacterial content of locations in or on healthy individuals, such as the skin,[77,127,128] the oral cavity,[77,129,130] and the gut.[77,131,132] These microbial habitats do not exist in isolation, but form a microbial landscape interconnecting various niches in each individual.[133] The physical links between the lower airways and areas such as the skin, oral cavity, and gastrointestinal tract provide a ready source of colonising agents for the CF lungs.[134] However, as discussed here, the interrelationships between the microorganisms occupying these different areas of the body may also be mediated indirectly, through their interactions with the host.

The characterisation of the membership and dynamics of the polymicrobial communities colonising areas such as the gut, oral cavity and skin is still in its relative infancy. Much of the complexity of these systems remains to be characterised, but technological advances, such as the development of next generation sequencing technologies, offer an opportunity to make significant gains in our understanding. These endeavours form part of a wider effort to construct an integrated "whole-body" view of the microbial communities associated with healthy people over time.[77] A natural progression of such an approach is to extend these nascent models to include pathogenic polymicrobial communities that exist within chronic infections, such as the CF lung.

There are, however, important methodological considerations to be made when undertaking such work that, if ignored, have the potential to significantly undermine the information that is derived. For example, in order to delineate the links between the microbes that colonise a niche, such as the gut or the CF lung, and parameters of disease, it is essential that we are able not only to identify the species that are present, but also the dynamics of these microbial populations over clinically appropriate timeframes. Culture-independent analysis in these contexts has typically involved the analysis of DNA extracted directly from a sample as a template for PCR amplification. However, such analysis is unable to distinguish between live and dead bacterial cells. Due to the persistence of DNA within clinical samples, such analyses may therefore be unable to detect rapid decreases in bacterial populations,[135] with bias potentially being introduced due to variations between species in the period of retention of amplifiable DNA.[136] One strategy that can be used to limit analyses to viable cells is the use of propidium monoazide (PMA) treatment of samples prior to nucleic acid extraction.[8] PMA intercalates into extracellular DNA or into DNA in cells whose membrane integrity has been lost. In contrast, PMA is excluded from intact cells. Subsequent exposure of a PMA-treated sample to a bright light source causes covalent cross-links to form, rendering the extracellular or dead cell DNA unable to act as a PCR template. The application of this process has been shown to effectively limit downstream DNA-based processing to signals from viable bacterial cells alone, with its use demonstrated in the metagenomic profiling of the bacterial communities in the CF lung.[8]

Other confounding factors which should be considered when interpreting PCR-based microbial community analyses are the biases introduced from primer selection, variations in nucleic acid extraction efficiencies between species, differential PCR amplification, the number of PCR cycles used, generation of chimeric sequences and the effect of *rrn* operon copy numbers and heterogeneity on diversity estimates.[137-140] In addition, although pyrosequencing has greatly enhanced our ability to monitor microbial communities, this technology throws up different challenges that must be addressed before analysing the data generated.[141] The use of single reads as primary data, in combination with the intrinsic error rate of pyrosequencing, means that diversity estimates are artificially inflated, sometimes by several orders of magnitude.[142,143] On a positive note however, computational methods to remove these artefacts should greatly reduce this problem in the future.[143]

The consideration of these factors will greatly strengthen the data obtained through the metagenomic profiling of microbiota. The challenge that remains for the study both of chronic infections affecting CF airways and of gut microbiota is to construct theoretical frameworks in which the implications of these data can be understood. To this end, studies that examine the behavior, and not just the species constituencies, of polymicrobial communities found in both healthy and diseased contexts, may help to construct such models. Ultimately, however, combining the molecular approaches outlined above with careful epidemiological analysis in longitudinal, well-designed,

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translational studies, will be necessary to generate these models. While in vitro models may afford new insights into the interactions between community members and the host, and molecular studies of patient samples can provide a description of the complexity of these communities, the powerful marriage of clinical and laboratory-based approaches will be required to more directly answer the question of how these communities influence human health and disease.

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References

1. Rogers GB, Carroll MP, Bruce KD. Studying bacterial infections through culture-independent approaches. J Med Microbiol 2009; 58:1401-18.
2. Rogers GB, Stressmann FA, Walker AW, Carrroll MP, Bruce KD. Lung infections in cystic fibrosis; deriving clinical insight from microbial complexity. Exp Rev Mol Diagnostics 2010; In press.
3. Bittar F, Richet H, Dubus JC, Reynaud-Gaubert M, Stremler N, Sarles J, et al. Molecular detection of multiple emerging pathogens in sputa from cystic fibrosis patients. PLoS ONE 2008; 3:2908.
4. Ecker DJ, Sampath R, Massire C, Blyn LB, Hall TA, Eshoo MW, et al. Ibis T5000: A universal biosensor approach for microbiology. Nat Rev Microbiol 2008; 6:553-8.
5. Rogers GB, Carroll MP, Serisier DJ, Hockey PM, Jones G, Bruce KD. Characterization of bacterial community diversity in cystic fibrosis lung infections by use of 16s ribosomal DNA terminal restriction fragment length polymorphism profiling. J Clin Microbiol 2004; 42:5176-83.
6. Sibley CD, Parkins MD, Rabin HR, Duan K, Norgaard JC, Surette MG. A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients. Proc Natl Acad Sci USA 2008; 105:15070-5.
7. Rogers GB, Carroll MP, Serisier DJ, Hockey PM, Kehagia V, Jones GR, et al. Bacterial activity in cystic fibrosis lung infections. Respir Res 2005; 6:49.
8. Rogers GB, Stressmann FA, Koller G, Daniels T, Carroll MP, Bruce KD. Assessing the diagnostic importance of nonviable bacterial cells in respiratory infections. Diagn Microbiol Infect Dis 2008; 62:133-41.
9. Rogers GB, Daniels TT, Tuck A, Carroll MP, Connett GJ, David GJ, et al. Studying bacteria in respiratory specimens by using conventional and molecular microbiological approaches. BMC Pulm Med 2009; 9:14.
10. Loux HR, Coplin WMV. Chronic phagedaena due to mixed infection. Ann Surg 1902; 35:586-97.
11. White GR. VIII, the identity of proteus infection and hospital gangrene: A case of mixed infection with *Aerogenes capsulatus* and *Proteus vulgaris*. Ann Surg 1902; 36:762-6.
12. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005; 308:1635-8.
13. Flint HJ, Duncan SH, Scott KP, Louis P. Interactions and competition within the microbial community of the human colon: Links between diet and health. Environ Microbiol 2007; 9:1101-11.
14. Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD, et al. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl Environ Microbiol 1999; 65:4799-807.
15. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, et al. Real-time PCR in clinical microbiology: Applications for routine laboratory testing. Clin Microbiol Rev 2006; 19:165-256.
16. Feinstein LM, Sul WJ, Blackwood CB. Assessment of bias associated with incomplete extraction of microbial DNA from soil. Appl Environ Microbiol 2009; 75:5428-33.
17. McIlroy SJ, Porter K, Seviour RJ, Tillett D. Extracting nucleic acids from activated sludge which reflect community population diversity. Antonie Van Leeuwenhoek 2009; 96:593-605.
18. Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: Uses, limitations and future applications in acute-care settings. Lancet Infect Dis 2004; 4:337-48.
19. Clarridge JE, 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev 2004; 17:840-62.
20. Juste A, Thomma BP, Lievens B. Recent advances in molecular techniques to study microbial communities in food-associated matrices and processes. Food Microbiol 2008; 25:745-61.
21. Malik S, Beer M, Megharaj M, Naidu R. The use of molecular techniques to characterize the microbial communities in contaminated soil and water. Environ Int 2008; 34:265-76.
22. Nocker A, Burr M, Camper AK. Genotypic microbial community profiling: A critical technical review. Microb Ecol 2007; 54:276-89.
23. Sontakke S, Cadenas MB, Maggi RG, Diniz PP, Breitschwerdt EB. Use of broad range16S rDNA PCR in clinical microbiology. J Microbiol Methods 2009; 76:217-25.
24. Razvi S, Saiman L. Microbiology of cystic fibrosis: Role of the clinical microbiology laboratory, susceptibility and synergy studies and infection control. In: Hodson M, Geddes D, Bush A, eds. *Cystic Fibrosis.* 3rd ed. London: Hodder Arnold 2007; 123-33.
25. Gilligan PH. Microbiology of airway disease in patients with cystic fibrosis. Clin Microbiol Rev 1991; 4:35-51.
26. Heijerman H. Infection and inflammation in cystic fibrosis: A short review. J Cyst Fibros 2005; 4:3-5.
27. Tunney MM, Field TR, Moriarty TF, Patrick S, Doering G, Muhlebach MS, et al. Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. Am J Respir Crit Care Med 2008; 177:995-1001.
28. Palmer KL, Aye LM, Whiteley M. Nutritional cues control *Pseudomonas aeruginosa* multicellular behavior in cystic fibrosis sputum. J Bacteriol 2007; 189:8079-87.
29. O'May CY, Sanderson K, Roddam LF, Kirov SM, Reid DW. Iron-binding compounds impair *Pseudomonas aeruginosa* biofilm formation, especially under anaerobic conditions. J Med Microbiol 2009; 58:765-73.
30. Rubin BK. Mucus, phlegm and sputum in cystic fibrosis. Respir Care 2009; 54:726-32.
31. Pedersen SS, Kharazmi A, Espersen F, Hoiby N. *Pseudomonas aeruginosa* alginate in cystic fibrosis sputum and the inflammatory response. Infect Immun 1990; 58:3363-8.
32. Barth AL, Pitt TL. The high amino-acid content of sputum from cystic fibrosis patients promotes growth of auxotrophic *Pseudomonas aeruginosa*. J Med Microbiol 1996; 45:110-9.
33. Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, et al. Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. J Clin Invest 2002; 109:317-25.
34. Yoon SS, Hennigan RF, Hilliard GM, Ochsner UA, Parvatiyar K, Kamani MC, et al. *Pseudomonas aeruginosa* anaerobic respiration in biofilms: Relationships to cystic fibrosis pathogenesis. Dev Cell 2002; 3:593-603.
35. Fegan M, Francis P, Hayward AC, Fuerst JA. Heterogeneity, persistence and distribution of *Pseudomonas aeruginosa* genotypes in cystic fibrosis patients. J Clin Microbiol 1991; 29:2151-7.
36. Grothues D, Koopmann U, von der Hardt H, Tummler B. Genome fingerprinting of *Pseudomonas aeruginosa* indicates colonization of cystic fibrosis siblings with closely related strains. J Clin Microbiol 1988; 26:1973-7.
37. Willner D, Furlan M, Haynes M, Schmieder R, Angly FE, Silva J, et al. Metagenomic analysis of respiratory tract DNA viral communities in cystic fibrosis and non-cystic fibrosis individuals. PLoS One 2009; 4:7370.
38. Bouchara JP, Hsieh HY, Croquefer S, Barton R, Marchais V, Pihet M, et al. Development of an oligonucleotide array for direct detection of fungi in sputum samples from patients with cystic fibrosis. J Clin Microbiol 2009; 47:142-52.
39. Brockhurst MA, Buckling A, Gardner A. Cooperation peaks at intermediate disturbance. Curr Biol 2007; 17:761-5.
40. Harrison F, Browning LE, Vos M, Buckling A. Cooperation and virulence in acute *Pseudomonas aeruginosa* infections. BMC Biol 2006; 4:21.
41. Nadell CD, Xavier JB, Levin SA, Foster KR. The evolution of quorum sensing in bacterial biofilms. PLoS Biol 2008; 6:14.
42. Nadell CD, Xavier JB, Foster KR. The sociobiology of biofilms. FEMS Microbiol Rev 2009; 33:206-24.
43. Xavier JB, Foster KR. Cooperation and conflict in microbial biofilms. Proc Natl Acad Sci USA 2007; 104:876-81.
44. Jenkinson HF, Lamont RJ. Oral microbial communities in sickness and in health. Trends Microbiol 2005; 13:589-95.
45. Sibley CD, Duan K, Fischer C, Parkins MD, Storey DG, Rabin HR, et al. Discerning the complexity of community interactions using a Drosophila model of polymicrobial infections. PLoS Pathog 2008; 4:1000184.
46. Frank DN, St. Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 2007; 104:13780-5.
47. Frank DN, Pace NR. Gastrointestinal microbiology enters the metagenomics era. Curr Opin Gastroenterol 2008; 24:4-10.
48. Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. Science 2001; 292:1115-8.
49. Mai V, Morris JG Jr. Colonic bacterial flora: Changing understandings in the molecular age. J Nutr 2004; 134:459-64.
50. Zoetendal EG, Cheng B, Koike S, Mackie RI. Molecular microbial ecology of the gastrointestinal tract: From phylogeny to function. Curr Issues Intest Microbiol 2004; 5:31-47.
51. Jernberg C, Sullivan A, Edlund C, Jansson JK. Monitoring of antibiotic-induced alterations in the human intestinal microflora and detection of probiotic strains by use of terminal restriction fragment length polymorphism. Appl Environ Microbiol 2005; 71:501-6.
52. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J 2007; 1:56-66.
53. Donskey CJ, Hujer AM, Das SM, Pultz NJ, Bonomo RA, Rice LB. Use of denaturing gradient gel electrophoresis for analysis of the stool microbiota of hospitalized patients. J Microbiol Methods 2003; 54:249-56.
54. Green GL, Brostoff J, Hudspith B, Michael M, Mylonaki M, Rayment N, et al. Molecular characterization of the bacteria adherent to human colorectal mucosa. J Appl Microbiol 2006; 100:460-9.
55. Vanhoutte T, Huys G, Brandt E, Swings J. Temporal stability analysis of the microbiota in human feces by denaturing gradient gel electrophoresis using universal and group-specific 16S rRNA gene primers. FEMS Microbiol Ecol 2004; 48:437-46.

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56. Armougom F, Raoult D. Use of pyrosequencing and DNA barcodes to monitor variations in Firmicutes and Bacteroidetes communities in the gut microbiota of obese humans. BMC Genomics 2008; 9:576.
57. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol 2008; 6:280.
58. Hoffmann C, Hill DA, Minkah N, Kirn T, Troy A, Artis D, et al. Community-wide response of the gut microbiota to enteropathogenic *Citrobacter rodentium* infection revealed by deep sequencing. Infect Immun 2009; 77:4668-78.
59. Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, et al. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. ISME J 2010; 4:232-41.
60. Bry L, Falk PG, Midtvedt T, Gordon JI. A model of host-microbial interactions in an open mammalian ecosystem. Science 1996; 273:1380-3.
61. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science 2006; 313:1126-30.
62. Hooper LV, Stappenbeck TS, Hong CV, Gordon JI. Angiogenins: A new class of microbicidal proteins involved in innate immunity. Nat Immunol 2003; 4:269-73.
63. Keilbaugh SA, Shin ME, Banchereau RF, McVay LD, Boyko N, Artis D, et al. Activation of RegIIIbeta/gamma and interferon gamma expression in the intestinal tract of SCID mice: An innate response to bacterial colonisation of the gut. Gut 2005; 54:623-9.
64. Klaasen HL, Van der Heijden PJ, Stok W, Poelma FG, Koopman JP, Van den Brink ME, et al. Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. Infect Immun 1993; 61:303-6.
65. Lopez-Boado YS, Wilson CL, Hooper LV, Gordon JI, Hultgren SJ, Parks WC. Bacterial exposure induces and activates matrilysin in mucosal epithelial cells. J Cell Biol 2000; 148:1305-15.
66. Smith EE, Buckley DG, Wu Z, Saenphimmachak C, Hoffman LR, D'Argenio DA, et al. Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. Proc Natl Acad Sci USA 2006; 103:8487-92.
67. Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via paneth cells. Proc Natl Acad Sci USA 2002; 99:15451-5.
68. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol 2008; 6:280.
69. Wat D, Gelder C, Hibbitts S, Cafferty F, Bowler I, Pierrepoint M, et al. The role of respiratory viruses in cystic fibrosis. J Cyst Fibros 2008; 7:320-8.
70. Macfarlane S. Microbial biofilm communities in the gastrointestinal tract. J Clin Gastroenterol 2008; 42:142-3.
71. Parsek MR, Singh PK. Bacterial biofilms: An emerging link to disease pathogenesis. Annu Rev Microbiol 2003; 57:677-701.
72. Fontaine L, Boutry C, Guedon E, Guillot A, Ibrahim M, Grossiord B, et al. Quorum-sensing regulation of the production of blp bacteriocins in *Streptococcus thermophilus*. J Bacteriol 2007; 189:7195-205.
73. Winstanley C, Fothergill JL. The role of quorum sensing in chronic cystic fibrosis *Pseudomonas aeruginosa* infections. FEMS Microbiol Lett 2009; 290:1-9.
74. Tsou AM, Frey EM, Hsiao A, Liu Z, Zhu J. Coordinated regulation of virulence by quorum sensing and motility pathways during the initial stages of *Vibrio cholerae* infection. Commun Integr Biol 2008; 1:42-4.
75. Wu L, Holbrook C, Zaborina O, Ploplys E, Rocha F, Pelham D, et al. *Pseudomonas aeruginosa* expresses a lethal virulence determinant, the PA-I lectin/adhesin, in the intestinal tract of a stressed host: The role of epithelia cell contact and molecules of the quorum sensing signaling system. Ann Surg 2003; 238:754-64.
76. de Sablet T, Chassard C, Bernalier-Donadille A, Vareille M, Gobert AP, Martin C. Human microbiota-secreted factors inhibit shiga toxin synthesis by enterohemorrhagic *Escherichia coli* O157:H7. Infect Immun 2009; 77:783-90.
77. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science 2009; 326:1694-7.
78. Croswell A, Amir E, Teggatz P, Barman M, Salzman NH. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric Salmonella infection. Infect Immun 2009; 77:2741-53.
79. Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML, Young VB. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. Infect Immun 2009; 77:2367-75.
80. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J 2007; 1:56-66.
81. Macfarlane S, Dillon JF. Microbial biofilms in the human gastrointestinal tract. J Appl Microbiol 2007; 102:1187-96.
82. Mandar R, Mikelsaar M. Transmission of mother's microflora to the newborn at birth. Biol Neonate 1996; 69:30-5.
83. Thompson-Chagoyan OC, Maldonado J, Gil A. Colonization and impact of disease and other factors on intestinal microbiota. Dig Dis Sci 2007; 52:2069-77.
84. Favier CF, Vaughan EE, De Vos WM, Akkermans AD. Molecular monitoring of succession of bacterial communities in human neonates. Appl Environ Microbiol 2002; 68:219-26.
85. Palmer C, Bik EM, Digiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. PLoS Biol 2007; 5:177.
86. Trosvik P, Stenseth NC, Rudi K. Convergent temporal dynamics of the human infant gut microbiota. ISME J 2010; 4:151-8.
87. Hasegawa M, Osaka T, Tawaratsumida K, Yamazaki T, Tada H, Chen GY, et al. Transitions in oral and intestinal microflora composition and innate immune receptor-dependent stimulation during mouse development. Infect Immun 2010; 78:639-50.
88. Martino DJ, Currie H, Taylor A, Conway P, Prescott SL. Relationship between early intestinal colonization, mucosal immunoglobulin A production and systemic immune development. Clin Exp Allergy 2008; 38:69-78.
89. Guarner F. Hygiene, microbial diversity and immune regulation. Curr Opin Gastroenterol 2007; 23:667-72.
90. Renders N, Verbrugh H, Van Belkum A. Dynamics of bacterial colonisation in the respiratory tract of patients with cystic fibrosis. Infect Genet Evol 2001; 1:29-39.
91. Rogers GB, Carroll MP, Connett GJ, Serisier DJ, Hockey PM, Kehagia V, et al. Bacterial community diversity in the CF lung. Pediatric Pulmonology 2005; 40:95-7.
92. Harris JK, De Groote MA, Sagel SD, Zemanick ET, Kapsner R, Penvari C, et al. Molecular identification of bacteria in bronchoalveolar lavage fluid from children with cystic fibrosis. Proc Natl Acad Sci USA 2007; 104:20529-33.
93. Stecher B, Hardt WD. The role of microbiota in infectious disease. Trends Microbiol 2008; 16:107-14.
94. Hentges DJ. Enteric pathogen—normal flora interactions. Am J Clin Nutr 1970; 23:1451-6.
95. Freter R, Brickner H, Botney M, Cleven D, Aranki A. Mechanisms that control bacterial populations in continuous-flow culture models of mouse large intestinal flora. Infect Immun 1983; 39:676-85.
96. Davidson JN, Hirsh DC. Use of the K88 antigen for in vivo bacterial competition with porcine strains of enteropathogenic *Escherichia coli*. Infect Immun 1975; 12:134-6.
97. Momose Y, Hirayama K, Itoh K. Competition for proline between indigenous *Escherichia coli* and *E. coli* O157:H7 in gnotobiotic mice associated with infant intestinal microbiota and its contribution to the colonization resistance against *E. coli* O157:H7. Antonie Van Leeuwenhoek 2008; 94:165-71.
98. Hopkins MJ, Macfarlane GT. Changes in predominant bacterial populations in human faeces with age and with *Clostridium difficile* infection. J Med Microbiol 2002; 51:448-54.
99. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe 2007; 2:204.
100. Salzman NH, Hung K, Haribhai D, Chu H, Karlsson-Sjoberg J, Amir E, et al. Enteric defensins are essential regulators of intestinal microbial ecology. Nat Immunol 2010; 11:76-82.
101. Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, Kremer M, et al. *Salmonella enterica* serovar *typhimurium* exploits inflammation to compete with the intestinal microbiota. PLoS Biol 2007; 5:2177-89.
102. Walk ST, Young VB. Emerging insights into antibiotic-associated diarrhea and *Clostridium difficile* infection through the lens of microbial ecology. Interdiscip Perspect Infect Dis 2008; 2008:125081.
103. CF Foundation. Patient registry annual data report 2007 (http://cff.org/UploadedFiles/research/ClinicalResearch/2007-patient-registry-report.pdf). 2007.
104. Machan ZA, Taylor GW, Pitt TL, Cole PJ, Wilson R. 2-heptyl-4-hydroxyquinoline N-oxide, an antistaphylococcal agent produced by *Pseudomonas aeruginosa*. J Antimicrob Chemother 1992; 30:615-23.
105. Hoffman LR, Deziel E, D'Argenio DA, Lepine F, Emerson J, McNamara S, et al. Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 2006; 103:19890-5.
106. Miller MH, Edberg SC, Mandel LJ, Behar CF, Steigbigel NH. Gentamicin uptake in wild-type and aminoglycoside-resistant small-colony mutants of *Staphylococcus aureus*. Antimicrob Agents Chemother 1980; 18:722-9.
107. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, et al. Small colony variants: A pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 2006; 4:295-305.
108. Vanden Broeck D, Horvath C, De Wolf MJ. *Vibrio cholerae*: Cholera toxin. Int J Biochem Cell Biol 2007; 39:1771-5.
109. Yazdankhah SP, Caugant DA. *Neisseria meningitidis*: An overview of the carriage state. J Med Microbiol 2004; 53:821-32.
110. Olivier KN, Weber DJ, Lee JH, Handler A, Tudor G, Molina PL, et al. Nontuberculous Mycobacteria in Cystic Fibrosis Study Group. Nontuberculous mycobacteria II: Nested-cohort study of impact on cystic fibrosis lung disease. Am J Respir Crit Care Med 2003; 167:835-40.
111. Voth DE, Ballard JD. *Clostridium difficile* toxins: Mechanism of action and role in disease. Clin Microbiol Rev 2005; 18:247-63.
112. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444:1027-31.

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113. Collins SM, Denou E, Verdu EF, Bercik P. The putative role of the intestinal microbiota in the irritable bowel syndrome. Dig Liver Dis 2009; 41:850-3.
114. Peterson DA, Frank DN, Pace NR, Gordon JI. Metagenomic approaches for defining the pathogenesis of inflammatory bowel diseases. Cell Host Microbe 2008; 3:417-27.
115. Lyte M. The microbial organ in the gut as a driver of homeostasis and disease. Med Hypotheses 2009; In press.
116. Forsythe P, Sudo N, Dinan T, Taylor VH, Bienenstock J. Mood and gut feelings. Brain Behav Immun 2010; 24:9-16.
117. Mahony JB. Detection of respiratory viruses by molecular methods. Clin Microbiol Rev 2008; 21:716-47.
118. Verberkmoes NC, Russell AL, Shah M, Godzik A, Rosenquist M, Halfvarson J, et al. Shotgun metaproteomics of the human distal gut microbiota. ISME J 2009; 3:179-89.
119. Li F, Hullar MA, Schwarz Y, Lampe JW. Human gut bacterial communities are altered by addition of cruciferous vegetables to a controlled fruit- and vegetable-free diet. J Nutr 2009; 139:1685-91.
120. Li W, Dowd SE, Scurlock B, Acosta-Martinez V, Lyte M. Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. Physiol Behav 2009; 96:557-67.
121. Lepage P, Colombet J, Marteau P, Sime-Ngando T, Dore J, Leclerc M. Dysbiosis in inflammatory bowel disease: A role for bacteriophages? Gut 2008; 57:424-5.
122. McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Dig Dis 1998; 16:292-307.
123. Yang L, Haagensen JA, Jelsbak L, Johansen HK, Sternberg C, Hoiby N, et al. In situ growth rates and biofilm development of *Pseudomonas aeruginosa* populations in chronic lung infections. J Bacteriol 2008; 190:2767-76.
124. Hollsing AE, Granstrom M, Vasil ML, Wretlind B, Strandvik B. Prospective study of serum antibodies to *Pseudomonas aeruginosa* exoproteins in cystic fibrosis. J Clin Microbiol 1987; 25:1868-74.
125. Mans JJ, von Lackum K, Dorsey C, Willis S, Wallet SM, Baker HV, et al. The degree of microbiome complexity influences the epithelial response to infection. BMC Genomics 2009; 10:380.
126. Cosseau C, Devine DA, Dullaghan E, Gardy JL, Chikatamarla A, Gellatly S, et al. The commensal *Streptococcus salivarius* K12 downregulates the innate immune responses of human epithelial cells and promotes host-microbe homeostasis. Infect Immun 2008; 76:4163-75.
127. Gao Z, Tseng CH, Pei Z, Blaser MJ. Molecular analysis of human forearm superficial skin bacterial biota. Proc Natl Acad Sci USA 2007; 104:2927-32.
128. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, NISC Comparative Sequencing Program, et al. Topographical and temporal diversity of the human skin microbiome. Science 2009; 324:1190-2.
129. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005; 43:5721-32.
130. Nasidze I, Li J, Quinque D, Tang K, Stoneking M. Global diversity in the human salivary microbiome. Genome Res 2009; 19:636-43.
131. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005; 308:1635-8.
132. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature 2009; 457:480-4.
133. Wilson M. Microbial Inhabitants of Humans: Their Ecology and Role in Health and Disease. Cambridge University Press 2005.
134. Kamei A, Koh AY, Gadjeva M, Priebe GP, Lory S, Pier GB. Analysis of acquisition of *Pseudomonas aeruginosa* gastrointestinal mucosal colonization and horizontal transmission in a murine model. J Infect Dis 2010; 201:71-80.
135. Fenollar F, Raoult D. Molecular diagnosis of bloodstream infections caused by non-cultivable bacteria. Int J Antimicrob Agents 2007; 30:7-15.
136. Rovery C, Greub G, Lepidi H, Casalta JP, Habib G, Collart F, et al. PCR detection of bacteria on cardiac valves of patients with treated bacterial endocarditis. J Clin Microbiol 2005; 43:163-7.
137. Bonnet R, Suau A, Dore J, Gibson GR, Collins MD. Differences in rDNA libraries of faecal bacteria derived from 10- and 25-cycle PCRs. Int J Syst Evol Microbiol 2002; 52:757-63.
138. Farrelly V, Rainey FA, Stackebrandt E. Effect of genome size and rrn gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Appl Environ Microbiol 1995; 61:2798-801.
139. Farris MH, Olson JB. Detection of Actinobacteria cultivated from environmental samples reveals bias in universal primers. Lett Appl Microbiol 2007; 45:376-81.
140. von Wintzingerode F, Gobel UB, Stackebrandt E. Determination of microbial diversity in environmental samples: Pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 1997; 21:213-29.
141. Gomez-Alvarez V, Teal TK, Schmidt TM. Systematic artifacts in metagenomes from complex microbial communities. ISME J 2009; 3:1314-7.
142. Kunin V, Engelbrektson A, Ochman H, Hugenholtz P. Wrinkles in the rare biosphere: Pyrosequencing errors lead to artificial inflation of diversity estimates. Environ Microbiol 2010; 12:118-23.
143. Quince C, Lanzen A, Curtis TP, Davenport RJ, Hall N, Head IM, et al. Accurate determination of microbial diversity from 454 pyrosequencing data. Nat Methods 2009; 6:639-41.