PERSISTENCE OF FACULTATIVE PATHOGENS

The Institute of Hygiene's Professor Dr Ulrich Dobrindt lays on facultative pathogens, bacterial adaptation and risk assessment

Facultative pathogens can colonise their hosts without causing infection. Comparative and functional analyses of asymptomatic colonisers will help us to understand why these bacteria can establish symptomatic infections, but also asymptomatically colonise or persist in the human body. Asymptomatic colonisation and persistence can interfere with proper risk assessment and outbreak surveillance of many pathogens. Whole genome-based analyses of colonising and infecting isolates supports the risk assessment and novel therapeutic approaches against bacterial infections, as Professor Ulrich Dobrindt discusses.

On the zoonotic potential of avian pathogenic *E. coli*

Escherichia coli bacteria usually colonise as harmless commensals in the gut of healthy humans and many animals. In addition, intestinal pathogenic *E. coli* can cause diarrhoea. Extraintestinal pathogenic *E. coli* (ExPEC) are the most common cause of community and hospital-acquired extraintestinal infections including urinary tract infection and bacteremia. ExPEC infections in ruminants and poultry include mastitis and colibacillosis. Human ExPEC and avian pathogenic *E. coli* (APEC) derive from the same phylogenetic lineages or share common evolutionary roots. Accordingly, APEC are considered a reservoir of virulence and resistance-associated genes for human pathogens, and may pose a zoonotic risk. The detection of certain human ExPEC lineages in poultry-derived products, but less frequently in meat products from other food animals, corroborates the above-mentioned hypothesis. Even if this zoonotic risk cannot be generalised, but holds only true for a certain amount of human infections caused by APEC, it will be highly relevant for public health, food animal production and food safety to monitor the introduction of often antibiotic resistant APEC lineages via contaminated food into the healthy human population.

In contrast to diarrhoeagenic *E. coli,* which are obligate pathogens, and thus usually directly associated with disease, human ExPEC and APEC are facultative pathogens. They persist as members of the normal intestinal microbiota without causing extraintestinal disease. To proof transmission of such APEC strains from poultry (products) or other environmental sources to humans, it is difficult to trace back clinical isolates to certain products due to the time period that lies between their acquisition and the establishment of an extraintestinal infection. Whole-genome sequencing (WGS) can support infection control surveillance and outbreak investigations also in such cases outside of an outbreak scenario. WGS allows the identification of strains based on single nucleotide polymorphisms (SNPs). By comparing bacterial genome sequences, the genetic relatedness of isolates from food products and patients can be analysed. One challenging and critical point in such traceback or case-control studies is to agree on the level of genetic relationship that convincingly proves a particular product as the source of a given human clinical isolate, e.g. the number of SNPs.

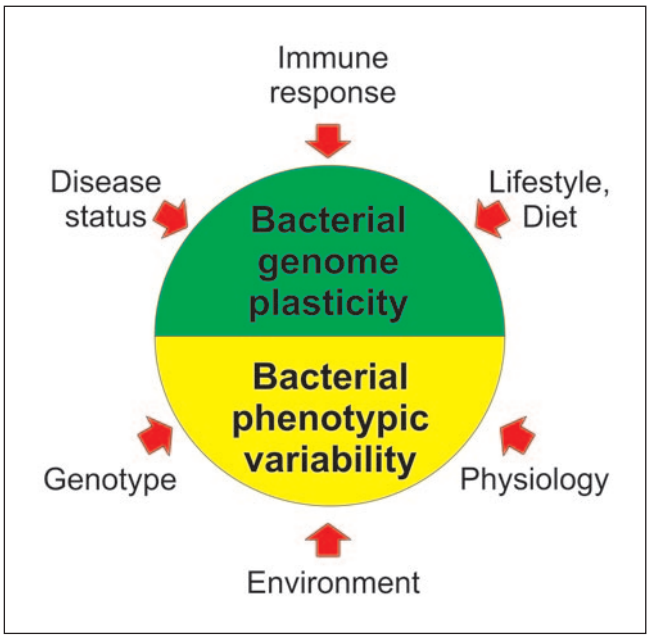

Fig. 1 Different host factors can affect genome plasticity and phenotypic variability of long term colonising or persisting bacteria. Host parameters (including the host innate and adaptive immune response, diet, lifestyle, disease status, physiology and genotype) drive bacterial adaptation and differential bacterial gene expression in response to the individual host situation or niche.

Close relationships between hosts and bacteria: Impact on personalised microbiomes and asymptomatic carriage

The close relationship between humans and their colonising microbes also becomes manifest in distinct human-associated microbiomes. Although in large parts unexplained, factors such as the environment, diet, host genetics and microbial exposure contribute to the shaping of the microbiota.[1] Similarly, long term colonising facultative pathogens living in close interaction with humans and animals have to adapt themselves to constantly changing growth conditions. They can often establish a lifestyle

that is at the intersection of commensalism and pathogenesis. These microbes are phenotypically variable and can manipulate host responses. This enables them to establish symptomatic infections, but also to asymptomatically colonise the human body. By switching between virulence and commensalism, they can cause recurrent or chronic health problems. Which factors direct whether or not bacteria enter the virulent or commensal lifestyle are often poorly understood. In addition, asymptomatic bacterial carriage can lead to mutations and altered gene expression in the host, which could contribute to the development of cancer or inflammatory diseases. The molecular basis of asymptomatic carriage of bacterial pathogens has not yet been fully investigated. We have to investigate in more detail bacterial traits, which promote asymptomatic carriage as well as the impact of host and environmental stimuli (e.g. nutrient availability, hormones, host susceptibility factors) on bacterial colonisation without overt induction of host responses. The expected results are relevant for many bacterial pathogens including food pathogens and will thus improve risk assessment and food safety.

Analysis of bacterial adaptation during close bacterium-host interaction

Urinary tract infection (UTI) is a good model to study microbial adaptation to the human host, because the different lifestyles of uropathogens and asymptomatic colonisers are clearly displayed. Uropathogens destroy the mucosal barrier in the kidneys and cause severe disease. On the other hand, asymptomatic bacteriuria (ABU) is the most common form of UTI and resembles commensalism. Patients can be asymptomatically colonised with the same *E. coli* strain for extended periods of time and do not develop symptoms. ABU is protective against recurrent, symptomatic infection.[2,3] The inability of ABU isolates to induce a marked host response has generally been explained by a lack of virulence.[4,5] However, ABU *E. coli* strains can also actively modulate host gene expression. This prevents the induction of the host immune response, thus enhancing their persistence.[6] By modulating host gene expression, these bacteria may prevent a marked immune activation, thereby maintaining their commensal lifestyle. A deeper understanding of the mechanisms by which these bacteria modulate the host's immune response will support the development of improved strategies to treat and prevent disease-associated host responses.

E. coli adaptation to long term asymptomatic colonisation of the urinary tract involves reductive genome evolution: symptomatic uropathogens can evolve towards commensalism in human hosts by a reduction of their overall genome content, virulence gene inactivation and modification of transcriptional regulators. Extended sequencing and characterisation of ABU isolates confirmed that long term growth in the urinary tract selects for reductive evolution, including the loss or specific inactivation of virulence genes.[7] Interestingly, our genome-wide analysis of a single bacterial strain's evolution in different deliberately colonised patients led to the surprising insight that hosts personalise their colonising bacteria. Whole genome comparison of the ancestral ABU *E. coli* isolate and of its descendants after therapeutic bladder colonisation of different patients indicated a small number of mutations affecting bacterial metabolism and virulence. Transcriptome and proteome analyses of these isolates revealed unique gene expression and adaptation patterns in each patient. These results suggest that adaptive bacterial evolution is driven by individual host environments, in addition to stochastic events. Nevertheless, we need to understand how hosts personalise their microbiota and to what extent host factors affect bacterial adaptation.

References

1 The Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 486:207-2014.

2 Sundén F, Håkansson L, Ljunggren E, Wullt B (2010) *Escherichia coli* 83972 bacteriuria protects against recurrent lower urinary tract infections in patients with incomplete bladder emptying. *J Urol.* 184:179-185.

3 Köves B, Salvador E, Grönberg-Hernández J, Zdziarski J, Wullt B, Svanborg C, Dobrindt U (2014) Rare emergence of symptoms during long-term asymptomatic *Escherichia coli* 83972 carriage without an altered virulence factor repertoire. *J Urol.* 191:519-528

4 Klemm P1, Roos V, Ulett GC, Svanborg C, Schembri MA (2006) Molecular characterization of the *Escherichia coli* asymptomatic bacteriuria strain 83972: the taming of a pathogen. *Infect Immun.* 74:781-785.

5 Zdziarski J, Brzuszkiewicz E, Wullt B, Liesegang H, Biran D, Voigt B, Grönberg-Hernández J, Ragnarsdóttir B, Hecker M, Ron EZ, Daniel R, Gottschalk G, Hacker J, Svanborg C, Dobrindt U (2010) Host imprints on bacterial genomes - rapid, divergent evolution in individual patients. *PLoS Pathog.* 6:e1001078.

6 Lutay N, Ambite I, Grönberg Hernández J, Rydström G, Ragnarsdóttir B, Puthia M, Nadeem A, Zhang J, Storm P, Dobrindt U, Wullt B, Svanborg C (2013) Bacterial control of host gene expression through RNA polymerase II. *J Clin Invest.* 123:2366-2379.

7 Salvador E, Wagenlehner F, Köhler CD, Mellmann A, Hacker J, Svanborg C, Dobrindt U (2012) Comparison of asymptomatic bacteriuria *Escherichia coli* isolates from healthy individuals versus those from hospital patients shows that long-term bladder colonization selects for attenuated virulence phenotypes. *Infect Immun.* 80:668-678.

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