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THE WAR ON CANCER

Does the future of cancer treatment lie in the large scale analysis of glycan binding biomolecules?

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AT THE GENOMIC LEVEL

The combination of comparative and functional genomics helps to prevent and combat bacterial infections of humans and livestock, as Professor Ulrich Dobrindt discusses

The Microbial Genome Plasticity – Molecular Infection Biology group at the Institute of Hygiene, University of Münster, is applying next generation DNA sequencing techniques to study genome plasticity of the bacterium *Escherichia coli* and its impact on (i) pathogenicity, (ii) antibiotic resistances, and (iii) bacterial adaptation in different niches and environments.

Normally, *E. coli* bacteria belong to the normal gut flora of humans and many animals. They persist as harmless commensals in the mucous layer of the intestinal tract. Several pathogenic variants, however, cause intestinal or extraintestinal infections. Intestinal pathogenic *E. coli* include many important foodborne pathogens and cause diarrhoea, whereas *E. coli* causing extraintestinal diseases are one of the major sources of urinary tract infection and bacteraemia in humans. Frequent extraintestinal infections in ruminants and poultry include mastitis and colibacillosis, respectively, and cause heavy economic losses due to mortality and morbidity. As certain human bacteraemia and avian pathogenic *E. coli* (APEC) isolates cannot always be clearly distinguished based on molecular epidemiology and their virulence-associated genome content, APEC are considered as a reservoir of virulence and resistance-associated genes for human pathogens and a zoonotic risk cannot be excluded.

New discoveries

E. coli is a heterogeneous species because its genome is highly dynamic and consequently, *E. coli* pathogens are remarkably diverse. The ability of certain *E. coli* variants to accumulate and express multiple virulence associated determinants increases their fitness and adaptability and determines their potential to cause disease. In addition to their role in the disease process, these virulence factors enable the pathogens to exploit their hosts in ways unavailable to commensals, and thus to spread and to persist in the bacterial community. A variety of virulence-associated and fitness factors have been extensively studied. However, the function and regulation of a large number of determinants requires further analysis to better understand their contribution to virulence.

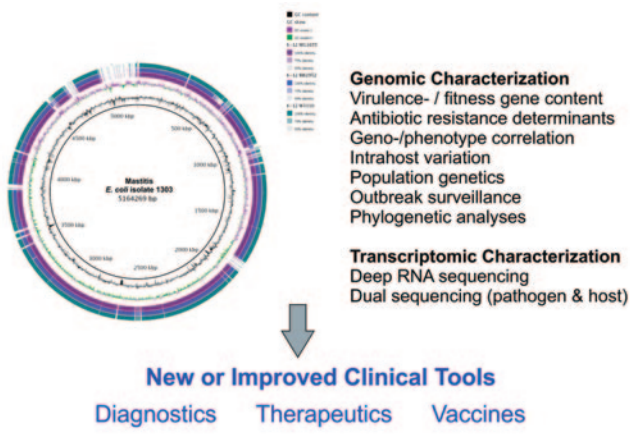

Fig. 1 Next generation sequencing and comparative genomics: powerful tools for infectious disease research. Whole genome sequence analyses help us to gain insight into the molecular pathogenesis and population structure of E. coli. *Data obtained from comparative genomic and transcriptomic analyses can be used to further develop new tools for diagnostic, preventive and therapeutic approaches*

Using next generation sequencing and functional as well as comparative genomic analyses, we characterise the virulence and fitness-associated genome content of *E. coli* pathogens and commensals (Fig. 1). We study mechanisms of bacterium-host interaction which contribute to host colonisation. Complete genome sequence data help to identify virulence traits. Furthermore, individual differences in genome content and structure may contribute to the development of improved diagnostic approaches against extraintestinal pathogenic *E. coli* (ExPEC) infection in humans and livestock (Fig. 2).

We study differential regulation of gene expression in commensals and pathogens by genome-wide transcriptome sequencing, metabolic analyses and interaction strategies with eukaryotes which may distinguish commensals from pathogens or different *E. coli* pathotypes (Fig. 3). Deep RNA sequencing also enables the discovery of small, non-coding RNAs (sRNAs), some of which seem to be key regulators that are involved in managing and fine tuning of bacterial virulence gene expression. The simultaneous analysis of the bacterial and eukaryotic transcriptomes is a challenging and recently expanding application of next generation sequencing. New insights into host-bacterium interaction or bacterial adaptability to, e.g. environmental changes or antibiotic treatment, can be achieved. The identification of new virulence or resistance-associated bacterial pathways, or differential regulation of common genes in pathogens and commensals, will enable us to design new therapies to specifically disrupt these virulence or resistance pathways. Similarly, the characterisation and modulation of sRNAs may improve antimicrobial therapies. Our studies on genome content and regulation of gene expression on bovine mastitis isolates identified several virulence and fitness-associated traits which are candidate diagnostic markers or targets for preventive and therapeutic approaches.

Studies and surveillance

Next generation sequencing and comparative analysis of complete genome sequences offer a high discriminatory power for the subtyping of bacterial isolates and allows the ability to distinguish even closely related isolates. The combination of genomic data and

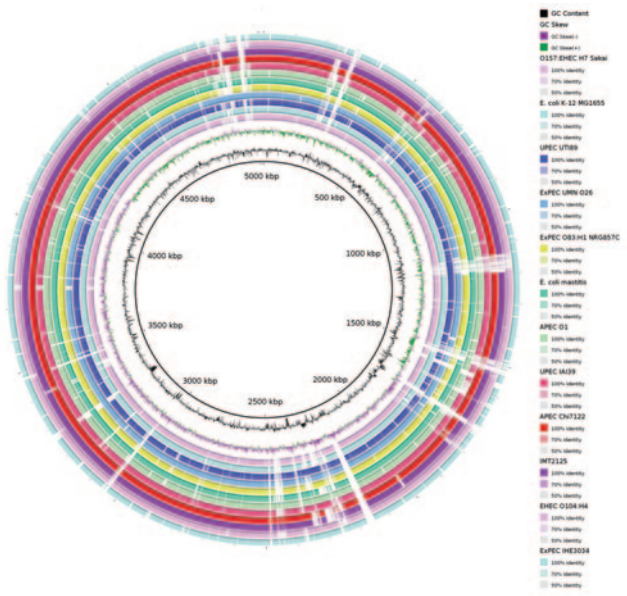

Fig. 2 Comparative analysis of complete genome sequence data of pathogenic and non-pathogenic E. coli *identifies virulence, resistance and fitness associated genomic information. The comparison of complete genome sequences of different* E. coli *isolates helps to identify genomic regions which are specific for individual strains or for pathotypes (e.g. uropathogenic* E. coli, *UPEC; avian pathogenic* E. coli, *APEC; bovine mastitis* E. coli; *enterohaemorrhagic* E. coli, *EHEC)*

temporal-geographic or disease or phenotype information allows the identification of so far unrecognised genetic relationships, and infer the sequence of evolutionary events. The comparative and functional genomic analysis of closely related clinical ExPEC isolates funded in the second ERA-Net Pathogenomics call allowed us to study bacterial adaptation strategies to long term colonisation and chronic infection. Our results showed that long term colonisation and adaptation is in part achieved by phenotypic attenuation, thus preventing activation of host mucosal inflammation as well as metabolic adaptation. These adaptation mechanisms appear to be unique for each host.

Because of the increasing number and the spread of (multi) resistant *E. coli* and their high genomic diversity, these strains constitute a serious problem regarding the treatment of these pathogens. The major tools used for combatting *E. coli* infections are antibiotics and vaccines. 'Traditional' vaccines, based on inactivated bacteria, protect against infection with the homologous strains but are less efficient against heterologous strains. Hence, vaccination for ExPEC is not widely practised because of the large pheno and genotypic variability of strains responsible for field outbreaks. The use of antibiotic therapy is problematic because of the increasing resistance to antibiotics. Moreover, to meet consumer demands and regulations, antibiotic use in animal production is expected to substantially decrease in the future. Consequently, there is an urgent need for alternative approaches to treat and prevent *E. coli* infection. One promising approach to control such infections is through the use of vaccines, providing that it is possible to design an efficient vaccine that is protective against a large number of strains. In the framework on an EMIDA ERA-Net consortium, we participated in the

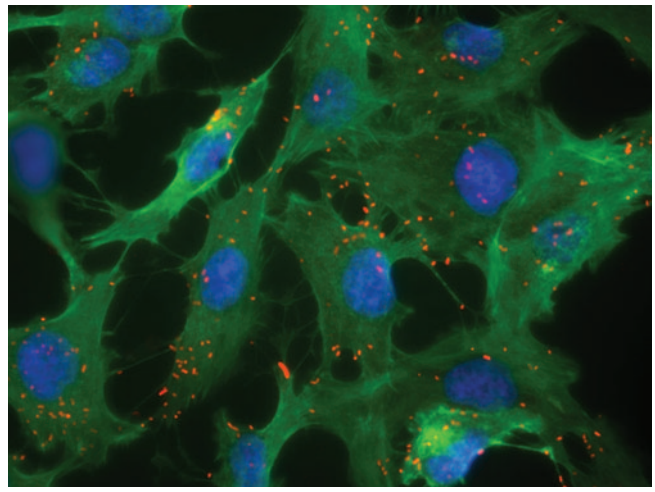

Fig. 3 Interaction of ExPEC with eukaryotic cells. Dual RNA sequencing enables the simultaneous investigation of host-pathogen interaction of both, the pathogen and the eukaryotic host, on the transcriptional level. Infecting ExPEC strain (red), host cell actin (green), host cell nucleus (blue)

development of such an APEC vaccine. The population structure of APEC and commensal chicken isolates has been analysed, and protective antigens preferentially expressed in pathogenic strains, as identified by comparative genomics, have been successfully tested regarding their ability to protect against heterologous infection. The work of our EMIDA ERA-Net consortium should improve vaccine development which should give a broad range of protection against the important APEC clones.

We anticipate that the use of next generation sequencing techniques to study the pathogenesis, resistance or adaptation of pathogenic *E. coli* causing disease in animals will continue. The continuation of research on virulence and resistance mechanisms of pathogenic *E. coli* will contribute to improved diagnostic, preventive and therapeutic approaches and, thus, also to consumer safety. We are well positioned to continue with next generation sequencing analyses in this field, and are also very much interested to go beyond the analysis based on single strains and pure cultures by analysing the role of probiotics and more complex bacterial populations during infection.

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