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## Oral Abstract Book

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# The extensive taxonomic and functional diversity of the Ethiopian village chicken microbiota

Laura Glendinning[1], Xinzheng Jia[2,3], Adebabay Kebede[4,5], Jianlin Han[3], Mick Watson[1], Olivier Hanotte[4,6]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]Foshan University, Foshan, China. [3]Chinese Academy of Agricultural Sciences, Beijing, China. [4]International Livestock Research Institute, Addis Ababa, Ethiopia. [5]Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia. [6]University of Nottingham, Nottingham, United Kingdom

## Abstract

Our understanding of the chicken microbiota has been greatly expanded due to studies using metabarcoding, culturomics, and metagenomics. However, most research has focused on commercial chickens raised in biosecure facilities; it is therefore likely that much microbial diversity has been missed, as free-range birds are known to have more diverse microbiota than conventionally housed birds.

In this study we characterised the microbiota of Ethiopian village chickens using metagenomic sequencing. These village chickens had predominantly indigenous genotypes, uncontrolled breeding, natural hatching, and a diverse diet from scavenging supplemented with limited crop residues. Shotgun sequencing was performed on DNA from 240 caecal samples from 26 villages in 15 districts. Short reads were assembled into contigs and then binned into putative genomes, before dereplication and quality control steps were performed to produce high-quality microbial metagenome assembled genomes (MAGs).

We constructed 9,977 strain-level MAGs and 1,790 species-level MAGs, representing diverse taxonomies, including 22 phyla from the Archaea and bacteria. We discovered 9682 strains, 1242 species, and 84 genera that were not present in previous chicken microbial genome datasets. These genomes encoded diverse carbohydrate degrading enzymes and metabolic pathways for the fermentation of various forms of fibre, highlighting their importance in fermenting indigestible carbohydrates. Pathways for methanogenesis, nitrogen, and sulphur metabolisms were also identified in these genomes.

Our findings demonstrate the richness of the microbiota in Ethiopian village chickens, highlighting the potential for further diversity discovery from rural chickens of other regions.

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## Human cytomegalovirus strain diversity and dynamics reveal the donor lung as a major contributor after transplantation

Büsra Külekci[1], Stefan Schwarz[2], Nadja Brait[3], Nicole Perkmann-Nagele[4], Peter Jaksch[2], Konrad Hoetzenecker[2], Elisabeth Puchhammer-Stöckl[1], Irene Goerzer[1]

[1]Center for Virology, Medical University of Vienna, Vienna, Austria. [2]Department of Thoracic Surgery, Medical University of Vienna, Vienna, Austria. [3]Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, Netherlands. [4]Division of Clinical Virology, Medical University of Vienna, Vienna, Austria

### Abstract

Mixed human cytomegalovirus (HCMV) strain infections are frequent in lung transplant recipients (LTRs). To date, the influence of the donor (D) and recipient (R) HCMV-serostatus on intra-host HCMV strain composition and replication dynamics after transplantation is only poorly understood.

Here, we investigated ten pre-transplant lungs from HCMV-seropositive donors, and 163 sequential HCMV-DNA positive plasma and bronchoalveolar lavage samples from 50 LTRs with multiviremic episodes post-transplantation. The study cohort included D+R+ (38%), D+R- (36%), and D-R+ (26%) patients. All samples were subjected to quantitative genotyping by short amplicon deep sequencing, and 24 thereof were additionally PacBio long-read sequenced for genotype linkages.

We find that D+R+ patients show a significantly elevated intra-host strain diversity compared to D+R- and D-R+ patients (P=0.0089). Both D+ patient groups display significantly higher replication dynamics than D- patients (P=0.0061). Five out of ten pre-transplant donor lungs were HCMV-DNA positive, whereof in three multiple HCMV strains were detected. In one recipient with viremia post-transplantation multiple donor strains reactivated, indicating that multi-strain transmission via lung transplantation is likely. Using long reads, we show that intra-host haplotypes can share distinctly linked genotypes, which limits overall intra-host diversity in mixed infections.

Together, our findings demonstrate donor-derived strains as a main source for increased HCMV strain diversity and dynamics post-transplantation, while a relatively limited number of intra-host strains may facilitate rapid adaptation to changing environments in the host. These results foster targeted strategies to mitigate the potential transmission of the donor strain reservoir with the allograft.

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# Using genomes of novel uncultured lineages to investigate archaeal evolution and habitat adaptation.

Paul Sheridan[1,2], Tom Williams[2], Cécile Gubry-Rangin[1]

[1]School of Biological Sciences, University of Aberdeen, Aberdeen, United Kingdom. [2]School of Biological Sciences, University of Bristol, Bristol, United Kingdom

## Abstract

**Background.** Novel microbial lineages, discovered using culture-independent techniques, offer us the opportunity to answer central questions in microbial ecology and evolution, such as how microorganisms have adapted to specific ecological niches. Concurrently, gene tree-species tree reconciliation techniques have enabled us to examine the mechanisms of genome evolution across large evolutionary timescales and predict the gene content of ancient organisms.

**Methods.** We reconstructed genomes representing novel branches of the archaeal tree of life from soil metagenomic sequences and used them in combination with gene tree-species tree reconciliation techniques to address fundamental questions in archaeal genome evolution. We examined two phyla: Thaumarchaeota, whose history involved relatively few ecosystem transitions and Thermoplasmatota, whose history involved many ecosystem transitions.

**Results.** In Thaumarchaeota, two ancient periods of extensive lateral gene acquisition cooccurred with expansion into terrestrial environments. Subsequent duplication of these novel genes, including those for carbohydrate transport and coenzyme metabolism, drove genome expansion and likely facilitated niche specialisation in soils. Whereas Thermoplasmatota was punctuated by several periods of extensive lateral acquisition. Importantly, functional genes, such as those for aerobic respiration and acid tolerance, that appeared conserved across diverse lineages and different habitats were in fact laterally acquired multiple times from different donors and maintained through convergent evolution, rather than vertically inherited from the common ancestor.

**Conclusions.** Our results suggest a previously under-appreciated importance of gene duplication and convergent evolution in archaeal habitat adaptation and highlight the importance of culture-independent genome sequencing to our understanding of genome evolution across the tree of life.

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# Using direct whole genome sequencing as an alternate approach to characterise *Campylobacter* species from human stool

Bilal Djeghout[1], Steven Rudder[1], Thanh Le-Viet[1], Gemma Kay[1], Ngozi Elumogo[1,2], Andrew Page[1], John Wain[1,3], Nicol Janecko[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Norfolk and Norwich University Hospital, Norwich, United Kingdom. [3]Faculty of Medicine, University of East Anglia, Norwich, United Kingdom

## Abstract

*Campylobacter*, the leading cause of bacterial gastroenteritis is difficult to culture, and modern rapid diagnostic methods hinder the characterisation of key genomic attributes. Direct whole genome sequencing of metagenomes offers an opportunity to by-pass culture and improve characterisation.

The aim of this study was to characterise *Campylobacter* from stool metagenomes of *Campylobacter*-infected patients using direct whole genome sequencing (WGS).

Twenty-four PCR-verified, stool samples with unknown quantities of *Campylobacter* were subjected to adapted DNA extraction methods and short-read sequencing using the Illumina Novaseq platform. A bespoke bioinformatics pipeline with tailored tools for metagenomes and *Campylobacter* characterisation was used.

*Campylobacter* genomes were partially assembled and characterised from 62.5% (15/24) of metagenomic assembled genomes (MAGs), of which ten samples contained *Campylobacter* genomes with more than 87% genome completeness. Of the 15 *Campylobacter* MAGs with sufficient read quality, 12 were identified as *C. jejuni*, one as *C. coli* and two as *C. fetus subsp. fetus*. Of the *C. jejuni*, six sequence types (ST) (ST5136, ST45, ST61, ST21, ST9897, ST2254) were identified in six genomes; remaining STs were unidentified. Resistance genotypes were detected in 7/12 *C. jejuni*, mediated by blaOXA-61 and blaOXA-65 genes. *C. coli* was identified as ST827 containing blaOXA-61 gene. *C. fetus subsp. fetus* contained insufficient reads for further characterisation. Virulence indicator genes were detected in *C. jejuni* and *C. coli* ranging from 1 to 127 genes.

This study successfully demonstrates the ability of tailored metagenomic and bioinformatic approaches to characterise key attributes of this low abundance foodborne pathogen in stool samples.

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# The phylogroup shift in commensal *Escherichia coli* population during international travel, as detected from metagenome assembled genomes

Matthew Davies[1,2], John Penders[2], Petra Wolffs[2], Willem van Schaik[1], Alan McNally[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Maastricht University, Maastricht, Netherlands

## Abstract

*Escherichia coli* is an important gut commensal that has potential to harbour antimicrobial resistance (AMR) genes. *E. coli* can be separated into eight genetically distinct phylogroups (A, B1, B2, C, D, E, F, G) that can vary by host specificity, virulence and pathogenicity. *E. coli* can vary drastically between countries, so understanding the role of international travel on resident *E. coli* is important in tackling the global spread of AMR.

Here, we created metagenome assembled genomes (MAGs) from longitudinal faecal samples of 179 Dutch travellers visiting North Africa, East Africa, South Asia or Southeast Asia. Bins were not dereplicated across samples to ensure strain-level variation was preserved, and *E. coli* MAGs were identified and annotated to phylogroup. The core genomes of the 213 *E. coli* MAGs and 420 reference *E. coli* were aligned, and a phylogenetic inference was performed.

Pre-travel, commensal *E. coli* are largely dominated by phylogroups B2 and A, but a drastic shift towards phylogroups A and B1 was detected, and this correlates with travellers acquiring extended-spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E). The within-traveller shift showed that no travellers acquired phylogroup B2 during travel, and the most common shift was a loss of B2 or D into A. These changes occurred similarly across travellers, regardless of acquisition of ESBL-E, and in each region travelled to.

It is epidemiologically important to understand the dynamics of *E. coli* during travel as discovering strains providing colonisation resistance to MDR *E. coli* could help develop strategies to prevent the spread of AMR.

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# Introducing the SeqCode: a nomenclatural code for uncultivated Archaea and Bacteria with DNA sequences as type.

Iain Sutcliffe

Northumbria University, Newcastle upon Tyne, United Kingdom

## Abstract

Presented on behalf of the SeqCode Initiative2.

Over the past two decades, genomics and phylogenomic methods have become fully integrated into prokaryotic systematics, whilst environmental genomics and microbiome studies have revealed a vast diversity of as-yet-uncultivated taxa. However, these as-yet-uncultivated taxa cannot be formally named under the International Code of Nomenclature of Prokaryotes (ICNP), as the International Committee on Systematics of Prokaryotes has recently restated its commitment to the primacy of strains as the types of species. In response to these developments, a new code of nomenclature, the International Code of Nomenclature of Prokaryotes Described from Sequence Data (colloquially the SeqCode), has been developed to allow naming of Archaea and Bacteria using DNA sequences as the nomenclatural type. The SeqCode will thus allow naming of the enormous uncultivated biodiversity discovered through the aid of metagenomic sequencing and single-cell genomics, and of fastidious prokaryotes that cannot reasonably be handled by culture collections or those where legal restrictions prevent the unrestricted distribution of strains between countries. The SeqCode provides an online registration system, the SeqCode Registry (https://seqco.de/) for curation and recording of names. Importantly, the SeqCode also recognises the priority of ICNP names provided they do not violate the priority of SeqCode names, thus minimizing divergence between the systems. In conclusion, the SeqCode will facilitate the naming of taxa in every biome on Earth and promote synergies between the field and laboratory disciplines of microbiology. More details and preprints of the SeqCode and its accompanying papers are available here: https://www.isme-microbes.org/seqcode-news.

2https://www.isme-microbes.org/organizing-committee


14–16 Meredith Street,
London, EC1R 0AB, UK
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