Abstract

5646

Isolation and characterization of non-*Helicobacter pylori Helicobacter* species infecting human stomach

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Non-*Helicobacter pylori Helicobacter* (NHPH), which differs from *H. pylori* in its corkscrew-like spiral morphology, has been known to infect humans since the 1980s. NHPH infection has been observed in patients with gastric diseases, including peptic ulcers, chronic gastritis, gastric cancers, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. *Helicobacter suis*, a bacterial species naturally hosted by pigs and monkeys, is the most prevalent NHPH in the human stomach. *H. suis* has been successfully isolated from pigs, but not from humans, evidence linking human *H. suis* infection to gastric diseases has remained incomplete. Recently, we successfully cultured *H.* suis from human gastric biopsies of patients with gastric diseases. Successful eradication of *H. suis* yielded significant improvements in endoscopic and histopathological findings. Oral infection of mice with *H. suis* clinical isolates elicited gastric and systemic inflammatory responses; in addition, progression of gastric mucosal metaplasia was observed 4 months post-infection. *H. suis* could be isolated from the stomachs of infected mice. Thus, we have certified the virulence of *H. suis* as a human gastric pathogen in accordance with Koch's postulates.

Comparative genomic analysis of *H. suis* using complete genomes of clinical isolates revealed that *H. suis* isolates from humans and pigs were genetically very similar, suggesting possible pig-to-human transmission. The genome of each *H. suis* isolate contained highly plastic genomic regions encoding putative strain-specific virulence factors, including type IV secretion system–associated genes. Since NHPH infection cannot be detected using routine diagnostic methods for *H. pylori* infection, we develop a serological diagnostic method for *H. suis* infection in humans. This study paves the way for epidemiological research aimed at identifying the causal relationships between NHPH including *H. suis* and gastric diseases.

5647

Pinpoint adaptive evolution emerging from >1000 genome comparison

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Aim: How do we (all forms of life) adapt to environments during evolution? Detecting adaptive variations through genome comparison within a species is difficult because most of the variations are neutral and those rare adaptive ones are linked to them. This difficulty may be overcome in *H. pylori*, which would separate linked variations by frequent and fine (~10 codons) mutual homologous recombination. It shows high genome diversity and rapid genome evolution presumably related to ever-changing interaction with its host. It yet shows discrete population genetic structure reflecting human's because of its rare familial transmission.

Methods: 1[1]. We collected its strains in various places in China, Korea and Japan, sequenced their genomes, split them by sequence sharing, and detected highly group-specific SNPs. **2**[2]. We collected its strains from gastric cancer patients and from duodenal ulcer patients, sequenced them and carried out GWAS between these two groups.

Results: 1[1]. All these sequence changes are on proteins involved in host interaction: host interaction proper (including a novel oncoprotein candidate, RkiP), outer membrane, transport and microbiome. To my surprise, they are very often at/by a residue critical for function such as human-mimicking glycosylation site in the flagellin. Genetic differentiation seems to have proceeded through changes in host adaptation. **2**[2]. The associated changes are also on host interaction proteins (including CtbP) and at/by a critical residue such as the one at the lactose binding pocket in TlpC receptor. The disease differentiation seems associated with host adaptative changes.

Conclusion: Genome comparison, either between genetic groups or between disease groups, in *H. pylori*, an organism with free homologous recombination, seems a powerful way of detecting adaptative changes in proteins at the codon-level resolution. I expect it will start a new era in understanding adaptative evolution and protein structure/function. This justifies global efforts towards 1,000,000 *H. pylori* genomes.

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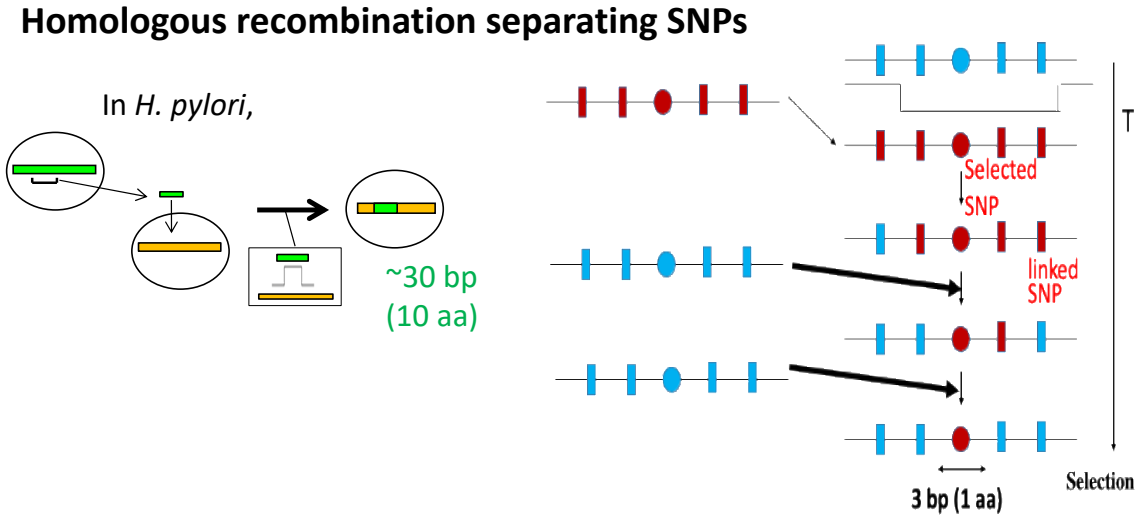

5648

Genome-wide association study of gastric cancer- and duodenal ulcer-derived *Helicobacter pylori* strains reveals discriminatory genetic variations and novel oncoprotein candidates

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Genome-wide association studies (GWASs) can reveal genetic variations associated with a phenotype in the absence of any hypothesis of candidate genes. The problem of false-positive sites linked with the responsible site might be bypassed in bacteria with a high homologous recombination rate, such as Helicobacter pylori, which causes gastric cancer. We conducted a small-sample GWAS (125 gastric cancer cases and 115 controls) followed by prediction of gastric cancer and control (duodenal ulcer) H. pylori strains. We identified 11 single nucleotide polymorphisms (eight amino acid changes) and three DNA motifs that, combined, allowed effective disease discrimination. They were often informative of the underlying molecular mechanisms, such as electric charge alteration at the ligand-binding pocket, alteration in subunit interaction, and mode-switching of DNA methylation. We also identified three novel virulence factors/oncoprotein candidates. These results provide both defined targets for further informatic and experimental analyses to gain insights into gastric cancer pathogenesis and a basis for identifying a set of biomarkers for distinguishing these H. pylori-related diseases (Microbial Genomics, 2021).

5649

Epigenome microevolution associated with DNA methyltransferases' sequence-specificity alteration in *H. pylori*

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Aim: *H. pylori* lives in half of our stomachs and causes gastric cancer. Its evolution is rapid because of high mutation rate and recombination rate, and its genomes are highly diverse. The genome diversity and rapid evolution are likely important for persistent infection conferring adaptability to the host environments.

Adaptive evolution has been regarded to proceed through selection from genomic sequence variations. Here, we propose an epigenome-driven adaptation model in which selection takes place from a diversity of epigenome states. Earlier, we knocked out a dozen of its DNA methyltransferases and analyzed methylome, transcriptome and adaptive phenotypes and demonstrated that many DNA methyltransferases serve as hub transcription factors of a gene regulation network[1]. Branches in this network might be replaced by others in a short evolutionary timescale by sequence-specificity changes in the methyltransferases[2].

To examine such epigenome-driven microevolution, we compared strains sampled from family members.

Methods: We analyzed genome and methylome by PacBio sequencer and transcriptome by RNA-seq with Illumina sequencer for 9 strains from 3 families.

Results: In one family, a unique Type III methylation motif was found in the strain from only one child. Its target genes and genes with expression change overlapped and included host-interaction genes and a 5mC DNA methyltransferase. In another family, we found changes in many methylation motifs, which include a set of related Type I methylation motifs. We were able to reconstruct evolution of the latter by a SNP, deletion and gene conversion in Type I specificity subunit genes. Expression changes were seen in host-interaction genes, a 5mC DNA methyltransferase, DNA polymerase, and ATP synthase.

Conclusion: Analyses of closely-related *H. pylori* strains from family members demonstrated microevolution of their methylome and transcriptome, which are associated with sequence-specificity changes in their DNA methyltransferases. These results support our hypothesis of epigenome-driven adaptive evolution.

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5650

Host and Bug Changes During *H. pylori*-driven Gastric Metaplasia and Dysplasia

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Chronic infection with *Helicobacter pylori* is the primary risk factor for developing stomach cancer. As disease progresses *H. pylori* must adapt to a changing host tissue environment that includes induction of new cell fates in the cells that line the stomach. In a transgenic mouse gastric metaplasia model, we found that strains from unrelated individuals differed in their ability to infect the stomach, to colonize metaplastic glands, and to alter the expression of the metaplasia-associated protein TFF3. We tested representative *H. pylori* isolates collected from the same patient during early and later stages of disease in a mouse model where we can rapidly induce disease-associated tissue changes. Only the later-stage *H. pylori* strains could robustly colonize the diseased stomach environment. We also found that the ability to colonize the diseased stomach was associated with genetic variation in a putative cell surface adhesin gene called *sabB*. Additional experiments revealed that SabB promotes binding to stomach tissue and is critical for stomach colonization by the late-stage strains. Thus, *H. pylori* diversifies its genome during disease progression and these genomic changes highlight critical factors for bacterial persistence.

5651

Ozan Gundogdu CHRO 2022 abstract.

Campylobacter pathogenesis and the chicken microbiome

Campylobacter is the most common global cause of human gastroenteritis, with the species *Campylobacter jejuni* accounting for over 80% of human infections. Despite this, the molecular drivers of *C. jejuni* disease are still poorly understood, complicated by the lack of adequate infection models. In addition, the increasing frequency of infection and the relentless rise of antimicrobial resistance mean that development of new interventions to reduce *Campylobacter* in the food chain is a global imperative. Here we present a holistic approach to combat *C. jejuni* using classical molecular microbiology and omics-based approaches to investigate the physiology and pathogenesis of *C. jejuni* and translate this knowledge to real life settings i.e., implement control strategies.

We demonstrate the presence of complete Type VI Secretion System (T6SS) operons in several *C. jejuni* strains and species and provide evidence of its role in pathogenesis conferring *C. jejuni* a competitive advantage within the host. We investigate how *C. jejuni* interacts with the intrinsic defence machinery of human intestinal epithelial cells (IECs), differentially regulating intracellular and extracellular ROS production in human IECs.

C. jejuni are distributed in most warm-blooded animals, and therefore the main route of transmission is generally foodborne, via the consumption and handling of meat products (particularly poultry). Using our fundamental knowledge of *C. jejuni* we investigate how and when *C. jejuni* appears within poultry via a comprehensive day to day microbiome analysis of the chicken ceca. We also investigate external factors from industrial farms on host-pathogen ecology of the microbiome and *C. jejuni*. Our results show that microbial communities in different industrial production systems are deterministic in elucidating the underlying biological confounders, and these recommendations are transferable to farm practices and diet manipulation leading to improved intervention strategies against *C. jejuni* within the food chain.

5654

The prophages of *Helicobacter pylori*

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Helicobacter pylori is a highly genetically diverse bacterium that infects the human stomach with uneven but high worldwide prevalence. Here, the research progress on *H. pylori* prophages is made. Prophages, integrated virus genomes, are key players of *H. pylori* mobilome. Lysogeny constitutes one of the bacteriophage cycle of reproduction. Although bacteriophages constitute the majority of all organisms in the biosphere, they were late discovered to be consistently present among *H. pylori* genomes. Indeed, advances in second-generation sequencing technologies are associated with an increasing number of reports of *H. pylori* prophages. Most of the prophages are remnant fragments that seem to no longer be able to excise from the host genome. These remnant prophages are similar to complete prophages in terms of synteny, diversity and gene content. Complete prophages present a high genome synteny, a structured population, and some are inducible, producing virus particles. The diversity of prophage genes allowed to distinguish between European populations, which is difficulty achievable using few bacterial genes. There is a strong phylogeographic signal within the phage genes, which is in agreement with a model of co-evolution between the virus and its bacterial host. The complete prophages have on average 34 genes, 28.7 Kb, 36.7% GC content, a pangenome made of 75 genes, a soft-core genome of 10 genes, and in more than half of the cases present a conserved integration site, pointing to vertical transmission. Additionally, prophages present five distinct populations: one African, one Asian, two European and one South-American. The Asian population is the most permeable to the importation of DNA fragments from other phages, in opposition with the genetic isolation signal found in the south-west European population. *H. pylori* phages are among the most recombinogenic phages known so far. These findings contributed to understand the complex co-evolution and interaction phage-bacteria.

5652

New Insights Into Mechanisms That Promote H. pylori-Induced Gastric Cancer

Richard Peek[1]

Epidemiological studies have determined that the attributable risk for distal gastric cancer conferred by *Helicobacter pylori* is approximately 90%. However, only a fraction of colonized persons ever develop neoplasia, and disease risk involves well-choreographed interactions between pathogen and host, which are dependent upon strain-specific bacterial factors, host genotypic traits, and/or environmental conditions. These observations, in conjunction with evidence that carriage of certain strains is inversely related to esophageal adenocarcinoma and atopic diseases, underscore the importance of understanding mechanisms that regulate biological interactions of *H. pylori* with their hosts that promote carcinogenesis. *H. pylori* strains are extremely diverse, freely recombining as panmictic populations. One strain-specific virulence determinant that augments the risk for gastric cancer is the *cag* pathogenicity island, a type 4 secretion system that injects the bacterial oncoprotein CagA into host epithelial cells. However, the use of high throughput sequencing has demonstrated that *H. pylori* does not simply exist as a monoculture within the human stomach but instead, is a resident of a distinct gastric microbial ecosystem. While *H. pylori* is the dominant species, the presence of other microorganisms provides a collaborative environment that augments carcinogenesis. Host polymorphisms within genes that regulate immunity and oncogenesis also heighten the risk for gastric cancer, in conjunction with *H. pylori* strain-specific constituents. Further,

environmental conditions such as iron deficiency can influence *H. pylori* phenotypes that lower the threshold for disease. Delineation of bacterial, host, and environmental mediators that augment gastric cancer risk has profound ramifications for both physicians and biomedical researchers as such findings will not only focus prevention approaches that target *H. pylori*-infected human populations at increased risk for stomach cancer, but will also provide mechanistic insights into inflammatory carcinomas that develop beyond the gastric niche.

5653

Helicobacter pylori multi-cagA genotype

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Infection with CagA positive *Helicobacter pylori* strains is linked to an increased risk for gastric diseases, including gastric cancer. Recent evidence indicates that dynamic expansion and contraction of *cagA* copy number may serve as a novel mechanism to enhance disease development. *H. pylori* can carry multiple tandem copies of *cagA* that can change dynamically. Isolates harboring more cagA copies produced more CagA, thus enhancing toxicity to host cells. Analysis of 314 *H. pylori* clinical strains isolated from patients in South Korea and the United States showed that 7.5% of clinical strains in the United States carried multiple cagA copies whereas none of the South Korean strains did. Comparative genomic analysis divided hpEurope into two groups: hpEurope/type-A and type-B. Only hpEurope/type-B displayed the multi-cagA genotype. Two direct cagA-flanking repeats of a genetic element termed CHA-ud were essential for the multi-cagA genotype in strain PMSS1 (hpEurope/type-B and cagPAI type-B). Furthermore, introduction of this genetic element into strain G27 (hpEurope/type-A and cagPAI type-A) was sufficient to generate the multi-cagA genotype.

To examine the effect of the immune response on *cagA* copy number change, we utilized a mouse model with different immune status. PMSS1 recovered from $Rag1^{-/-}$ mice, lacking functional T or B cells, retained more *cagA* copies. PMSS1 recovered from $IL\text{-}10^{-/-}$ mice, showing intense inflammation, had fewer cagA copies compared to those recovered from wild-type mice. Moreover, cagA copy number of PMSS1 recovered from wild-type and $IL\text{-}10^{-/-}$ mice was positively correlated with the capacity to induce IL-8 secretion at four weeks of infection. This study shows that *H. pylori* PMSS1 in mice with less intense immune response possess higher *cagA* copy number than those infected in mice with more intense

immune response and thus the multi-*cagA* genotype, along with *cagY* recombination, functions as an immune-sensitive regulator of *H. pylori* virulence.

5655

With *Helicobacter pylori* in the stomach and around the world

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Emerging evidence shows that the human microbiota plays a larger role in disease progression and health than previously anticipated. *Helicobacter pylori*, the causative agent of gastric cancer and duodenal and gastric ulcers, was early associated with gastric disease, but it has also been proposed that the accompanying microbiota in *Helicobacter pylori*-infected individuals might affect disease progression and gastric cancer development. However, the presence of any specific microbiome pattern in gastric cancer development is still debatable due to the variation within and between studied populations, non-harmonized clinical scenarios, and methodological differences.

In this presentation I will aim to highlight a number of the methodological consideration that we believe are imperative to discuss in order for the field to advance in our understanding of the gastric microbiota and its presumed changes with the histological and functional precancerous alterations. To support this reasoning, I will use data from our recent metatranscriptomic analysis of gastric biopsies from a population with high *H. pylori* prevalence and high gastric cancer risk.

I will also present our ongoing work of expanding the worldwide collection of *Helicobacter pylori* whole genome sequences, including making them publicly available.

5656

CHECKS AND BALANCES: DIRECT INTERACTION OF SMALL RNAS CJNC140 AND CJNC110 OPTIMIZES EXPRESSION OF KEY PATHOGENIC PHENOTYPES OF CAMPYLOBACTER JEJUNI

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Non-coding small RNAs (sRNAs) represent a new frontier in gene regulation, yet the knowledge of these important regulators for *Campylobacter jejuni* pathogenesis remains sparse. By utilizing gene knockout, RNAseq, northern blotting, electrophoretic mobility shift assays (EMSA), computational analysis, and phenotypic assays, our lab has begun to elucidate the functions of two critical sRNAs, CjNC110 and CjNC140, in *C. jejuni* pathogenesis. Our results indicate that CjNC140 displays a primarily inhibitory role in motility, autoagglutination, L-methionine concentration, AI-2 production, hydrogen peroxide resistance, and early chicken colonization. With the exception of motility, all of these results directly contrast the positive regulation of these phenotypes displayed by CjNC110. RNAseq and northern blotting further demonstrate that expression of CjNC140 increases in the absence of CjNC110, while levels of CjNC110 decrease in the absence of CjNC140. Using EMSA, our results demonstrate a direct interaction between the two sRNAs via GA- (CjNC110) and CU- (CjNC140) rich stem-loops, as well as between CjNC140 and *p19*, a key iron transport gene. Computational analysis reveals both CjNC140 and CjNC110 are highly conserved in *C. jejuni* and supports CjNC140 as a functional homolog of the iron regulatory sRNA, RyhB. In summary, our results demonstrate two highly conserved trans-encoded sRNAs that work in tandem to maintain homeostasis of gene expression and optimize phenotypes critical for chicken colonization by *C. jejuni.*

4606

Rapid Identification of *Campylobacter jejuni* using Single-cell Raman Spectroscopy Combined with Conditional Generative Adversarial Network

Kaidi Wang, Xiangyun Ma, and Xiaonan Lu*

Aim: Rapid detection of *Campylobacter jejuni* is crucial to ensure food safety[1]. Raman spectroscopy has been widely used in the detection and identification of foodborne pathogens as it is reliable, label-free, and easy to perform[2]. However, Raman spectra for single-cell analysis are hindered by relatively low signal-to-noise ratio (SNR) and analytical speed. This study aimed to identify *C. jejuni* using Raman spectroscopy at single-cell level and accelerate the detection using a conditional generative adversarial network (CGAN).

Methods: We investigated the identification of *C. jejuni* from other common foodborne bacteria, including *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella enterica*. Single-cell Raman spectra of these bacteria were collected using a homebuilt confocal Raman spectroscopic system (671-nm laser) combined with a polydimethylsiloxane (PDMS)-based microfluidic device. A CGAN was developed to improve the SNR of single-cell Raman spectra and reduce the spectral collection time. Identification of *C. jejuni* from other foodborne pathogens was performed using a convolutional neural network.

Results: SNR of single-cell Raman spectra of *C. jejuni* increased from 3.35 to 20.27 after spectral recovery using CGAN and the processed Raman spectra recovered most spectral features. An identification accuracy of 96.2% was achieved to identify *C. jejuni* from other common foodborne bacteria using CGAN-recovered Raman spectra, compared to 76.4% using unprocessed spectra. CGAN could accelerate data acquisition time by one order of magnitude (i.e., 30 s v.s. 3 s) via improving the SNR by a factor of ~ 6.

Conclusion: We proposed a rapid and reliable approach to identify *C. jejuni* at single-cell level using Raman spectroscopy combined with CGAN, providing a powerful tool for epidemiological surveillance of *Campylobacter* in the agri-food chain.

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4639

Geno- and phenotypic comparison of antimicrobial resistance in *Campylobacter* spp. isolates from Vietnam and Germany.

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Aim: *Campylobacter* spp. is the most frequent cause of bacterial food-borne gastroenteritis. The majority of *Campylobacter* infections are self-limiting, however, antibiotics were required in 30 % of the reported German campylobacteriosis cases. In this study we compared antibiotic resistances of German (DE) and Vietnamese (VN) *Campylobacter* isolates by comparative geno- and phenotyping methods.

Methods: A total of 494 *Campylobacter* isolates of poultry from Vietnam and Germany were characterized by microdilution using European standardized and customized plate formats. In-house pipelines based on Abricate and the NCBI AMRFinderPlus database were applied to analyze whole genome sequencing data. In case of discrepancies between geno- and phenotype, strains were experimentally reanalyzed, additional web tools were used and raw read mapping against suspected gene variants via the Geneious software were performed.

Results: VN *Campylobacter* isolates displayed more resistances against antimicrobials than DE isolates. The resistance determinants, *erm*(B) for macrolide, *catA* genes for chloramphenicol, *fexA* and *optrA* for florfenicol and *lnu*(C) for lincomycin resistance were exclusively present in VN strains. The gene *aph(2'')-Ii1* conferring gentamicin resistance was only present in one DE strain. Different variants of the *tet*(O) gene and further tetracycline genes *tet*(L) and *tet*(W) as well as streptothricin, streptomycin and kanamycin resistance determinants were differently distributed among DE and VN *Campylobacter* populations. Some of the resistance determinants were putatively located on plasmids or in operon structures on the chromosome. Hence, their spread might be enhanced by co-transfer during natural transformation and by conjugative plasmid transfer.

Conclusion: Most geno- and phenotypic results were concordant. However, several differences in prediction of resistances by genotype suggest the need for some improvements, such as consideration of mosaic genes, other missing gene variants in databases, mutations leading to truncated genes and by deciphering yet unknown resistance mechanisms.

4643

Deciphering the association of cecal microbiota with *Campylobacter* colonization status in broiler chickens

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Aim:

Campylobacter spp. are major food safety concern and are transmitted to humans mainly via contaminated poultry meat. We previously found that some commercial broiler farms consistently produced *Campylobacter*-negative flocks while others had not, even though the farms operated under similar production and management practices. We hypothesized that this colonization difference might be associated with the microbiota compositions of the chicken gut, and the aim of this study is to determine the difference in the microbiota compositions between *Campylobacter*-negative and -positive chicken flocks.

Methods:

Six commercial broiler farms were selected for this study based on their known *Campylobacter* status (three negative and three positive) over multiple production cycles. For each farm on each production cycle (2-3 cycles total), 60 whole ceca (15/each house, four houses in total) were collected from five-week-old broilers. Forty ceca per farm were processed for *Campylobacter* isolation using selective media and for DNA extraction needed for gut microbiota (16S rRNA gene-based) analysis.

Results:

Campylobacter status of all the farms was confirmed to be the same as known before. Cecal microbiota species richness, phylogenetic diversity, community structure, and composition of the *Campylobacter*-positive farms were noticeably different from the *Campylobacter*-

negative farms. Taxonomic analyses revealed that genera such as *Rikenella*, *Campylobacter*, and *Helicobacter* increased in relative abundance, whereas, *Lactobacillus*, *Blautia*, and *Escherichia* decreased in relative abundance in the *Campylobacter*-positive group. Pearson's correlation analysis indicated that *Lactobacillus, Parabacteroides*, and *Lachnospiraceae(f)* were significantly positively correlated with *Campylobacter* absence.

Conclusion:

The results indicate that cecal microbiota diversity and composition significantly differed between the *Campylobacter*-positive and -negative broiler farms. Our findings also suggest that some endogenous cecal microbiota, such as *Lactobacillus*, may influence *Campylobacter* colonization in broilers and may be further explored as microbiota-based interventions to control *Campylobacter* in poultry.

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4702

A Stochastic, mathematical model for analysing phase variation induced phenotypic switching.

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Our aim is to model phenotypic diversity introduced through phase variation by producing mathematical system models to understand the role of selection and bottleneck events on bacterial populations. Which we will then apply to in situ and animal models to confirm the validity of our method. Phase variable events are stochastic and constant and allow genes to switch to an expressing state or non-expressing state through changes in repeat tract lengths. Through using ordinary differential equations, the ON or OFF state of a gene can be predicted at the generation level. The application of selection on to these genes influences what phenotypes survive from generation to generation. While introducing non-selective bottlenecks can act to stochastically change the population, to either increase or decrease phenotypic diversity. Through the use of computer and mathematical modelling we can predict the forces of selection acting upon phase variable genes in complex systems. This allows us to identify which phase variable genes are acted upon under selective barriers and what phase variable systems are important for bacterial function within hosts. We have validated our modelling in silico and on limited in situ examples showing that predicting of selection and bottleneck events can be predicted to some accuracy.

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4798

Evolutionary Principles of Bacterial Signaling Capacity and Complexity

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ABSTRACT Microbes rely on signal transduction systems to sense and respond to environmental changes for survival and reproduction. It is generally known that niche adaptation plays an important role in shaping the signaling repertoire. However, the evolution of bacterial signaling capacity lacks systematic studies with a temporal direction. In particular, it is unclear how complexity evolved from simplicity or vice versa for signaling networks. Here, we examine the evolutionary processes of major signal transduction systems in *Campylobacterota* (formerly *Epsilonproteobacteria*), a phylum with sufficient evolutionary depth and ecological diversity. We discovered that chemosensory system increases complexity by horizontal gene transfer (HGT) of entire chemosensory classes, and different chemosensory classes rarely mix their components. Two-component system gains complexity by atypical histidine kinases fused with receiver domain to achieve multistep or branched signal transduction process. The presence and complexity of c-di-GMP-mediated system is related to the size of signaling network, and c-di-GMP pathways are easy to rewire, since enzymes and effectors can be linked without direct protein-protein interaction. Overall, signaling capacity and complexity rise and drop together in *Campylobacterota*, determined by sensory demand, genetic resources, and coevolution within the genomic context. These findings reflect plausible evolutionary principles for other cellular networks and genome evolution of the *Bacteria* domain.

IMPORTANCE Bacteria are capable of sensing and responding to environmental changes by several signal transduction systems with different mechanisms. Much attention is paid to model organisms with complex signaling networks to understand their composition and function, but how a complicated network evolved from a simple one or vice versa lacks systematic studies. Here, we tracked the evolutionary process of each signaling system in a bacterial phylum with robust "eco-evo" framework and summarized the general principles of signaling network evolution. Our findings bridge the gaps in bacterial signaling capacity from highly sophisticated to extremely streamlined, shedding light on rational design of genetic circuitry. This study may serve as a paradigm to examine the complex construction of other cellular networks and genome evolution.

KEYWORDS chemotaxis, c-di-GMP, *Campylobacter*, *Helicobacter*

4818

Control of *Campylobacter* concentrations during poultry processing at three New Zealand processing plants

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Aim: Campylobacteriosis is the most frequently notified enteric disease in New Zealand, with poultry recognised as the dominant source for human infection. A longitudinal study was undertaken across primary and secondary processing at three broiler poultry processing facilities to evaluate the efficacy of processing steps in controlling *Campylobacter*.

Methods: Five rinsate samples were collected from carcasses at eight primary processing steps and from seven secondary processing product types, at each of six sampling visits per processing plant. In total, 1350 rinsate samples were enumerated for *Campylobacter*.

Results: There was an almost six-log reduction in *Campylobacter* concentrations on carcasses across primary processing, with *Campylobacter* not detected from 76% of samples at the end of primary processing. Compared with a 2013 study from one of the processing plants, *Campylobacter* concentrations were similar at the start of processing,

but were approximately two-log lower at the end of primary processing, indicating a significant improvement in pathogen control steps since 2013. For secondary processing products, *Campylobacter* concentrations were higher on whole birds from one plant which has separate primary and secondary processing premises. At each plant, all portions except drumsticks had higher concentrations than the weight equivalent of whole bird carcasses. While *Campylobacter* concentrations were previously found to be higher on skin than meat, the current study did not consistently find lower counts on skinless product.

Conclusion: Findings will enable the poultry industry to identify key areas to target to further improve processing procedures to reduce *Campylobacter* prevalence and concentrations on poultry meat, toward a reduced risk for consumers. Results also provide a benchmark to compare the efficacy of future interventions.

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4836

Evaluation of different risk mitigation strategies for *Campylobacter* along the raw milk supply chain

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Aim: A quantitative microbiological risk assessment (QMRA) model for *Campylobacter* in raw milk was developed to support risk managers in controlling this pathogen along the supply chain. Using this model, the effect of different risk mitigation scenarios were evaluated.

Methods: The QMRA applies data from samples taken every two weeks from a small dairy farm in Germany from 2021. The samples examined were rectal feacal samples, udder swabs, raw milk, milk filters, milking parlor swabs and boot socks. These were microbiologically analyzed for *Campylobacter* according to ISO 10272-1:2017 [1] and ISO 10272-2:2017 [2].

The developed stochastic QMRA model estimates the human exposure to *Campylobacter* from raw milk and the number of human cases. Probability distributions (e.g. pert, triangle, lognormal, normal) were used to represent the data whenever possible and probabilistic risk estimation was performed using Monte Carlo simulations (100,000 iterations).

Results: A baseline model was developed using the classic model for the dose-response of *Campylobacter*. Based on this model it was estimated that two cases will occur for one thousand consumed raw milk servings with a mean portion size of 250 ml. Using a novel dose-response model based on data from raw milk outbreaks, this number of cases would increase to 197.

Different scenario analysis demonstrated that the initial concentration of *Campylobacter* in cow faeces had the highest impact on the risk. Another influential parameter was the cleaning of udders. These could therefore be important targets for establishing control measures.

Conclusion: This is the first QMRA for *Campylobacter* in raw milk that explicitly includes steps for contamination during milking and consequently gives an early insight into the supply chain. Risk managers can use the model results as a basis for establishing new control points along the raw milk supply chain.

Reference:

1. ISO 10272-1:2017. Microbiology of the food chain – horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method
2. ISO 10272-2:2017. Microbiology of the food chain – horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Colony-count technique

4842

Determination of viable-but-non-culturable *Campylobacter jejuni* in chicken using quantitative PCR combined with propidium monoazide pretreatment

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Aim: Many bacterial species can enter a starvation mode of metabolism or a physiologically viable-but-non-culturable (VBNC) state under stress conditions. Several human pathogenic bacteria including *Campylobacter* have been reported to enter this dormancy state under unfavorable conditions. VBNC state of pathogens can pose risks to food safety and public health because they cannot be detected using the routine microbiological plating assay, but resuscitate under favorable conditions to develop virulence. Rapid and accurate determination of VBNC *Campylobacter* is critical to further understand the induction and resuscitation of the dormancy state of this microbe in the agri-food system.

Method: In this study, a method combing propidium monoazide (PMA) pretreatment with real-time polymerase qualitative chain reaction (qPCR) was developed for reliable determination of VBNC *C. jejuni.*

Results: A standard curve with a background of heat-inactivated cells was obtained with a linear quantification range of 3.43 to 8.43 log CFU/mL and a correlation coefficient of 0.9999, indicating a good quantitative capacity. It was then used to monitor the induction process of VBNC *C. jejuni* under osmotic stress. Over 10% *C. jejuni* population was successfully induced into the VBNC state after 48-h treatment. In addition, the applicability of this method was evaluated by detecting spiked VBNC *C. jejuni* in chicken breast samples. The limit of detection was determined to be 3.12 log CFU/g in chicken products.

Conclusion: In conclusion, PMA-qPCR is a rapid, specific, and sensitive method for the detection and quantification of VBNC *C. jejuni* in poultry products. This study aids in assessing the prevalence of VBNC *C. jejuni* in the agro-ecosystem and provides reliable data for risk assessment.

4882

The glycoconjugate vaccine against *Campylobacter jejuni*

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Aim: We are developing an *E. coli*-vectored glycoconjugate vaccine, exploiting the *Campylobacter jejuni* (*Cj*) heptasaccharide from the N-linked protein glycosylation pathway as an antigen, to reduce this pathogen in chickens and combat *Cj*-induced human diarrhea.

Methods: Vaccination and challenge studies were conducted to determine vaccine efficacy in broilers[1] and in a mouse diarrhea model that mimics human disease[2]. In both models, vaccine efficacy was investigated by enumerating *Cj* levels, measuring vaccine-induced immune-responses, determining antibody-carbohydrate specificities and comparing *Cj*-specific opsonophagocytic activities. In broilers, analyses of the microbiome composition, host genetics, serum and IgY glycosylation patterns were also conducted to investigate potential differences in vaccine responder versus non-responder birds.

Results: Vaccination with the N-glycan-based vaccine significantly reduced *Cj* levels in chickens and mice and induced an N-glycan-specific immune response[1, 2]. In chickens, the observed responder/non-responder effect can be attributed to variations in the microbiome and/or to different IgY glycosylation patterns subsequently contributing to up to 10 log drops in *Cj* colonization levels observed in responder birds; while host genetics had no influence on vaccine efficacy[1]. In mice, the N-glycan response was specific for the heptasaccharide and antibodies did not recognize the N-glycan lacking the glucose branch that is transferred by the *Cj* glycosyltransferase, PglI. Furthermore, some recent *Cj* isolates were not recognized by N-glycan-specific serum, but possessed an intact *pglI* gene. Here, loss of PglI function could be attributed to one amino acid deletion in the active site (DXDD) motif, an alteration that can be found in ~4% of all available PglI sequences[2].

Conclusion: Our *E. coli*-based *Campylobacter* N-glycan vaccine is easy to administer, cost efficient and an effective means to combat this pathogen at the source and to potentially battle *Campylobacter*-induced diarrhea in humans.

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4954

Platforms of Gastrointestinal-bacterial Ultrasensitive Native-sample Detection with Aptasensors in Modularization (GUNDAM): The Opening Chapter

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Aim: *Helicobacter pylori* and *Campylobacter jejuni* have become informed threat to human. Infection of *H. pylori* is associated with gastritis, gastric ulcers, and even gastric carcinoma. *C. jejuni* is identified as the leading cause of bacterial diarrhea. he difficulty in monitoring these bacteria lies in that they are usually in low abundance to be pathogenic. Thus, a rapid, ultrasensitive, accurate, and non-invasive detection method is expected.

Methods: Modular biosensors were developed. In general, these biosensors are composed of: (1) a bacteria-specific enriching module enabled by whole-bacteria aptamers; (2) a bifunctional bacteria-binding and actuating module; (3) a transducing module.

Results: To date, several novel transducing mechanisms have been introduced in these biosensors. These include: Fluorescence recovery from Fe^{3+}-quenched carbon dots or fluorescein (module 3) actuated by siderophore-Fe^{3+} scavenging (module 2); Zero-background chemiluminescence mediated by luciferin-modified bifunctional module 2 reacting with recombinant luciferase of module 3; Colorimetric determination enabled by color change from colorless to brownish red actuated by siderophore-modified module 2 chelated Fe^{3+} (module 3). These mechanisms transduce the density of captured bacteria, live or dead, into excited fluorescence, chemiluminescent glow or absorptance signals, establishing their correlation for quantification. Under the ideal buffer condition, the limit of detection (LOD) for *H. pylori* and *C. jejuni* is as lowest as 1 CFU/mL[1] and 3 CFU/mL[2], respectively. As the matrices of native samples, where *H. pylori* and *C. jejuni* reside in, are commonly complicated with impurities, microfluidics has been used to attenuate

interference. Microfluidics coupling the biosensors achieved culture-independent platforms, with an actual LOD of 10 CFU/mL for both bacteria validated.

Conclusion: These low LOD are calculated values, indicating a challenge in real sensing of trace bacteria below 10 CFU/mL. Thus, biosensing technology for single-cell bacteria detection should be developed, expecting new tricks up its sleeve.

Keywords: *Helicobacter pylori*; *Campylobacter jejuni*; Modular biosensors; Microfluidics; Ultrasensitive detection

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4962

The pangenomics of *Campylobacter* populations isolated during poultry processing at three New Zealand processing plants

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Aim: Campylobacteriosis is the most frequently notified enteric disease in New Zealand (NZ). Poultry are recognised as the dominant source for human infection. The genetic diversity of *Campylobacter* on chicken carcasses may include variants that are more resistant to processing treatments. We investigated the effect of antimicrobial steps on the genotypes of *Campylobacter jejuni* and *C. coli* isolates during primary processing at three broiler poultry processing facilities.

Methods: 279 *Campylobacter* isolates were obtained from 270 carcass rinsate samples collected from three defined processing steps at six sampling events per processing plant. Isolate numbers varied by plant, sampling step and sampling event. 180 *C. jejuni* and *C.*

coli isolates from the "Post-manual evisceration", "Post-Acidified Sodium Chlorite" and "Whole bird" processing steps were selected from three sampling events per plant for whole genome sequencing, and subsequent genomic analysis with Nullarbor2[1] and other tools[2-9].

Results: The 180 sequenced isolates (144 *C. jejuni*, 35 *C. coli* and 1 mixed isolate) comprised 13 different sequence types (STs; 5 *C. coli* and 8 *C. jejuni* STs). The internationally rare *C. jejuni* ST2927 was the most common (22.3%), arising from two flocks from the same farm, and has not been identified in NZ before. The number of virulence genes detected were fewer for *C. coli* (68–73) than for *C. jejuni* (82–113). There was an association between ST and virulence genes detected, but no association with processing step. A single antimicrobial resistance (AMR) gene (variants of bla_{OXA}) was present in most isolates, while *tetO* (resistance to tetracycline) was also present in two ST6964 isolates. No association was found between sampling point and ST, AMR gene/alleles, or virulence genes present.

Conclusion: Genomically, there was no evidence that ST2927 might be more of a concern than other endemic STs for the poultry industry or clinically.

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RESEARCH ARTICLE

The evolutionary path of chemosensory and flagellar macromolecular machines in *Campylobacterota*

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Abstract

The evolution of macromolecular complex is a fundamental biological question, which is related to the origin of life and also guides our practice in synthetic biology. The chemosensory system is one of the complex structures that evolved very early in bacteria and displays enormous diversity and complexity in terms of composition and array structure in modern species. However, how the diversity and complexity of the chemosensory system evolved remains unclear. Here, using the *Campylobacterota* phylum with a robust "eco-evo" framework, we investigated the co-evolution of the chemosensory system and one of its important signaling outputs, flagellar machinery. Our analyses show that substantial flagellar gene alterations will lead to switch of its primary chemosensory class from one to another, or result in a hybrid of two classes. Unexpectedly, we discovered that the high-torque generating flagellar motor structure of *Campylobacter jejuni* and *Helicobacter pylori* likely evolved in the last common ancestor of the *Campylobacterota* phylum. Later lineages that experienced significant flagellar alterations lost some key components of complex scaffolding structures, thus derived simpler structures than their ancestor. Overall, this study revealed the co-evolutionary path of the chemosensory system and flagellar system, and highlights that the evolution of flagellar structural complexity requires more investigation in the *Bacteria* domain based on a resolved phylogenetic framework, with no assumptions on the evolutionary direction.

5511

Gull species as a source of *Campylobacter* in a densely populated urban area

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Aim: Wild birds, such as gulls, can act as reservoirs of infectious agents and play an important role in the dissemination and maintenance of zoonotic agents, including *Campylobacter*. Yellow-legged gull (*Larus michahellis*) populations have increased dramatically, becoming a problem for human health because of its generalist insalubrious scavenging feeding habits, and its increasing direct and indirect interactions with human populations, compared with Audouin's gull (*Larus audouinii*). In Barcelona there is an important urban colony of yellow-legged gulls, whilst Audouin's gulls have been reported to breed in the city only once in recent years, in 2013. We aimed to gain insight into *Campylobacter* epidemiology by characterizing the isolates recovered from these gull colonies.

Methods: We obtained cloacal swabs from 429 gulls from Barcelona (Yellow-legged gulls, n = 331; n = 98 Audouin's gulls) across different time periods (2009-2018). We used a selective agar (mCCDA) for the isolation of *Campylobacter*. We assessed the virulence potential (by screening 14 putative virulence genes), the antimicrobial susceptibility (MIC, 6 antimicrobials) and the genetic diversity (by PFGE and MLST) of the recovered *C. jejuni* isolates.

Results: The prevalence of virulence-associated genes was high, indicating that most isolates from both colonies had a high virulence potential. On the contrary, despite most of the isolates were pansusceptible, a higher proportion of isolates from yellow-legged gulls, compared with those from Audouin's gulls, showed resistance to antimicrobials of relevance in human medicine. Also,despite the high genetic diversity among isolates, MLST analyses showed that

several *C. jejuni* genotypes (sequence types, STs) have been associated with human gastroenteritis.

Conclusion: Overall results suggest a potential anthropogenic origin of those strains and highlight the public health risk that these seagull species studied may pose, especially in a human populated area such as Barcelona.

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5529

Investigating the role of FlhF involved in flagellar synthesis in *Campylobacter jejuni*

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Aim: FlhF is a key protein required for complete flagellar synthesis, and its deletion results in the complete absence of flagella and thus motility in *Campylobacter jejuni*. This study is to explore the mechanism of FlhF involved in flagellar synthesis in *Campylobacter jejuni*.

Methods: In brief, the transcriptional function of FlhF was explored by EMSA and ChIP-qPCR here. The overall influence of FlhF on flagellar gene expression was analyzed by RNA-Seq &qRT-PCR. We further explored whether FlhF directly regulates early flagellar regulatory factors by EMSA.

Results: FlhF has an overall influence on the transcription of flagellar genes with an *flhF* mutant displaying down-regulation of most flagellar-related genes. FlhF can directly bind to the *flgI* promoter to regulate its expression, which has significant expression change in an *flhF* mutant. The possible binding site of FlhF to the *flgI* promoter was explored by continuously narrowing the *flgI* promoter region and performing further point mutations. Meanwhile, FlhF can directly bind to the promoters of *rpoD, flgS,* and *fliA* encoding early flagellin regulators, thereby directly or indirectly regulating the synthesis of class I, II, and III flagellar genes, respectively.

Conclusion: In summary, this study demonstrates that FlhF may directly regulate the transcription of flagellar genes by binding to their promoters as a transcriptional regulator. This will help in our attempts to understand the mechanistic role of FlhF in flagellar

biosynthetic and bacterial flagellation. We hope this study will be used as the foundation for future studies on FlhF function.

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5530

Contributions of phase variation to chickens colonisation, gastrointestinal spread and liver invasion by *Campylobacter jejuni*

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Aim: *Campylobacter jejuni*, usually found in the gastrointestinal tract (GI) of chickens, can cross the GI epithelial barrier and invade internal tissues[1]. *C. jejuni* can adapt to the unpredictability of selection pressures and host environments, including phage infection and immune responses, through phase variation (PV) mediated by mutations in mononucleotide repeat tracts[2]. Our aim was to assess the contributions of PV to invasion of chicken tissues by comparing *C. jejuni* isolates and whether the PV status differs between infection sites.

Methods: The contributions of PV to chicken colonisation and extraintestinal spread was examined in two *in-vivo* experiments using broiler chickens. Chickens were orally gavaged with either cc353 and cc464 chicken isolates or *C. jejuni* M1 and NCTC11168 strains. Colony sweeps from the inoculum and homogenates of caeca, ileum, spleen and liver of infected birds, 7 and 14 days post-infection were analysed by fragment length analyses to determine the PV status of 11-33 PV genes for each sample. Combined and individual PV gene states were examined.

Results: Hierarchical clustering indicated that infection group was the major determinant of the overall patterns of variation in PV expression. Examination of individual PV genes detected consistent or partially consistent variation in PV states between the inoculum and all sampled host sites and some limited evidence of site-to-site variation. Specifically, we observed ON switches in *maf1* or *maf4* genes for all strains except M1 where these putative flagella-modification and agglutination-associated genes are

absent. Conversely, homologues of the known or putative LPS-modification genes, *cj1295* and *cj1296*, were observed to switch OFF.

Conclusion: *C. jejuni* lineages are capable of extraintestinal spread in chickens, a process that may contribute to human infections. Specific PV genes appear to contribute to host colonisation, but not extraintestinal spread of different *C. jejuni* lineages.

Funding: This study is part of the CampAttack project funded by the BBSRC.

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5535

Production of *Campylobacter jejuni* in biofilms and sensitivity of various disinfectant substances

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Aim: The purpose of the study was to produce biofilm and investigate how biofilm was affected by six different disinfectants that are often used in farms, slaughterhouses and food premises.

Methods: Biofilm was produced by *C. jejuni* in mixed culture with six other bacterial species in a static model by microtiter plates and a dynamic model with pieces of plastic water pipes for chickens. The disinfectants tested were hypochlorous acid, hydrogen peroxide, peracetic acid, 70% ethanol, buffered acids and peroxymonosulphate. A total of 3264 microtiter wells and 765 pieces of water pipes were analyzed in order to make an overall assessment of which disinfectant had best effect on removing biofilm with *C. jeuni*.

Results: All disinfectants reduced the amount of bacteria. The largest mean reduction were found after treatment of hydrogen peroxide both in the microtiter plates and water pipes. The least effect was found after treatment of buffered acids. *C. jejuni* could be not be detected from any of the water pipes pieces after treatment with hydrogen peroxide, hypochlorous acid, and peracetic acid. Less effective at killing *C. jejuni* in biofilm were buffered acids where *C. jejuni* could be detected from 53% of the water pipes after treatment. *C. jejuni* could be isolated from 15% and 16%, respectively, of the water pipes treated with peroxymonosulfate and 70% ethanol, respectively.

Conclusion: The results indicate that the disinfectant must be chosen based on the specific environment as well as the bacteria it aims to eliminate, since the effectiveness differs between different types of biofilms and the agents present. It was also shown that the effect of mechanical cleaning cannot be replaced by disinfectants alone. However,

hydrogen peroxide had the greatest ability to disinfect several different environments with different types of biofilms and can serve as a complement to multidisciplinary cleaning routines.

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5536

Survival of different sequence types of *Campylobacter jejuni* and risks during handling in the kitchen

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Aim: The purpose was to study differences between sequence types of *C. jejuni* in survival in the freezer and transmission in the kitchen.

Methods: Broiler chicken fillets were artificially contaminated before freezing with two different concentrations and sequence types of *C. jejuni*. ST-257, a common ST in chickens and cattle and, ST-918, a frequently isolated ST in fresh retail broiler meat and isolated from 25% of the patients in a Swedish outbreak. Simulated tested risk factors in the kitchen were hands before and after washing, utensils and cutting board before and after wiping with a dishcloth.

Results: Both ST-257 and ST-918 were reduced in the freezer (-18°C), with a reduction being greatest during the first four days of freezing. There was a difference in the ability to withstand the stress of freezing, with ST-918 decreasing to a lesser extent compared with ST-257, indicating a possible difference in survival and their ability to cause disease. In the kitchen, the meat juice probably poses a greater risk than undercooked meat, since the juice had similar concentrations of *C. jejuni* as the uncooked meat and the juice can easily spread to other surfaces in the kitchen. Simulated hands and cutting board pose the highest risk for transmission of *C. jejuni* from poultry meat to humans. Although the amount of *Campylobacter* was significantly lower after washing the gloves, there was a remarkably large amount (26%) of *Campylobacter* left after washing. A difference was found between

the different sequence types with 32% of ST-918 and 20% of ST-257 that could be quantified from the gloves.

Conclusion: There are differences between *Campylobacter* sequence types in their ability to withstand freezing stress and *Campylobacter* remaining in the environment. Hands after washing and on cutting boards after wiping is the most likely source of cross-contamination in the kitchen.

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5539

Genetic and Phenotypic Variation of *Campylobacter jejuni* NCTC11168 during Laboratory Passage

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Aim: *Campylobacter jejuni* strain NCTC11168 was commonly used as a standard strain for flagellar biosynthesis research. In this report, two distinguished phenotypic isolates (CJ1Z, mutant, lawn; CJ2S, wt, normal colony) appeared during the laboratory passages for NCTC11168.

Methods: Phenotypic assessments including motility plates, transmission electron microscopy, biofilm-formation assay, autoagglutination assay and Genome re-sequencing for these two isolates (CJ1Z, mutant, lawn; CJ2S, wt, normal colony) were carried out in this study.

Results: Scanning by electron microscopy showed the flagellum was lost in CJ1Z. Phenotypic assessments and Genome re-sequencing for these two isolates were carried out

in this study. The capacity of the bio-film formation, colony auto-agglutination and isolate motility significantly reduced in the mutant CJ1Z. Comparative genomic analysis indicated a unique native nucleotide insertion in flhA (nt, 2154) which caused the mutation of I719Y, I720Y and the early truncation in FlhA.

Conclusions: To the best of our knowledge, FlhA was proved to be functionally influence the expression of the flagella in *C. jejuni*, but the function of the C terminal of FlhA was first described in this study.

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5542

Species Classification and Novel Plasmid Identifications in *Arcobacter cryaerophilus* and *Arcobacter cryaerophilus-like* Organisms

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Aims: To determine the genetic and plasmid features of *A. cryaerophilus* based on the whole-genome sequence and classify the species of *A. cryaerophilus.*

Methods: Average Nucleotide Identity (ANI) and *in silico* DNA-DNA hybridization (*is*DDH) were used for the species classification for *A. cryaerophilus* strains. The genomic characteristics were determined using various bioinformatics software.

Results: Two clades (four subclades) were identified among *A. cryaerophilus* strains with the phylogenetic analysis. The phylogenetic tree indicated these strains exhibited a high intra-species genomic diversity. No clustering was assorted with the host or geographic location among these genomes. A novel large multiple drug-resistant plasmid (named pCNAC48 with 161,992 bp in length) was identified. Two antibiotic-resistance islands were found in the plasmid with lengths of 7,950 bp and 25,137 bp and G+C content of

38.23% and 32.39%, respectively. Ten drug resistance genes and some transposable elements were cross-distributed among the islands in the plasmid. Antimicrobial susceptibility tests indicated these resistance genes in the plasmid were functional. Plasmid conjugation and curing experiments proved pCNAC48 was stable in *A. cryaerophilus* strains ICDCAC48.

Conclusions: Our results indicated that *A. cryaerophilus* should be reclassified into four new species at the genomic level. In this study, we obtained the genetic characteristics of *A. cryaerophilus* from different sources and exhibited a high intraspecies genomic diversity between the strains. A multidrug-resistant megaplasmid was identified and the characteristics of the plasmid were elucidated, contributing effectively to filling up the knowledge gap on this foodborne pathogen. The potential of plasmids to mediate horizontal transfer among *Arcobacter* and other species warrants further consideration by researchers interested in the risks to public health from these organisms.

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5553

Application of TraDIS to define the core essential genome of *C. jejuni* and *C. coli*

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Aim: Due to the lack of an effective vaccine to prevent *Campylobacter*, combined with a rapid increase in antimicrobial resistant strains, there is an urgent need to identify new targets for intervention. Our aim was to create a cross-species essential gene list (*i.e.* those genes that are necessary for growth and/or survival), to identify possible targets.

Methods: Comprehensive transposon mutant libraries were created in six *C. jejuni*, four *C. coli*, one *C. lari* and one *C hyointestinalis* strain, allowing for those genes that cannot tolerate a transposon insertion to be identified as essential. Comparison of essential genes and core genome analysis highlighted essential genes common across multiple strains and/or species.

Results: Comparison of *C. jejuni* and *C. coli*, the two species that cause the most disease, identified 386 essential genes. Genes of interest highlighted members of the purine pathway being essential for *C. jejuni* whilst the potassium uptake system KtrA/B being essential in *C. coli*. When adding in two more species (*C. lari* and *C. hyointestinalis*) the number of essential genes reduced to 341. Within the 341 essential genes, there are many genes that have been found to be essential in other bacteria. However, there are 25 genes which have no known function with 16 of these associated with the membrane. These surface-associated, *Campylobacter*-specific essential genes may provide attractive targets for intervention.

Conclusion: The essential gene lists will help to prioritise targets for the development of novel therapeutics and preventative measures.

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5554

Host adaptation and the emergence of globally disseminate

Abstract

Bacteria can adapt in new hosts and respond to different selective pressures but not all bacterial species are found in all hosts and environments. The genetic mechanisms that promote these adaptations are not fully understood. This project investigates the genomic and phenotypic adaptations that promote colonization/proliferation of bacteria of the genus *Campylobacter* and explores variation at the species, lineage and gene level. Pangenomic comparative analyses revealed core and accessory gene variation highlighting the importance of gene gain and loss in the evolution of this species and the use of genomics in identifying molecular markers to monitor lineage specific *in vivo* infection experiments. *Campylobacter* are highly recombinogenic, thus a focus has been given on quantifying recombination in the genome. A detailed analysis of recombination has shown the proportion of the mobile genetic elements (mobilome) in the *Campylobacter* genus and pinpointed genes associated with host adaptation. Additionally, analysis of *Campylobacter* resistomes between species, lineages, hosts and environments revealed multidrug resistant (MDR) genomic islands (GIs) and the involvement of plasmids in horizontal gene transfer (HGT). This work has provided evidence of interspecies recombination between different species that share the same hosts and the genes associated with them and broadened understanding of how genomic plasticity can allow these versatile bacterial pathogens to adapt into new niches and environments.

5533

An Investigation into the Critical Factors Influencing the Spread of *Campylobacter* during Chicken Handling in Chinese Commercial kitchens

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Aim: This study aimed to determine the critical factors for *Campylobacter* cross-contamination in Chinese commercial kitchens during chicken handling.

Methods: Five commercial kitchens were visited to detect *Campylobacter* occurrence from 2019 to 2020. Chicken samples (n=363) and kitchen surfaces samples (n=479) were collected, total bacterial counts and *Campylobacter* spp. were detected. 75 isolates of *Campylobacter jejuni* were characterized by WGS.

Results: 77.41% of chicken carcass samples and 37.37% of kitchen surfaces showed *Campylobacter* spp. contamination. After cleaning, boards, hands, and knives still showed high bacterial loads including *Campylobacter* spp., which related to poor sanitary conditions. The results of WGS indicated that *Campylobacter* cross-contamination occurred during chicken preparation. Improper handling practices were the reason for surfaces having a contamination of *Campylobacter*.

Conclusion: Sanitary condition of surfaces, ability of biofilm formation of isolates, and improper handling practice were the critical point contributing to spread of *Campylobacter* in kitchen environment.

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5567

Detection of *Campylobacter* DNA in commercial chicks; implications for control on farms.

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Aim: There is an urgent need for greater surveillance of *Campylobacter* in broiler (meat) chickens on farms in order to better understand this major route of transmission for human disease and design effective interventions. There are many challenges that preclude the use of traditional culture techniques, including the inability to culture *Campylobacter* from chicks in commercial settings <2 weeks of age. The aim of this study was to use parallel sequencing to determine the longitudinal epidemiology of multiple *Campylobacter* variants amongst live broiler chicks on farms.

Methods: We developed a parallel sequencing method using the short variable region of the *Campylobacter porA* gene to detect the presence of multiple *Campylobacter* variants simultaneously from fresh faecal samples, and validated the results using 16S bacterial profiling and nucleotide sequencing of isolates from culture-positive flocks[1].

Results: We detected *Campylobacter* DNA in all of 34 flocks tested from 3 European countries, irrespective of culture status, and from chicks as young as 1 day of age. *Campylobacter* was typically detected at low levels but with high diversity, until a flock became culture positive where upon one or two variants became dominant[2]. The findings have since been repeated amongst additional Swiss[3] and UK flocks.

Conclusion: The results indicate that *Campylobacter* is prevalent amongst broiler flocks at a much earlier age than can be detected by culture, giving new opportunities for intervention strategies on farms. In addition, more work is needed to understand why *Campylobacter* variants become prevalent in some flocks and not others; modelling approaches suggest this may be host rather than pathogen driven[4].

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5590

Evidence on linking Bacterial Etiologies with stunted childhood growth and overgrowth of small intestinal bacterial taxa impacting vulnerable child gut health

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Aim: Campylobacter & *E. coli* are potential human pathogen which may lead to contaminate the farmed poultry and its products as much as 100%. Sometimes it can cause gastroenteritis outbreaks particularly originating from contaminated poultry consumption. These are the most common cause of increased morbidity and mortality developing countries especially in children. The epidemiology of gastroenteritis due to poultry meat and its products is progressively increasing on account of the facts that chicken meat has now become the most abundantly produced and consumed due to its cheaper price, preference, product value addition and complete absence of microbiological quality testing and regulatory set up in the country. Almost all domains of poultry industry including feed milling, farming, health care labs, and retail poultry meat marketing and processing industry contribute. Environmental enteric dysfunction (EED), a least understood condition, widespread among malnourished children resulting stunting growth, associated with poor sanitation, certain gut infections, and micronutrient deficiencies.

Methods: In this study total of 64 stool sample were collected from children aged ≤5 years zfrom 2 urban and 2 semi-urban settings of Pakistan. Fecal samples were analyzed for presence or absence of diarrheagenic *E. coli* pathotypes and phylogroups. Selected stool samples were then analyzed for microbiome analysis using 16S ribosomal RNA (rRNA) gene sequencing to determine correlation between change in microbiota with the presence of different *E. coli* groups.

Results: Our results showed that out of 64 E. coli isolates 39.68% were typable (n=25) among which EPEC was most prevalent (52%; n=13), followed by EAEC (20%; n=5), EIEC (12%; n=3), EHEC (8%; n=2) and ETEC 2 (8%; n=2). Moreover, diarrheagenic

isolates associated with stunted growth were found to be multidrug resistant and also belonged to diverse phylogenetic groups.

Conclusion: Microbiome analysis showed that microbial diversity was significantly decreased in stunted children stool samples as compared to that of normal children. Relative abundance analysis showed that *Escherichia-Shigella* and *Streptococcus* were found to be associated with stunted growth in children as they were highly prevalent in NT and EHEC pathotypes of E. coli. This warrants to take measures both at the regulatory as well as industrial level for an effective control of public health losses.

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5601

The prophages of the genus *Helicobacter*

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The genus *Helicobacter* comprises species that colonize either the gastric mucosa (gastric *Helicobacter*), or the liver/intestinal tracts (enterohepatic *Helicobacter*) of distinct classes of the subphylum *Vertebrata*, including mammals, birds and reptiles. *Helicobacter pylori* is the type species and was the first described species in the early eighties of last century. *H. pylori* colonizes the stomach of the human host, causing gastritis, peptic ulcer and gastric cancer. Previously, we have described the first complete *H. pylori* prophage and showed that prophage genes tend to belong to the same phylogeographic group as *H. pylori*, reinforcing co-evolution and phage-host interactions persistence. Prophage sequences found in *H. acynonichis* and *H. felis* share homology with *H. pylori* prophages, but the co-evolutionary scenario between prophages and their *Helicobacter* hosts remains undetermined. To address this question we have retrieved 343 *Helicobacter* genomes from the Pathosystems Resource Integration Center, comprising 43 different species. These genomes were mined for the presence of prophages using the software PHASTER, and BLAST search using reference prophages as query. PHASTER identified 483 incomplete, 29 questionable and 6 intact prophages, resulting in 1.5 (± 1.0) prophages present per genome, with a minimum of zero and maximum of five; while BLAST search identified 40 prophages. This result shows that most prophages of *Helicobacter* genus are in a massive decay process, which usually involve loss of prophage DNA segments, genome rearrangement, among other inactivation mechanisms. Currently we are analyzing the prophage sequences of *Helicobacter* genus and we intend to present a congruence analysis between the phylogenetic trees of the host genome and prophage genome to evaluate the dependency and agreement between the phylogenies, as well as searching for specific events, such as duplication, host jumps, tropism of prophage integration, and integration site.

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5644

Complementary Ribo-seq approaches map the translatome and provide a small protein census in *Campylobacter jejuni*

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Aim: Knowing the molecules encoded by bacterial pathogens and how their expression is regulated is essential to understand how they survive, colonize, and cause disease. RNA-seq approaches that map transcriptomes, including in our differential RNA-seq maps for the *Campylobacter jejuni* and *Helicobacter pylori* (1,2), have revealed a wealth of new transcripts in bacterial pathogens. However, RNA-seq does not provide direct evidence for coding potential and misses, in particular, small proteins (≤ 50-100 amino acids) translated from small open reading frames (sORFs), a poorly annotated component of bacterial genomes with emerging roles in bacterial physiology and virulence. Here, we present an integrated ribosome profiling (Ribo-seq) approach to refine the "translatome" of *C. jejuni*.

Methods: In addition to conventional Ribo-seq, we employed translation initiation site (TIS) profiling to map start codons and reveal internal sORFs. We also established a new Ribo-seq approach for mapping of translation termination sites (TTS). These methods were combined with extensive independent validation via epitope tagging and western blotting, as well as mass spectrometry and functional characterization.

Results: Our refined map confirms previously-predicted leaderless ORFs/leader peptides, re-annotates start or stop codons of 35 genes, reveals isoforms generated by internal start sites, and adds 42 novel sORFs in diverse contexts to the *C. jejuni* annotation (sRNAs, in 5′UTRs, internal/out-of-frame/antisense in ORFs). Our new TTS approach revealed stop codons not apparent from the reference genome in virulence-associated loci. We validated expression of almost 60 annotated and novel sORFs, including *cioY*, which we show encodes a conserved, 34 amino acid component of the CioAB terminal oxidase.

Conclusion: Overall, we provide a blueprint for integrating several Ribo-seq approaches to refine and enrich bacterial proteome annotations, and have made our *C. jejuni* translatome data, including annotation updates, easily accessible in an interactive browser (CampyBrowse).

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5645

Campylobacter jejuni developed the resistance to bacteriophage CP39 by phase variable expression of *06875* encoding the CGPTase

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Aim: Bacteriophage is an antimicrobial alternative for treating pathogens in food production. However, the development of phage resistance is a main concern for the phage application. This study characterized the phage CP39 and investigated the phage resistance of CP39 in *Campylobacter jejuni* NCTC12662.

Methods: We applied WGS for CP39 genomic characterization and co-cultured the phage with C. jejuni strain NCTC 12662. After 36 h, bacterial DNA was collected and send for WGS. SNP were analyzed for NCTC 12662 sequence after phage infection, to identify the point mutations in the CPS *loci*.

Results:

Phage CP39 belonged to the *Myoviridae* family by the WGS and phylogenetic analysis. Phage CP39 was confirmed as a capsular polysaccharide (CPS)-dependent phage by primary *C. jejuni* phage typing, that the phage could not be adsorbed by the acapsular mutant Δ*kpsM*, but showed the same lytic ability in both the wild-type strain NCTC 12662 and the Δ*motA* mutant lacking motile flagella filaments. The *06875* gene encoding CDP-glycerol:poly (glycerophosphate) glycerophosphotransferase (CGPTase) in the CPS loci was related to the phage CP39 adsorption by SNP analysis and rapid phage resistance was observed during the phage infection with high mutation frequency of *06875* (32%). The mutation of the *06875* gene caused the phase variable expression of non-functional protein, which allowed the bacteria against the phage infection by modifying the CPS.

Conclusion: Our study confirmed the *06875* gene responsible for the CPS-phage adsorption for the first time, and demonstrated that the phase variable expression as a main mechanism for the bacteria to defend phage CP39.

4608

The *Campylobacter jejuni* Type VI secretion system displays roles in intrabacterial antagonism and human host-cell interaction.

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Aim: The multi-protein Type Six Secretion system (T6SS) apparatus is encoded by >25% of gram-negative bacteria[1]. Various roles have been ascribed to the T6SS in different bacteria including bacterial antagonism, stress resistance and host-cell modulation[2,3]. Analysis of 5,829 published *C. jejuni* genomes identified T6SS operons in 19.5% of genomes, particularly phylogenetic groups associated with human infection and chicken isolates[4]. Previous investigations of the *C. jejuni* T6SS have explored roles in the chicken gut and links to human infection; however, the potential antibacterial and anti-host properties of the *C. jejuni* T6SS are still relatively unknown[5-7]. With such a high prevalence in clinical isolates, we aim to further our understanding of the T6SS by investigating potential functions that aid pathogenesis.

Methods: To investigate the *C. jejuni* T6SS, structural knockout mutants (*tssD* and *tssBC*) were generated in T6SS-positive *C. jejuni* strain 488. Preliminary investigations examining

temporal expression and secretion of T6SS components were performed using the 488 strain. Phenotypic assays were conducted using 488 wild-type and T6SS mutant strains to further characterise the T6SS. Human intestinal epithelial cell (IECs) lines, T84 and Caco-2, were infected with 488 wild-type and T6SS mutant strains to determine the *C. jejuni* T6SS influence on host-cell infection. Co-culture experiments were performed to examine T6SS involvement in bacterial interaction.

Results: In both T84 and Caco-2 cells, T6SS mutants displayed reduced ability to interact and invade. Additionally, the mutants elicited weaker induction of IL-8 after infection. During co-culture, structural mutants no longer displayed a selective advantage against T6SS-negative prey strains.

Conclusion: T6SS-positive *C. jejuni* 488 displays interstrain antibacterial activity not observed with non-functional T6SS structural mutant strains. Results from IEC assays indicate that the *C. jejuni* T6SS plays a role in human host-cell interaction. These findings warrant further investigation to help fully elucidate the function of the *C. jejuni* T6SS.

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4611

Quorum sensing and biofilm formation of *Campylobacter jejuni* are inhibited by decanoic and lauric acids

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Aim: *C. jejuni* continues to pose a significant burden to public health while tremendous efforts have been put to reduce *Campylobacter*-associated foodborne illnesses[1]. Biofilm formation mediated by quorum sensing is suggested to be critical to the survival of *C. jejuni* in the agroecosystem[2-4]. This study identified two naturally occurring fatty acids with quorum sensing inhibitory effects from twelve low-cost GRAS compounds, decanoic and lauric acids. The inhibition effect of those two fatty acids on *C. jejuni* biofilm formation and motility was investigated.

Methods: This study investigated the quorum sensing inhibitory effect of twelve natural-origin compounds on *C. jejuni* via *V. harveyi* AI-2 assay. The most potent *C. jejuni* AI-2 inhibitors among the screened compounds, decanoic and lauric acids, were selected to test their inhibitory effects on biofilm formation and bacterial motility. Three *C. jejuni* strains were cultivated in 96-well plates with and without the supplementation of the fatty acids, and the total biomass of formed *C. jejuni* biofilms was quantified by crystal violet staining assay. The soft agar plate assay was used to assess the effect of fatty acids on *C. jejuni* motility.

Results: Decanoic acid and lauric acid were identified to be effective in inhibiting AI-2 activity of *C. jejuni*. Both fatty acids at 100 ppm inhibited ~90% AI-2 activity of *C. jejuni* without bacterial inactivation. The biofilm biomass of two *C. jejuni* strains was reduced by

10%-50% after treatment with both fatty acids. In addition, both fatty acids effectively reduced the motility of all tested *C. jejuni* strains.

Conclusion: These findings bring new insights into using fatty acids for *C. jejuni* control in agri-food systems. This quorum sensing quenching approach can aid in developing alternative strategies to reduce the prevalence of *C. jejuni* in agri-food-related settings.

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4828

Helicobacter pylori: Does asymptomatic infection mean no gastroscopic lesions?

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Objectives: We determined the common clinical characteristics of patients infected with *Helicobacter pylori(H. pylori)* and investigated the relationship between *H. pylori* infection and clinical symptoms and gastroscopic manifestations, focusd on the clinical manifestations in asymptomatic patients.

Methods: We obtained the physical examination data of patients who underwent the ^{14}C urea breath test (^{14}C-UBT) between January 2018 and December 2020 at our Hospital. Basic demographic data, questionnaire data on clinical symptoms, and clinical examination data of the patients were also collected, and the correlation analysis was performed.

Results: A total of 2,863 participants were included in the study. The overall *H. pylori* infection rate was 26.30%. The clinical symptoms between *H. pylori*-positive patients and *H. pylori*-negative patients did not differ significantly ($P > 0.05$). However, *H. pylori*-positive patients exhibited more severe gastroscopic manifestations ($P < 0.001$). The ^{14}C-UBT disintegrations per minute (DPM) values in *H. pylori*-positive patients correlated with their serum pepsinogen and gastrin-17 levels. With an increase in the DPM value, more combinations of clinical symptoms appeared in the patients. Among *H. pylori*-positive patients, DPM levels in asymptomatic patients were lower than those in symptomatic patients ($P < 0.001$). However, gastroscopic manifestations did not vary significantly between asymptomatic and symptomatic patients ($P > 0.05$).

Conclusion: Patients infected with *H. pylori* showed no specific gastrointestinal symptoms. Patients with asymptomatic infection showed lower DPM levels, but their gastroscopic manifestations were similar to those of patients with symptomatic infection, and their lesions were more severe than *H. pylori*-negative people. This study provide a theoretical basis for considering the requirement of eradication therapy in patients with asymptomatic infection.

4837

Campylobacter jejuni induces the unfolded protein response in human intestinal epithelial cells

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Aim: Gastroenteritis is a global problem with *Campylobacter jejuni* being the most common bacterial cause[1]. Despite its importance, the mechanisms by which *Campylobacter* infection promotes inflammation and disease in humans remain unclear. We recently found that *C. jejuni* activates the unfolded protein response (UPR) in human intestinal epithelial cells (IECs), a conserved pathway in eukaryotic cells important for restoring homeostasis and relieving stress in the endoplasmic reticulum but is also implicated in inflammation[2-4]. Our aim is to investigate cellular and molecular drivers of *C. jejuni*-activated UPR to define its key features and to better understand *C. jejuni* infection and disease.

Methods: To investigate whether *C. jejuni* activates the UPR in T84 and Caco-2 IECs and potential bacterial determinant(s) that are responsible for UPR activation, both cell lines were infected with *C. jejuni* 11168H, 81-176 and 488 wild-type strains and 11168H mutant

strains. RNA and protein are extracted from infected cells and further processed for transcriptional and translational methods. In addition, thapsigargin and UPR inhibitors were used to examine the impact of UPR on *C. jejuni* pathogenesis.

Results: The UPR induction through PERK and IRE1α pathways was demonstrated by increased transcriptional and translational level of CHOP and spliced XBP1 respectively in infected IECs compared to uninfected IECs. *C. jejuni* 11168H *kpsM* and *flaA* mutant strains showed less expression of CHOP compared to the wild-type strain. Thapsigargin pretreatment reduced intracellular survival of *C. jejuni* and UPR inhibitor pretreatment increased the number of intracellular *C. jejuni*.

Conclusion: *C. jejuni* induces the UPR through PERK and IRE1α pathways in human IECs. We demonstrated UPR is activated in infected IECs as host cellular defense mechanism which might be linked to inflammation. These findings will provide insights into the cellular and molecular drivers of *C. jejuni*-driven UPR activation to better understand *Campylobacter* infection and disease.

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4955

Campylobacter oralis sp. nov., a redefined novel *Campylobacter* species containing IBD-associated plasmids and *csep1* gene

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A new species of the *Campylobacter* genus is described, isolated from the human oral cavity and gastrointestinal tract. This bacterium was previously known as *Campylobacter concisus* genomospecies 2. Genome analysis and phenotypic characteristics demonstrated that strains of *C. concisus* genomospecies (GS) 2 belong to a novel species within the genus *Campylobacter*. A total of 245 *C. concisus* genomes including 85 GS1 and 160 GS2 strains, were analysed. DNA-DNA hybridization (DDH) values between strains of GS1 and GS2 *C. concisus* were from 42.8 to 66%. Whole-Genome Average Nucleotide Identity (ANI) between GS1 and GS2 *C. concisus* strains were from 88.6 to 89.4. Both the DDH and ANI values between GS1 and GS2 *C. concisus* strains were clearly below the cut off values for defining the same bacterial species (70% and 95). The average GC contents of GS1 and GS2 *C. oncisus* strains were 37.3% and 39.2% respectively. GS2 *C. concisus* can also be differentiated from GS1 *C. concisus* based on 23S rRNA gene, core genome, phenotypes, specific genes as well as MALDI-TOF mass spectrometry. These data show that GS2 *C. concisus* is a novel *Campylobacter* species. For this species, the name *Campylobacter oralis* sp. nov. is proposed, with the complete genome sequenced strain P15UCO-S2 as the type strain. Previously reported inflammatory bowel disease (IBD)-associated *Campylobacter* molecular markers are either predominantly present in *C. oralis* or only present in *C. oralis*.

Keywords: *Campylobacter oralis*, *Campylobacter concisus*, genomospecies, *Campylobacter*

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4965

Two campylobacteriosis outbreaks might be caused by asymptomatic cook carrier were identified in Beijing

Problem Statement: Two campylobacteriosis outbreaks might be caused by asymptomatic cook carrier were identified in Beijing. In the outbreak 1, all of the patients were co-workers of one factory and the diarrhea happened after the same meal supplied from one meal delivery service company. In the outbreak 2, 3 diarrheal students from the same school visited the local hospital. Epidemic investigation found more diarrheal students in the school and they had one meal together.

Approach: Fresh stool samples were collected in the above two outbreaks. The entire samples were transported to the laboratory for further examination. Real time screening for 10 major pathogens including *V. cholerae, V. parahaemolyticus, C. jejuni, C. coli, Salmonella, Shigella, Diarrheagenic E. coli,* Norovirus, Rotavirus, and Enteric Adenovirus. According to the PCR results, the *Campylobacter* isolation was performed for the entire samples. The Pulsed-field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), whole-genome sequence (WGS) and antibiotic susceptibility tests for the *Campylobacter* isolates were carried out in this study.

Results: In the outbreak 1, 14 *C. jejuni* isolates were obtained from 12 patients and 2 asymptomatic cooks. All of these isolates had same PFGE pattern and the same susceptibility pattern, and belong to ST-6959. WGS analysis indicated this outbreak was caused by one highly clonal *C. jejuni*. In the outbreak 2, 7 *C. jejuni* isolates were obtained from 6 patients and 1 asymptomatic cook. All of these isolates had same PFGE pattern and the same susceptibility pattern, and belong to ST11329. One *Campylobacter coli* was obtained from the asymptomatic cook at the same time. The whole-genome multilocus sequence typing (wgMLST) analysis for 7 *C. jejuni* isolates was performed with fast-GeP using the genome of the strain from the cook as reference. The *ad hoc* wgMLST analysis found that, among the 1665 reference coding sequences (CDSs), 1662 CDSs were complete and shared by the 7 genome sequences. The neighbor-net phylogeny of 7 *C. jejuni* isolates using SplitsTree4 based on the shared loci was constructed. Through the phylogenomic tree, we found the

asymptomatic cook might be the source of this outbreak.

Conclusion The results indicated that two outbreaks were was caused by highly clonal *C. jejuni*, respectively. The asymptomatic cook is most likely to be the source of this outbreak. Routinely inspection and surveillance for *Campylobacter* is need for the food producing staff, particularly for the cook in the cafeteria in the school or other public food services.

4967

Identification and analysis of iron uptake systems in a large database of *C. jejuni* and *C. coli* isolates

Campylobacter jejuni and *C. coli* are gram-negative, helical, microaerobes, considered to be the leading bacterial cause of human gastroenteritis. However, they can live commensally in many animals, notably chickens. The acquisition of the crucial nutrient, iron, which is limited in host niches, is key for successful intestinal colonisation across a range of hosts (1). A database of 18,784 *C. jejuni* and *C. coli* isolates were examined for the presence and allelic variation of known iron utilisation systems and their transporters. This study has shown that the haem, Fe^{2+} and rhodotorulic acid systems are 100% conserved across a large collection of isolates with the main variation occurring in the enterobactin and lactoferrin iron utilisation systems. There was a high level of allelic variation within the *cfrB* enterobactin receptor and multiple protein variants were identified, functional and non-functional. Functional *cfrB* was identified without its cognate esterase *cj1376* in ~44% of isolates. The presence of the *ctuA* lactoferrin iron uptake receptor correlated with the *tonB1-exbB1-exbD1* transporter system. Using alphafold co-evolution software, a model of this interaction was produced. The model, along with conserved regions in *ctuA* in every isolate in the database has allowed the identification of a potential TonB1 binding box in CtuA.

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5512

Virulence and infectious assessment of *Campylobacter jejuni* 63A – a novel isolate from California gull excreta

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Abstract

Campylobacter jejuni is one of the most common foodborne zoonotic pathogens worldwide, especially in the poultry industry. The common route of infection is the fecal-oral route and by consumption of contaminated food or water. Wild birds are also a major source of *C. jejuni*, however, there is limited information on the virulence and infectivity of wild bird derived *C. jejuni* or its human health risks associated with exposure to wild bird infested surface waters. This study focused on understanding the importance of *Campylobacter* spp. isolated from wild birds. A limited survey on the presence of *Campylobacter* spp. in wild birds was initiated and identified a novel *C. jejuni* (63A) isolated from California gull excreta. An initial MLST analyses of 63A classified it as a ST2654 sequence type. Whole genome analyses revealed high similarity of DNA sequences (97-99%) with other pathogenic *Campylobacter* spp. strains, particularly those isolated from patients suffering from Guillain-Barre´ syndrome (GBS). Furthermore, analyses revealed that the lipooligosaccharid (LOS**),** capsular polysaccharide (CPS), and cytolethal distending toxin (*cdtABC*) operons/genes were all present and were 98-99% DNA/amino acid sequence identity with the same clinical isolates collected from GBS patients. The unique LOS genes (*neuA1*, *neuB1*, *neuC1* and *cstIII*) to GBS patient isolates were identified and categorized 63A as group 1 LOS locus type. The presence of intact CPS and *cdtABC* genes in 63A along with their high sequence similarity to clinical isolates was strikingly different from many other wild-bird isolates. These findings suggest the high possibility of significant virulence associated with the new isolate. 63A was also not limited to only California gull, as we detected 63A in additional waterfowl such as sandhill cranes and snow geese, highlighting its ability to be vectored by diverse avian species. Lastly, an *in vivo* chick-model of infection was further employed to confirm that 63A was capable of colonizing the chick gastrointestinal tract with an

estimated median infectious dose of 7.16×10^7 CFU, further suggesting that 63A isolate has zoonotic potential and can infect humans. Taken together, *Campylobacter* spp. isolated from waterfowl, may pose a significant human health risk associated with surface waters used for recreation.

Keywords: *Campylobacter jejuni* 63A, virulence, genome, infectivity, host specificity, waterfowl

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5555

Prevalence, Whole genome sequence and antimicrobial resistance of Campylobacter spp. Isolates in pets in Shenzhen，China

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Abstracts: The prevalence of campylobacter spp. in pets is an potential concern of human health. However, little is known about the pet-related campylobacter spp. in China. A total of 325 fecal screened for campylobacter spp. by culture, and a questionnaire was filled out by pet owners. Totally 110 campylobacter spp. were identified by MALDI-TOF MS. The highest prevalence was detected in C.upsaliensis (30.2%,98/325), followed by C. helveticus(2.5%,8/325)and C. jejuni(1.2%,4/325). The prevalence of campylobacter in dog and cat were 35.0% and 30.1%, respectively.All 110 isolates were tested for antimicrobial resistance against a panel of 11 antimicrobials by agar dilution method. The highest resistance rate occurred in Ciprofloxacin(97.27%), followed by Nalidixic acid(89.09%), and Tetracycline(70.0%). Multidrug resistance(MDR) was found in 70.9%(78/110) of the isolates, two main multiresistance patterns were detected: Quinolones/Tetracyclines/clindamycin (40.0%, 44/110) and Maroclides/Quinolones/ clindamycin (32.7%, 36/110).

Furthermore, whole genome sequence was performed to 87 C. upsaliensis, 8 C.helveticus, and 4 C. jejuni. Antimicrobial resistant genes were determined by blasting with Resfinder database. 36.8%(32/87) of C.upsaliensis carried aminoglycoside resistance gene, 10.3%(1/87)carried tetracycline resistance gene and 1.1%(1/87) C.upsaliensis isolates harbored beta-lactam resistance gene.

Virulence genes were determined by blasting with the virulence factor database(VFDB). All 87 C.upsaliensis strains carried virulence factor cadF, porA, pebA, LOS, CDT and CiaB, and 23.0%(20/87)of C.upsaliensis harbored type IV secretion system (T4SS).

According to the phylogenetic tree drawn by K-mer method, we found 8 isolates in clade 3 which were phenotypically resistant to up to 9 antimicrobials, and carried both tetracycline resistance and aminoglycoside resistance gene. Moerover,100% of the eight isolates carried virulence factor flaA in contrast to only 5.1%(4/79)of none-clade 3 isolates carried flaA gene. We also found the C.jejuni in pets source were highly related with the clinial sources.

To the best of our knowledge, this study was the first report of campylobacter spp. in pets in Shenzhen, South China. It shown that pets constitute an important reservoir and a potential source of campylobacter spp. strains.

Keywords: *Campylobacter upsaliensis;* Whole-genome sequencing; Antibiotic resistance

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Biographical note of the first author Changyan Ju, female, born in 1982, majoring in analysis of genetic characteristics of Campylobacter and Arcobacter; Yanping Ma, female, born in 1991, MD, specialty is pathogenic microbiology.

5562
Phenotypic Characterization of Clinical *Campylobacter jejuni* Isolates

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Aim: Campylobacteriosis is one of the most widespread infectious diseases worldwide. Given the incidence and prevalence of this zoonosis, it is important to understand the mechanisms that the bacteria *Campylobacter jejuni* has developed to withstand harsh environmental conditions encountered along the food chain.

Methods: A total of 5 *C. jejuni* strains (H249, H518, H529, H660 and H661) isolated from human faeces with different genotypes were characterized through different phenotypic methods, including the tolerance to oxygen at 12 h and 24 h, and the oxidants 5 mM hydrogen peroxide (H_2O_2) and 0.05 % (v/v) cumene hydroperoxide (CHP) at 15 min, 30 min and 60 min, as well as the ability to form biofilm on polystyrene and stainless steel at different temperatures (25ºC, 30ºC and 37ºC) and atmospheres (aerobiosis and microaerobiosis), and the motility performance.

Results: Different phenotypes were observed among the strains. All strains behaved similar to both oxidants with a loss of viability over time, despite showing different aerotolerance: strains H249 and H661 were aerotolerants, while strains H518, H529 and H660 were hyper-aerotolerants, although the strain H249 was the only one completely removed after exposure to CHP at 60 min. All isolates were motile, except strain H660, that was the highest biofilm former on polystyrene, while strain H249, that swarmed the longest distance (4.8 cm), was the lowest biofilm former on that material. On stainless steel, the strains H660 and H661 (motile up to 3.8 cm) showed the highest biofilm formation, but the strain H529 (motile up to 1.9 cm) showed the lowest ability.

Conclusion: Results showed a relation between the ability of *C. jejuni* to survive to environmental harsh conditions, and the aerotolerance and motility. However, the multi-phenotypes observed among the different *C. jejuni* strains suggest that the survival mechanisms adopted by the bacteria are diverse and complex.

5566

Genome Detectives: The Power of Engaging Citizen Scientists in the Curation of Nucleotide Sequencing Data.

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Aim: The open-access web-based PubMLST.org platform[1] has served the microbiology community for over 20 years but has expanded rapidly in the last five years, with a four-fold increase in the number of bacterial isolates deposited. Despite the extensive open-source PubMLST.org infrastructure to store, catalogue and analyse genomes and associated information (provenance and phenotype 'metadata'), a major challenge is the curation of new variants of the thousands of genes, which is necessary to facilitate whole genome sequence analyses. To address this problem, we have employed citizen science to support characterisation and curation of bacterial genomes.

Methods: The Genome Detectives project was designed as a user-friendly workflow for members of the general public to identify key gene characteristics through a series of task-based questions using the Zooniverse platform (www.zooniverse.org). To ensure data integrity, each gene requires a minimum of 20 user classifications and review by a database curator.

Results: Within the first month, more than 500 citizen science volunteers made more than 5,000 gene classifications. The project is continuing to grow, doubling in numbers again in the second month. Data analyses show the collective outcome of 20 citizen-science user classifications per gene correlate well with decisions taken by experienced database curators.

Conclusion: At the time of writing, there were more than 100,000 *Campylobacter* isolates with associated data, and approaching 2 million high quality, curated alleles on the PubMLST database. The citizen-science approach is a powerful force for scientific inquiry where computer power is currently lacking and has created unprecedented ability to answer

large-scale scientific questions related to the molecular surveillance of *Campylobacter*, whilst also connecting non-scientists to the authentic process of science.

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5573

Prevalence and Genetic Characteristics of *Arcobacter* spp. in China

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Aim: *Arcobacter* is a globally emerging foodborne and zoonotic pathogen that can cause diarrhea in humans. The aim of the present study was to assess the prevalence and genetic characteristics of *Arcobacter* spp. from different sources in China.

Methods: *Arcobacter* spp. was isolated from animal fecal, food and diarrheal patients. Antibiotic susceptibility test were performed with agar dilution methods. Genetic characteristics were analyzed based on the genome sequenced using an Illumina MiSeq. SPSS26.0 was used for statistical analysis, and the chi-square test (χ^2) was used to compare the count data between groups.

Results: The isolation ratios from retail raw chicken meat, beef, pork seafood and vegetables were 72% (237/329), 40% (12/30), 43% (13/30), 53% (16/30) and 39% (12/31), respectively. However, the isolation ratios from the fecal samples of poultry, sheep and diarrheal patients were 8% (4/50), 8% (9/120) and 2.1% (20/937). Whole genome sequence were performed for 172 isolates (70 *A.butzleri*, 83 *A. cryaerophilus* and 19 *A. skirrowi*) and totally 257 qualified genomes were analyzed in this study. The genomes of these three species were estimated to vary from 1.5 Mb to 2.5 Mb in length, with a G+C content of around 15%-29%. Compared with *A. butzleri* and *A. skirrowii*, high genetic diversity of *A. cryaerophilus* was identified. Some β-lactam, tetracycline and aminoglycoside resistance genes were found in *A. butzleri* and *A. cryaerophilus*. Virulence factors were identified in almost all of the strains. Type VI secretion system (T6SS) gene cluster was first identified in *Arcobacter* in this study.

Conclusion: The detection rates from different sources vary greatly. The results obtained in this study will benefit the further analysis of *Arcobacter* in China.

5580

Campylobacter jejuni energy taxis to L-fucose

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Aim: *Campylobacter jejuni* (*Cj*) is a leading cause of bacterial diarrhea worldwide and infections in infants are associated with growth-stunting in low-to-middle income countries (LMICs). *Cj* was once considered asaccharolytic, but genes encoding L-fucose metabolic enzymes are found in >60% of *Cj* sequenced genomes (1). Breastfed infants expel 4-5 mg free L-fucose per gram of feces through digestion of human milk oligosaccharides by their intestinal microbiota (2); and we detected significantly less L-fucose-metabolizing *Cj* in exclusively breastfed versus non-breastfed infants in LMICs (3). We hypothesize that L-fucose-metabolizing *Cj* swim toward the free sugar and that FucX plays a key role in this process (1). We demonstrated that FucX is an L-fucose dehydrogenase, upregulated in the presence of L-fucose and mucin, that binds preferentially to its $NADP^+$ cofactor even in the absence of L-fucose; and this enzyme is sufficient for L-fucose chemotaxis when introduced into strains lacking the pathway for sugar metabolism (4). The aim of this study is to investigate the role of FucX in L-fucose chemotaxis.

Methods: Tube-based chemotaxis assay, NADP/NADPH ratio measurement.

Results: My studies indicate that FucX influences L-fucose chemotaxis by decreasing cellular $NADP^+$/NADPH ratios which subsequently affect the energy taxis components, CetABC which respond to shifting gradients of electron acceptors and donors. This shift in $NADP^+$ levels results in flagellar rotation toward L-fucose and this swimming behavior can be complemented with a homologous L-fucose dehydrogenase from *Burkholderia multivorans*.

Conclusion: Taken together, these studies provide an explanation for why fucose metabolizing *C. jejuni* isolates might swim away from intestinal epithelial cells when they encounter mucin and free fucose and then are subsequently cleared by the host. Future studies will examine these relationships in mouse models of *C. jejuni* diarrheal disease and growth-stunting.

5588

Campylobacter in the database of knowledge gaps - DISCONTOOLS

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Aim: DISCONTOOLS, DISease CONtrol TOOLS, is an open-access database developed under EU-funded FP7. It provides information of research gaps to improve infectious disease control in order to reduce the burden of diseases.

Methods: The information in the database is updated every fifth year.

Results: Prevention and control of campylobacteriosis can be applied directly to humans or animal reservoirs. Handling and consumption of poultry meat is regarded as the most significant risk factor for human campylobacteriosis. In spite of extensive research, there are still gaps in knowledge, e.g. about transmission and pathogenicity. Recently, new animal models using gnotobiotic mice have proved useful, serving as models of human disease. More knowledge, in particular at field level, is needed in development of easy-to-administer vaccines that protect against all relevant genotypes. Current diagnostics and commercially available diagnostic kits focus to a large extent on culturable *Campylobacter*, and there is a need for culture-independent assays. Control of *Campylobacter* from other

sources than poultry/poultry meat, could require development of new infrastructure for sewage and water treatment, and better control of recreational exposures.

Conclusions:

Identified Gaps/Needs:

- Further development of diagnostic kits for rapid detection and quantification
- Development of methods for quick differentiation between live/dead bacteria
- More insight into the mechanisms of pathogenicity
- Further research and evaluation of animal models mimicking human disease
- Alternative pharmaceuticals for treatment of severe cases of human campylobacteriosis
- Better understanding of the transmission routes to broiler flocks and how to control non-foodborne transmission
- Effective commercial vaccines to prevent colonization of poultry
- Comprehensive knowledge about bacteriocins, bacteriophages, feed additives and antimicrobial peptides as preventive therapies in poultry
- More precise information about the true numbers of human cases
- Improved techniques for linking human cluster isolates to sources
- Better information to the public in terms of consumer education and hygiene training in order to prevent campylobacteriosis

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5595

Whole genome-based surveillance of German clinical *Campylobacter jejuni* isolates identifies dominant clusters

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Aim: Campylobacteriosis continues to cause the highest number of notifiable disease cases among bacterial food-borne infections in Germany. The majority of human infections does not have an obvious epidemiological link and is therefore considered sporadic. Here we present results of a nationwide whole genome-based Campylobacter surveillance program, which has been established in 2020, but also included retrospective samples. For the collection of clinical Campylobacter isolates numerous primary laboratories contributed nationwide.

Methods: *Campylobacter* spp. from clinical cases were cultivated under microaerophilic conditions on CCDA and species determination was carried out by PCR. Whole genome sequencing was performed with Illumina NextSeq, yielding paired end sequence reads. Analysis was performed with Ridom SeqSphere Software and open access online tools.

Results: With 1713 sequenced samples, the German molecular surveillance program of Campylobacteriosis represented ~ 3% of the notified 57421 cases in 2021. 1364 isolates (79.6%) belonged to the species *C. jejuni*, followed by 241 isolates *C. coli* (14.1%), and 2 isolates (0.1%) *C. fetus*. Currently, the surveillance database includes 3352 *C. jejuni* isolates from 2012-2022. We detected 23 *C. jejuni* cgMLST clusters with at least 20 isolates, sharing the same SeqSphere cgMLST complex type (CT) with a maximum allele distance of 4. These clusters cover 31% of all *C. jejuni* isolates in the database. The four largest clusters, each including at least 80 isolates, are CT1542, CT53, CT2151 and CT543. Interestingly, CT543 is also one of the major recurring lineages described in a study from Luxembourg (1). All of the larger clusters include isolates from several years and are distributed across multiple federal states.

Conclusion: A nationwide whole genome-based surveillance program has been successfully established for Campylobacteriosis in Germany (2). Several larger *C. jejuni* clusters of >20 isolates have been detected, which are geographically diverse and span across multiple years. Some cgMLST complex types prevalent in Germany have also been reported elsewhere in Europe (1). Whether the isolates within each cluster originate from the same source is currently being evaluated in an integrated approach together with epidemiologists, consumer protection experts and bioinformaticians.

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4612

Diverse sensory repertoire of paralogous chemoreceptors Tlp2, Tlp3 and Tlp4 in *Campylobacter jejuni*

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Campylobacter jejuni colonization and pathogenicity require the bacteria to sense the chemicals in its environment and move toward the target host tissues in order to cause disease. The first and the most critical step in this process is initiated by membrane-bound sensory proteins, chemoreceptors, which recognise chemical signals and trigger a response directing a cell to move toward an attractant or away from a repellent, as well as direct the microbe toward a susceptible host cell.

Aim: Here, we illustrate that the molecule - sensing repertoire of *C. jejuni* chemoreceptor family Tlp2, Tlp3 and Tlp4 includes sugars and amino- and organic acids, commonly found in animal intestinal tract.

Methods: In this study, we have used different techniques to investigate the involvement of Tlp2, Tlp3 and Tlp4 in the chemotaxis signalling pathway via small molecule arrays, glycan arrays, and surface plasmon (SPR) as well as chemotaxis assays of wild type and isogenic mutant strains.

Results: We demonstrate that the receptors sense this wide variety of chemicals via a single binding pocket with high and low affinity binding sites where one or more molecules can be bound at the same time. We also reveal that Tlp2, Tlp3 and Tlp4 receptors may have arisen through gene duplications followed by a divergent evolutionary drift.

Conclusion: Diverse sensory repertoire could provide *C. jejuni* with the ability to modulate responses to attractant and repellent signals and allow adaptation for host-pathogen interactions.

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4958

High-dose dual therapy versus culture-based susceptibility-guided therapy as a rescue regimen for *Helicobacter pylori* infection: a randomized controlled trial

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Background: An alternative rescue regimen is needed for *H.pylori* infection in the face of increasing antibiotic resistance.
Objectives: To compare the efficacy, safety, patient compliance, and cost between high-dose dual therapy (HDDT) and culture-based susceptibility-guided therapy (CB-SGT) as a rescue regimen for *Helicobacter pylori* infection.
Methods: One hundred forty-six patients with a history of eradication failure were enrolled and randomly assigned to receive HDDT or CB-SGT. HDDT consisted of esomeprazole 20 mg and amoxicillin 750 mg, both given four times per day (qid). CB-SGT consisted of esomeprazole 20 mg twice daily (bid), amoxicillin 1000 mg bid plus clarithromycin 500 mg bid, metronidazole 400 mg bid, or levofloxacin 500 mg daily (qd) for sensitive patients, in that order. For patients with triple resistance, a bismuth-containing regimen with a high dose of metronidazole was chosen, including esomeprazole 20 mg bid, bismuth 220 mg bid, amoxicillin 1000 mg bid, and metronidazole 400 mg qid. All regimens were given for 14 days.
Results: The eradication *H. pylori* rates achieved with HDDT in the intention-to-treat, per-protocol, and modified intention-to-treat analyses were all 84.9% (62/73, 95% confidence interval [CI]: 76.5%–93.9%), compared with 83.6% (61/73, 95% CI: 74.9%–92.3%), 84.7% (61/72, 95% CI: 76.2%–93.2%), and 84.7% (61/72, 95% CI: 76.2%–93.2%) with CB-SGT, respectively. Patient adherence was high in both groups. The HDDT had a lower cost and rate of side effects (P<0.001) compared with CB-SGT.
Conclusions: Compared with the CB-SGT, HDDT showed similar efficacy and patient compliance with fewer side effects and a lower cost. Thus, HDDT offers an alternative empirical rescue regimen for *H. pylori* infection.
Keywords: high-dose dual therapy,rescue regimen,*Helicobacter pylori*,eradication rate

5518

Prevalence and diversity of *Campylobacter* carriage by migratory birds at three major habitats in China

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A variety of *Campylobacter* species have been reported in wild birds, posing a potential avian-human transmission pathway[1-5]. Currently there has been little surveillance data on *Campylobacter* carriage in migratory birds in China. In this work, fresh fecal droppings from individual migratory birds were collected from bird wintering/stopover sites in three provinces in China, namely Xingkai Lake in Heilongjiang, Cangzhou in Hebei, and counties along the Yarlung Tsangpo River in Tibet, from October 2020 to March 2021. Nucleic acid was extracted and tested for *Campylobacter* with PCR-based methods. Overall 73.1% (272/372) of the samples were positive for *Campylobacter* 23S rRNA under real time RT-PCR condition. The detection in Heilongjiang (90.7%) and Hebei (86.3%) was higher than that in Tibet (65.9%, $p < 0.05$). A subset of positives was further characterized with 16S amplicon sequencing[6]. 85.1% (103/121) were identified to *Campylobacter* species with >98.65% identity, including *C. jejuni/C. coli* (15, 14.6%), *C. lari* (10, 9.7%), an unknown species (54, 52.4%) with NCBI accession number CP059600, and non-identifiable species (24, 23.3%). This unknown species was the predominant *Campylobacter* in *Anser fabalis*/*Anser albifrons* of Heilongjiang (16/17, 94.1%) and in *Anser indicus* of Tibet (30/48, 62.5%). *C. jejuni/C.coli* was exclusively detected in Tibet (14/48, 29.2%), except for one case in Heilongjiang. Hebei, which

exhibited diverse migratory bird types, showed almost even distribution of the *Campylobacter* species listed above, except *C. jejuni/C.coli*. Phylogenetic analysis was performed on *atpA* gene to further elucidate their genetic relations. Resistance to fluoroquinolone and macrolide was also evaluated with real time PCR targeting gyrA T86I and 23S A2075G mutations, respectively[7]. This work demonstrated that *Campylobacter* was highly prevalent and diverse in migratory birds in China, including the important pathogenic species *C. jejuni/C. coli* and an unknown *Campylobacter* species. Further characterization is required to understand the transmission risk.

Keywords: *Campylobacter*; migratory birds; China; molecular epidemiology

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5527

The Mla phospholipid transport system alter the *Campylobacter jejuni* outer membrane properties

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Aim: Gram-negative bacteria outer membrane (OM) is an asymmetric lipid bilayer with lipopolysaccharides (LPS) in the outer layer and phospholipids (PL) in the inner layer. This unique structure makes the OM impermeable to external agents, including antibiotics and bile salts. One system that plays an important role in maintaining OM asymmetry is the Mla protein system (MlaABCDEF), involved in PL transport (Giordano et al. 2020). In this study, we determined the effect of proteins encoded by *mlaEFDB* operon genes on OM properties and virulence of *C. jejuni* 81-176.

Method: We investigated the effect of mutations in *mlaEFDB* genes on hydrophobicity, OMV production and adhesion, invasion of *C. jejuni* into epithelial cells and biofilm formation ability.

Results and Conclusion: Loss of some Mla pathway proteins alters OM hydrophobicity, OMV production and *C. jejuni* 81-176 virulence.

This work was supported by the National Science Centre, Poland (UMO-2018/30/M/NZ6/00429).

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5537

Successful application of *Helicobacter pylori* personalized treatment via novel gastric string test and qPCR

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Aim: To evaluate (1) the effectiveness of the strategy combining gastric string-test and quantitative PCR (qPCR) in diagnosing *Helicobacter pylori* (*H. pylori*) infection; (2) identifying clarithromycin and levofloxacin resistance via qPCR SNPs detection; and (3) the eradication rate of *H pylori* via the guidance of SNPs identification.

Methods: This is an open-label, comparative study in which we enrolled subjects tested by ^{13}C- urea breath tests (UBT) and string-qPCR test. ^{13}C-UBT positive patients were given 14 days susceptibility guided bismuth quadruple therapy contained proton pump inhibitor (PPI), colloidal bismuth tartrate, plus amoxicillin, clarithromycin, levofloxacin or furazolidone based on string-qPCR SNP results. In the control group, *H. pylori* eradication rate was analyzed from ^{13}C-UBT positive patients who were treated retrospectively via PPI, colloidal bismuth tartrate, amoxicillin and clarithromycin for 14 days between 2020 to 2022.

Results: 50 ^{13}C-UBT positive patients were assigned to susceptibility guided-therapy

and 30 ^{13}C-UBT negative subjects were recruited as a negative control. A total of 50 patients who received the empirical therapy were included as the control group. The string-qPCR test and ^{13}C-UBT had a 92.0% and 100% consistency in the positive and negative results, respectively. According to the SNPs analysis, the clarithromycin and levofloxacin resistances were 21.0% and 23.4%, respectively. Both the susceptibility-guided and the empiric therapy were successful with per-protocol eradication rates of 93.5% and 88.0% (P =0.358), respectively.

Conclusion: The combination of string-test and qPCR can effectively detect *H. pylori* and its resistance to clarithromycin and levofloxacin. Both susceptibility-guided therapy based on string-qPCR results and empirical therapy showed good eradication rates, indicating that string-qPCR test has an excellent practicality in real-world clinical settings.

5540

Genetic Characteristics of Lipooligosaccharide (LOS) and Capsular Polysaccharide (CPS) of Campylobacter jejuni from Different Sources in China

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Aim: To determine the distribution of two important virulence factors (LOS and CPS) in *C. jejuni* isolated from different sources in China and to develop a rapid screening method for Guillain–Barré syndrome (GBS)-associated strains.

Methods: Whole-genome sequencing was carried out for 494 *C. jejuni* strains**.** OrthoMCL software was used to define the LOS/CPS gene clusters. CPS genotyping was performed with serotype-specific sequence alignment using BLAST software. Real-time PCR was developed with the unique sequences of specific CPS types.

Results: Nine novel and 29 previously confirmed LOS classes were identified; LOS classes A, B, and C were the most common (48.2%, 238/494) among the 494 strains. Twenty-six capsular types were identified in 448 strains. HS2, HS4c, HS5/31, HS19 and HS8/17 were the most frequent CPS genotypes (58.7%, 263/448). Strains of 17 CPS genotypes (strain number >5) had one or two prevalent LOS classes ($p<0.05$). Multiplex real-time PCR for rapid identification of HS2, HS19 and HS41 was developed and validated with strains of known serotypes.

Conclusion: These results describe the genetic characteristics of the important virulence factors in *C. jejuni* in China. The multiplex real-time PCR developed in this study will allow for enhanced surveillance of GBS-associated strains in China.

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5547

Two novel Campylobacter species from cat and sheep in China

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Nine independently novel bacterial strains, designated XJK22-1、XJK33-1、XJK49-2、XJK56-3、XJK62-3、XJK7-1、S13-1、SYS25-1 and SYS28-3, were isolated from the feces of cat and sheep in 2019 and 2020 in Beijing, China. Cells were 2–3 μm long and ≤ 0.5 μm wide, Gram-stain-negative, microaerobic, motile, oxidase-positive, and urease-negative. Phylogenetic analyses based on 16S rRNA gene sequences indicated that these nine isolates belong to the genus Campylobacter, but formed two robust clades that was clearly separate from the currently recognized species and respectively isolated from the cat and sheep. Both these strains shared low 16S rRNA gene sequence similarity, digital DNA-DNA hybridization relatedness and average nucleotide identity values with *Campylobacter upsaliensis* CCUG 14913^T, *C. lanienae* NCTC 13004^T and against each other, which are below the cut-off values generally recognized for isolates of the same species. The genomic DNA G+C contents of type strains XJK22-1^T and SYS25-1^T were 34.99 mol% and 32.43 mol%, respectively. Electron microscopy showed that these cells were spiral-shaped, with bipolar unsheathed flagella. Comparing the phenotypic and phylogenetic features among the nine strains and their related organisms, strains XJK22-1^T and SYS25-1^T

represent two novel species within the genus Campylobacter, for which the names *Campylobacter upsaliensis like* sp. nov. (Type strain XJK22-1^T) and *Campylobacter lanienae like* sp. nov. (Type strain SYS25-1^T) are proposed.

Keywords: novel bacterial strains; phylogenetic analyses; 16S rRNA gene; phenotypic features

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5548

Evaluation of BioNumerics Core Genome and Whole Genome Multilocus Sequence Typing for *Campylobacter* Outbreak Detection

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Aim

Pulsed-field gel electrophoresis (PFGE) and 7-gene multilocus sequence typing (7-MLST) have been historically used to differentiate sporadic from outbreak *Campylobacter* isolates. Whole genome sequencing (WGS) has been shown to provide superior resolution and concordance with epidemiologic data when compared with PFGE and 7-MLST during outbreak investigations. In this study, we aim to evaluate epidemiologic concordance for high quality single nucleotide polymorphism (hqSNP), core genome (cg)MLST, and whole genome (wg)MLST to cluster or differentiate outbreak-associated and sporadic *C. jejuni* and *C. coli* isolates.

Methods

237 *C. jejuni* and five *C. coli* isolates epidemiologically linked to 16 outbreaks and 73 sporadic isolates were sequenced using the Illumina MiSeq. Sequences were analyzed using wgMLST and cgMLST (Oxford scheme) in BioNumerics v7.6.3. These sequences were also analyzed with hqSNP analysis using LYVE-SET v1.1.4f (github.com/lskatz/lyve-SET).

Results

Sporadic *C. jejuni* and *C. coli* isolates were mostly differentiated from outbreak-associated isolates by cgMLST (69/73), wgMLST (72/73), and hqSNP (72/73) analyses. There was a high correlation (Baker's Gamma Index > 0.965) between cgMLST and wgMLST analyses of the isolates; however, wgMLST generally had higher allele differences. The correlation was sometimes lower (Baker's Gamma Index > 0.630) comparing hqSNP analysis to these allele-based methods.

Conclusions

We demonstrated that *C. jejuni* and *C. coli* isolates clustered in concordance with epidemiologic data using WGS-based analysis methods. Discrepancies between allele and SNP based approaches may reflect the differences between how genomic variation (SNPs and indels) are captured between the two methods. Further study will be completed to understand these discrepancies.

5561

Optimization for *Campylobacter* separation from different cleanliness samples in slaughterhouse with different combinations of antibiotics

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Aim: To compare the positive rate and bacterial load of *Campylobacter* in the slaughterhouse with different antibiotic combination medium, so as to obtain the optimal combination of antibiotics for the isolation and purification of *Campylobacter* from samples of different cleanliness.

Methods: Collect samples of different cleanliness in the whole industry chain of slaughterhouse and use different combinations of antibiotic culture medium to experiment on pure culture of *Campylobacter*, low cleanliness, medium cleanliness and high cleanliness samples respectively. The antibiotic combination is divided into three categories, six antibodies (polymyxin B, trimethoprim, rifampicin, cycloheximide, cefoperazone and amphotericin B); four antibodies (polymyxin B, trimethoprim, rifampicin, cefoperazone); three antibodies (polymyxin B, trimethoprim, cefoperazone).

Results: The best suitable antibiotic combination for low cleanliness samples is six antibodies; medium pollution samples have the highest detection positive rate and bacterial load in the four antibodies; low pollution samples have the highest detection positive rate in four antibodies, three antibodies for highest bacterial load.

Conclusion: Isolation of *Campylobacter* from samples of different cleanliness was studied in this test. The CCDA medium containing different antibiotic combinations was selectively used to identify *Campylobacter* more conveniently and effectively. The selection of antibiotics in the current separation and purification method of *Campylobacter* was improved and perfected.

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5565

Campylobacter jejuni flagellar regulatory protein FlhF is involved in the defense mechanism of oxidative stress

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Aim: *Campylobacter jejuni* is a worldwide prevalent food-borne zoonotic pathogen. As a microaerophilic bacterium, it has evolved a variety of response mechanisms to resist oxidative stress, including flagella and their mediated motility. As a key protein in the regulation of *C.jejuni* flagella, FlhF plays an important role in the process of flagellar assembly. Therefore, in this study, we aimed to investigate the biological effect of FlhF on the oxidative stress phenotype of *C.jejuni* and its mediation mechanism of oxidative stress.

Methods: The oxidative stress resistance of *flhf* mutants was examined by comparing the survival of logarithmic growth phase bacteria (WT/Δ*flhf*) under H_2O_2 (1 mM 30 min) stimulation. RNA samples were extracted for RNA-seq to analyze the regulatory mechanism.

Results: Under H_2O_2 conditions, the *C.jejuni flhf* mutant had significantly reduced viability and weaker resistance to H_2O_2 compared with the wild type. Through RNA-seq analysis, it was found that under H_2O_2 conditions, the *flhf* mutant resulted in significant down regulation of oxidative stress-related genes.

Conclusion: *Campylobacter jejuni* flagellar regulatory protein FlhF helps to resist oxidative stress.

5572

Isolation and Molecular Sub-typing of *Campylobacter jejuni* and *Campylobacter coli* in China

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Aim: One effective isolation methods for *C. jejuni* and *C. coli* was developed and evaluated. Genetic and sub-typing characteristics of the *C. jejuni* and *C. coli* isolates from different sources were investigated.

Methods: The isolation method was optimized and the $eLOD_{50}$ was determined by inoculation experiments with different strains in different samples. *C. jejuni* and *C. coli* isolates were sequenced using an Illumina MiSeq. The multilocus sequence typing was determined based on each genome with the open-access PubMLST.org website database. Genetic population were obtained with cgMLST and cgSNPs analysis based on the genomes.

Results: Enrichment culture method with the optimized Preston broth (named ICDC-CAMPY broth) combined with the filtration is one effective method for isolation of *C. jejuni* and *C. coli*. The LOD_{50} for each food items was less than 10^1CFU/25g (ml). The isolation ratio of *Campylobacter* from the diarrheal patients was 12.1% (23/190). Two hundred and thirty STs were identified among 600 *C. jejuni* isolates. Over the identified STs, 115 STs had only one isolate. Four STs (2993, 6913, 11329 and 6959) were identified as the outbreak related and the other most commonly STs were ST22, ST354, ST464, ST51 and ST2328. The top five STs from the sporadic diarrheal patients were ST2328, ST22, ST354, ST51 and ST5. 137 STs were identified among 604 *C. coli* isolates. The top five STs were ST1586, ST860, ST1145, ST854, ST825 and ST872. Two STs (9227 and 1068) were identified as the outbreak related and the most commonly detected STs in sporadic diarrheal patients were ST860, ST1145, ST1625, ST5510 and ST829. Three major genetic clades were identified in *C. jejuni* and *C. coli*, respectively.

Conclusion: *C. jejuni* showed a high level of diversity within the same region and same sources. Most of the clinical *C. jejuni* isolates were CC22.

5574

A family case infection and genomic characterization of *Campylobacter jejuni* associated with perimyocarditis

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Aim: *Campylobacter* spp. is the leading cause of foodborne gastrointestinal infections in humans worldwide. Although post-infection complications are rare, they can occur mainly in young children, elderly and immunocompromised people. Here, it is described the case of a family that had contact with the same source of *C. jejuni* contamination, in which only the little siblings were infected by the bacteria and only the 11-year-old male suffered later from perimyocarditis. The *C. jejuni* isolates from the children were sequenced for comparative genomics in the background of the perimyocarditis case.

Methods: The isolates were molecularly typed using pulsed field gel electrophoresis (PFGE), and whole-genome sequenced. Raw reads were filtered and assembled for the bioinformatics analysis that included calculation of Average Nucleotide Identity (ANI) values, Multilocus sequence typing (MLST), genome annotation, searching for antimicrobial resistance (AMR) genes and point mutations conferring resistance, virulence genes and plasmids, pangenome construction and comparison of phase-variable (PV) genes.

Results: Both isolates were grouped in the same PFGE type, Sequence Type (ST)-148 and clonal complex CC21. The genetic relatedness among the two isolates resulted in ANI values greater than 99.99%. The virulence and AMR pattern of the two isolates were identical and one gen coding for a methyl-accepting chemotaxis signal transduction protein was found to be exclusive for the perimyocarditis-associated isolate. Interestingly, differences were observed in the ON/OFF state of 7 genes (*kdpB*, *flgR*, *wlaN*, *cj1144c*, *cj1305c*, *cj1310c* and *cj1420c*) in both isolates.

Conclusion: The *C. jejuni* strains suffered little but significant genomic changes after infecting the children indicating that PV occurs during human colonization, which modulates bacteria virulence, through human host adaptation, that ultimately is related to

complications following a campylobacteriosis episode depending on the host status. This highlights the importance of the relation between host and pathogen in severe complications of *Campylobacter* infections.

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5579

Distribution characteristics of the *sabA, hofC, homA, homB* and *frpB-4* genes of *Helicobacter pylori* in different regions of China

Background

Helicobacter pylori (H. pylori) encodes numerous outer membrane proteins (OMPs), with considerable geographic heterogeneity and related to different clinical outcomes. This study aimed to investigate the distribution characteristics of five important OMP genes (sabA, hofC, homA, homB and frpB-4) in different regions of China.

Materials and method

A total of 266 strains were isolated from 348 stomach biopsy specimens in Shandong, Guangxi, Heilongjiang, Hunan, and Qinghai provinces. The presence of sabA, hofC, homA, homB and frpB-4 gene was detected by polymerase chain reaction (PCR) from H. pylori genomic DNA.

Results

Among the strains in five regions, the prevalence of frpB-4 was 100% and that of hofC was 97.7%. The prevalence of homB in the isolates from Qinghai (45.5%) was significantly lower than that in Shandong (75.3%), Guangxi (76.9%) and Hunan (69.6%) ($P<0.05$). The frequency of homA in Shandong (30.1%) was significantly lower than in Guangxi (57.7%) and Qinghai (63.6%) ($P<0.05$). The prevalence of the sabA gene in Shandong, Guangxi, Heilongjiang, Hunan and Qinghai provinces was 21.9%, 59.7%, 45.9%, 52.2%, and 18.2%, respectively ($P<0.05$). The sabA “on” status was significantly more frequent in isolates from Guangxi (46.8%), Heilongjiang (37.8%), and Hunan (47.8%) than Qinghai (3.0%) ($P<0.05$). The presence of homA and sabA genes may be negatively correlated with the development of gastritis. There was no significant association between the frpB-4, hofC, homB gene and clinical outcomes.

Conclusion

The prevalence of homA, homB, and sabA genes and the sabA “on” or “off” status have significant geographical differences among five provinces in China. The presence of homA and sabA genes may be protective factors of gastritis.

5581

Capsular Genotype and Lipooligosaccharide Class Genomic Characterizations of *Campylobacter jejuni* From Food Animals in China

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Aim: The aim is to assess the genomic pathogenic characterizations of *C. jejuni* isolates from food animal origins in China.

Methods: 1609 *C. jejuni* isolates sampled from 2005 to 2019 in China were analyzed using capsular genotyping. Strains from cattle and poultry were further characterized by LOS classification and multilocus sequence typing (MLST), compared with the isolates from human patients worldwide with enteritis and GBS. Correlation between LOS class and disease associated capsular genotype in food animal isolates were analyzed.

Results: Disease associated capsular genotypes and LOS classes over-represented in human isolates were also dominant in animal isolates, especially cattle isolates. Based on the same disease associated capsular genotype, more LOS class types were represented by food animal isolates than human disease isolates. Importantly, high-risk lineages CC-22, CC-464, and CC-21 were found dominated in human isolates with GBS worldwide, which were also represented in the food animal isolates with disease associated capsular types.

Conclusion: This is the first study providing genetic evidence for food animal isolates of particular capsular genotypes harbor similar pathogenic characteristics to human clinical

isolates. Collective efforts for campylobacteriosis hazard control need to be focused on the zoonotic pathogenicity of animal isolates, along the food chain “from farm to table”.

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5583

Phylogenetic analysis of *Campylobacter* species in a multi-host system from a wildlife-rural interface in Uganda

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Aim: The transmission of microorganisms has been observed between different host species, including wild, domestic animals and humans, with sometimes significant health impacts. Still, the understanding of when, where, and why cross-species transmission (aka 'spillover') occurs is often unknown. Therefore, identifying which organisms are present in different hosts in the same space and time is an essential step in testing theories about why spillover occurs. This project aims to identify *Campylobacter* species and their relatedness within a multi-host system living in a rural area of Uganda.

Methods: The study uses faecal samples from anonymous human patients, humans in the community with animal contact data, livestock animals (cattle and goats), wild unhabituated and habituated gorillas, the latter with increased exposure to humans and domestic livestock. The samples were analyzed using shotgun high throughput sequencing and bioinformatics tools[1,2,3,4,5], and then the alignment file from GTDB-Tk[6] was copied to create a phylogenetic tree using the software IQ-TREE 2[7]. The tree visualization was done on the iTOL website (https://itol.embl.de).

Results: Thirty-seven *Campylobacter* draft genomes were recovered, with completeness which ranged from 39.71 to 98.09%. Thirty-four draft genomes had completeness of more than 50%. Phylogenetic analyses identified genomes with differing levels of similarity to

"Candidatus *C. infantis", C. lanienae, "C. troglodytis"* and other genomes in distinct clades, with different host associations.

Conclusion: We identified species specific, and potentially novel *Campylobacter* by sampling multiple hosts in the same location and time.

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5598

Campylobacter spp. in Shellfish in Croatia

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Aim: The aim of this study was to assess the presence of thermotolerant *Campylobacter* species in shellfish production and harvesting areas of the Istrian aquatory (North Adriatic Sea), regarding the growing conditions (in terms of location and sea temperature) and shellfish species, as factors influencing increased public health risk when consuming shellfish.

Methods: The study included samples of the most economically important species of live bivalve molluscs: mussels (*Mytilus galloprovincialis*), oysters (*Ostrea edulis*) and queen scallops (*Aequipecten opercularis*) that were collected in a period from August to October 2021, on a weekly basis at nine locations of the Istrian County, Croatia. Isolation of *Campylobacter* was done according to standard ISO 10272-1:2017 method, and species were identified using multiplex PCR. Isolates identified as *C. jejuni* and *C. lari* were genotyped using multilocus sequence typing (MLST).

Results: Among 108 examined samples of bivalve molluscs, mussels dominated and were the only ones found positive for the presence of *Campylobacter* (25.6%). The majority of *Campylobacter* positive samples, 85% of them (n = 17), were detected when the sea temperature was between 20 °C and 27 °C. In total, 19 *C. lari* and one *C. jejuni* strains were isolated. *C. lari* isolates found in this study belong to 13 sequence types (STs), and 9 of them are newly described in this research. Two out of the four previously described *C. lari* STs that were found in this study were previously detected in human stool. The only *C. jejuni* isolate was found to be sequence type 1268, which belongs to ST-1275 clonal complex that is almost exclusively found in seabirds but can sporadically cause infection in humans.

Conclusion: Based on the obtained results, introducing surveillance of thermotolerant *Campylobacter* in shellfish in the Republic of Croatia is advised as an improvement for public health safety.

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5599

Antimicrobial resistance profile of a *Campylobacter jejuni* strain causing perimyocarditis

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Aim: Campylobacteriosis is a foodborne illness caused by bacteria of the genus *Campylobacter* that is considered the most frequently reported foodborne illness worldwide. Despite, campylobacteriosis is usually a self-limiting illness, the use of antibiotics recommended for severe cases has led to the development of antibiotic-resistant *Campylobacter* strains that limit drugs usefulness. We described the first familiar case that had contact with the same source of *C. jejuni* contamination, in which only the little siblings were infected by *C. jejuni* and only one of them suffered from perimyocarditis.

Methods: The strains isolated from the children were molecularly typed and sequenced to study the genomic traits that may be related to perimyocarditis. Antimicrobial resistance genes and virulence genes were analysed using the workflow Wombat. The former were searched using a combination of ABRicate and AMRFinder tools, whereas the latter were analysed using tBLASTn against an in-house database of 76 virulence genes of interest.

Results: ABRicate included all three CmeABC efflux pump genes (*cmeA*, *cmeB* and *cmeC*) along with the *cmeR* repressor, whereas AMRFinder identified the *blaOXA-193* gen (resistance to β-lactam) in the two strains. Besides, both isolates harboured the same 58 genes as *C. jejuni* NCTC 11168 genome, and *virB11*, *ggt*, *cgtB*, *cst-II* and the T6SS coding genes were not present in any of the strains.

Conclusion: The antimicrobial resistance and virulence patterns of both isolates were identical, indicating that the strains were highly similar. Therefore, these results make difficult to draw conclusions in the genomic features that could be linked to perimyocarditis.

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5600

Phase variable genes identification in a *Campylobacter jejuni* strain causing perimyocarditis

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Aim: Campylobacteriosis is a foodborne illness caused by bacteria of the genus *Campylobacter* that is considered the most frequently reported foodborne illness in the EU since 2005. The use of antibiotics is only recommended for severe cases as campylobacteriosis is usually a self-limiting. However, complications after infection were reported, such as Guillain–Barré syndrome and Miller Fischer syndrome and perimyocarditis. We described the first familiar case that had contact with the same source of *C. jejuni* contamination, in which only the little siblings were infected by *C. jejuni* and only one of them suffered from perimyocarditis.

Methods: The strains isolated from the children were molecularly typed and sequenced to study the genomic traits that may be related to perimyocarditis. The study included the identification of differences between both isolates and specially the analysis of phase variable genes using PhasomeIt.

Results: Two hypothetical proteins were only found in the isolate not associated with perimyocarditis, whereas a methyl-accepting protein was exclusive for the isolate linked to the perimyocarditis case. The remaining differences corresponded to phase variable genes involved in flagella, chemotaxis, LOS and capsule, for which different ON/OFF states were observed.

Conclusion: The different ON/OFF states found in both isolates indicate that phase variation ocurred in *C. jejuni* after host infection and this bacteria behaviour can be later associated with the different infection results in the little siblings.

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5643

Prevalence of *Campylobacter* spp. contamination in poultry, porcine, bovine, and ovine liver in Ireland

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Aim: Campylobacteriosis is a self-limiting, notifiable disease caused by the *Campylobacter* genus, a gram-negative bacterium that typically resides in the gastrointestinal tract of many wild and domesticated animals, including pigs, poultry, sheep, and cattle. Though it is well-established that the primary reservoir for *Campylobacter* infection in humans is poultry muscle, the prevalence/disease burden of this foodborne pathogen in both poultry and non-poultry species retail liver in Ireland is less well understood.

Methods: 25 g samples were tested according to the ISO 10272-1: 2017 detection method with the addition of Butzler as a second selective media. Isolates were confirmed using Maldi-Tof.

Results: In this pilot study, a total of 190 samples were analysed, comprising of bovine, ovine, porcine and poultry liver sourced from four abattoirs in the Leinster region in the first half of 2022. Detection results confirmed high prevalence of *Campylobacter spp.* in poultry liver (35/50, 70%) and lower-than-expected prevalence in bovine (4/45, 4.4%) and porcine (8/50, 16%) liver when compared to the results of other similar studies. The most important finding of this study was the high prevalence of *Campylobacter spp.* detected in ovine liver (38/45, 84.4%). Speciation of positive isolates revealed an overall predominance of *C. jejuni* (67/92, 72.8%), found to be more prevalent than *C. coli* in all species except porcine. The second selective agar (Butzler) represented an enhancement of the methodology and improved overall recovery for *Campylobacter* spp. in porcine and poultry liver of 4% (2/50) and 12% (6/50), respectively.

Conclusion: These results indicate the substantial burden of *Campylobacter spp*. in liver for human consumption across multiple species, particularly highlighting ovine liver as a poorly recognised reservoir of potential human disease

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4677

Campylobacter species diversity in infants and environmental reservoirs of households in Eastern Ethiopia

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Aim: Early infection of children with *Campylobacter* is associated with EED/stunting in children (1, 2). Our earlier cross-sectional study, as a part of ongoing *Campylobacter* Genomics and Environmental Enteric Dysfunction (CAGED) project, conducted in rural eastern Ethiopia demonstrated that 88% of child stools harbored multiple *Campylobacter* species (average of 12 species/positive child)(3). This study is assessing the *Campylobacter* species diversity in children and environmental samples in the same region, as a first step towards identifying reservoirs associated with infants' infection.

Methods: Child stools (n=1,164) were collected monthly from birth until 12 months of age. Environmental (livestock feces [cattle, chicken, goat and sheep]) and human (mother and sibling stools) samples were collected biannually (n=232 per sample type). Species-specific quantitative PCR (*cpn60* and *hipO* target) was used to assess the prevalence and diversity of *Campylobacter* species (4). Gene targets for two thermophilic and 13 non-thermophilic *Campylobacter* species were included. Specificity and sensitivity of the primers were validated in-house.

Results: To date, *Campylobacter* was detected in 91% (106/116) of households, and 44% (445/1,002) of samples were positive for at least one *Campylobacter* species. Candidatus *C. infans* (5) and *C. jejuni* were predominant in human (36-56% and 6-16%, respectively) and environmental (6-14% and 6-19%, respectively) samples, followed by *C. upsaliensis* (6-15%), *C. lari* (3-6%) and *C. concisus* (2-4%). Other *Campylobacter* species were rarely detected (<2%). The prevalence of *C. upsaliensis* and *C. infans* correlated between mother, sibling and child stools (r^2<0.40; P<0.009). The prevalence of *C. jejuni* in cattle feces correlated with other livestock feces (chicken, goat and sheep; r^2>0.25; P<0.01).

Conclusion: Our study demonstrated that non-thermophilic *Campylobacter* were predominant in Ethiopian households. Children harbored multiple species of *Campylobacter* and may get infected through multiple reservoirs. Studies assessing the temporal prevalence of *Campylobacter* species in child stools and their reservoirs are underway.

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4678

Possible exposure pathways of *Campylobacter* to children within households from rural eastern Ethiopia

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Aim: Recent studies have suggested potential associations between *Campylobacter* colonization and EED/stunting in children (1–3). However, little is known about how children in developing countries are exposed to *Campylobacter,* which greatly limits the development of effective interventions in reducing *Campylobacter* infections and consequently EED/stunting in children. As part of the EXposure assessment of *CAM*pylobacter infections (EXCAM) project, this study is assessing the *Campylobacter* load in children associated environmental samples to understand the exposure pathways of *Campylobacter* infections of children in Eastern Ethiopian households.

Methods: Human (areola swabs, mother, sibling and child hand rinse, and breast milk) and environmental (fomite, soil, food consumed by children, drinking and bathing water) samples were collected (n=157 per sample type; 76 households) when the child ranged between 3-14 months of age. A genus-specific (targeting 16S RNA) viability quantitative PCR was used to determine *Campylobacter* load in above samples (4). *Escherichia coli* (indicator of potential fecal contamination) was quantified using EC-MUG and Chromocult.

Results: Preliminary results confirmed that PMAxx treatment allowed detection of live *Campylobacter*; however, it reduced the qPCR sensitivity (4 Ct value difference between PMAxx treated *versus* non-treated viable *Campylobacter*). Studies to quantify *Campylobacter* in different samples are underway. Overall, *E. coli* was detected in 58% of all samples. Especially 67, 88 & 87%, (2.1-log CFU/pair of hands) in child, sibling and mother hand rinse, respectively and 92 and 71% (2.4-log CFU/L) in bathing and drinking water, respectively. *E. coli* load in fomites (59%; 1.6-log CFU/fomite), breast milk (13%; 1.2-log CFU/ml), mother and sibling hands were associated with fecal contamination of the child (r^2<0.38; P<0.007), and thus, might serve as potential sources of *Campylobacter* exposure.

Conclusion: Our data highlighted high exposure risks to fecal bacteria through a variety of objects. Results will be integrated with behavioral observations to estimate intensity of exposure and main sources.

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4700

Prevalence of *Campylobacter* in Infants and potential reservoirs in Eastern Ethiopian households using real-time PCR

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Aim: In Ethiopia, diarrhea is the second prevailing cause of infant mortality in children <5 [1,2]. Our previous cross-sectional study, as a part of ongoing *Campylobacter* Genomics and Environmental Enteric Dysfunction (CAGED) project, suggested an association between high prevalence of *Campylobacter* infections during early childhood and development of adverse health effects, including stunting and EED in low resource settings [3]. Here, we aim to assess the temporal colonization of infants with *Campylobacter* in rural Eastern Ethiopian households.

Methods: A longitudinal study involving 115 infants was conducted from December 2020-June 2022. Participants were selected randomly from ten kebeles in Haramaya woreda, Ethiopia. Child stools (n=1,164) were collected monthly from birth until 12 months of age. Environmental (soil, water), livestock feces [cattle, chicken, goat, sheep]) and human (mother and sibling stools) samples were collected biannually (n=1,721) from the same households. Genus-specific real-time PCR (Taqman; gene target: 16S RNA) was used to detect *Campylobacter* [4,5].

Results: A total of 78% (n=1,592/2,035), 95% CI[0.76,0.80] of field samples were positive for *Campylobacter*. To date, 67% (n=852/1267),95%CI[0.65,0.69] human, 96% (n=629/654),95%CI[0.94,0.97] livestock and 98% (n=111/113),95%CI[0.94,0.99] environmental samples were positive at the genus level. *Campylobacter* was most prevalent in sibling stools samples (86%, n=129/150),95%CI [0.79,0.91] compared to child (62.7%; n=612/976),95%CI[0.59,0.65] and mother (78.7%; n=111/141),95%CI [0.71,0.85] stools. A positive relationship between *Campylobacter* presence and increased child age was observed (r=0.45; p<0.001). Prevalence of *Campylobacter* was similar across

livestock species tested with an overall average of 96%. Majority of soil samples (98%; n=111/113),95% CI [0.94,0.99] were also positive for *Campylobacter*.

Conclusions: *Campylobacter* is highly prevalent in children and their associated environment. Preliminary results suggest 30% of children <1 month were exposed to *Campylobacter* while in children >6 months' exposure increased to 82.9%. Analysis to understand associations between *Campylobacter* presence with diarrhea, stunting, and EED and reservoirs of child infection are ongoing.

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4701

Co-resistance molecular mechanism between Erythromycin and DDAB in *Campylobacter jejuni*

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Aim: Disinfectants and antibiotics are used to eradicate or prevent the growth of microbials, raising concerns about the development of co-resistance to both antimicrobial types. Although co-resistance to disinfectants and antibiotics has become increasingly concerned, little is known of how the co-resistance evolve. This study aims to evaluate the co-resistance mechanism at the genomic, transcriptomic and metabolome levels of *Campylobacter jejuni* (*C. jejuni*).

Methods: we explore co-resistance mechanism to didecyl dimethyl ammonium bromide (DDAB) and erythromycin (ERY) at the genomic, transcriptomic and metabolome levels in *C. jejuni*.

Results: Multidimensional analysis show that co-resistance mechanisms, in particular CmeABC efflux-related mutations, as well as the relative expression of specific genes (e.g., *gltA*, *mqo*, *sucD*, *pycA*, *sucC*, *eno*, *ackA*, *ldh*, *trpD* and *trpB*), are principal contributors to

co-resistance. Moreover, co-resistance shift the metabolite pathways of D-Glutamine and D-glutamate metabolism and alanine, aspartate and glutamate metabolism in the mutants.

Conclusion: This work suggests that amino acid metabolism may affect evolutionary trajectories of co-resistance.

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4704

Research progress of Campylobacter efflux pump

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Abstract: Campylobacteriosis is an important global public health problem caused by Campylobacter and is a leading cause of human gastroenteritis worldwide, causing many socioeconomic impacts. For Campylobacter-induced enteritis macrolides mainly erythromycin or streptomycin are the drugs of choice, and fluoroquinolones such as ciprofloxacin are also commonly used[1]. In recent years, Campylobacter resistance to antibiotics has become a serious global threat, both in developed and developing countries. To cope with the selective pressure imposed by antibiotic use, Campylobacter has evolved multiple antibiotic resistance mechanisms, including modification or mutation of antimicrobial targets, modification or inactivation of antibiotics, and drug efflux pumps. Bacterial antibiotic efflux pumps are key players in antibiotic resistance. However, the super efflux pump variant(named RE-CmeABC) is much more potent in conferring Campylobacter resistance to fluoroquinolones and expands the mutant selection window of ciprofloxacinand as well as reduces intracellular accumulation of antibiotics[2-3]. Furthermore, RE-CmeABC is horizontally transferable, shifts antibiotic MIC distribution among clinical isolates, and is increasingly prevalent in Campylobacter jejuni isolates, suggesting that it confers a fitness advantage under antimicrobial selection. Unlike other specific resistance determinants that only allow bacteria to resist a particular antimicrobial, the acquisition of a functionally enhanced efflux pump will empower bacteria with simultaneous resistance to multiple classes of antibiotics. The findings of super efflux pump variant reveal a new mechanism for enhanced multidrug resistance and an effective strategy utilized by bacteria for adaptation to selection from multiple antibiotics. These findings open a new direction for us to understand how bacteria adapt to antibiotic

treatment. Therefore innovative strategies are needed to curb the rise and spread of antibiotic-resistant Campylobacter.

Keywords: Campylobacter; antibiotic resistance; efflux pump

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4708

Effect of Natural Active Substance on Drug Resistance of Campylobacter

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Abstract: *Campylobacter jejuni* is one of the major food-borne pathogens causing bacterial diarrhea and can survive continuously in an environmentally nonculturable state (VBNC) [1]。The important risk factors leading to its transmission through the food chain lead to the spread of multidrug-resistant and extensively drug-resistant *Campylobacter*, and the phenomenon of reduced utility of antibiotics. The ability of *C. jejuni* to adhere to common surfaces such as stainless steel and plastics in the food and poultry processing industries over a wide temperature range of 4 - 42 ° C [2], Therefore, the search for natural antibiotic replacement substances with low or no toxicity, high biodegradability and good antibacterial properties at extreme temperatures and pH has begun. Some natural active ingredients extracted from traditional Chinese medicines can be widely used for the development of antibiotic replacement. Studies have reported that eugenol and the paradigm cinnamaldehyde mixture can down-regulate the C. jejuni virulence genes flaA, virB and wlaN as well as the proinflammatory cytokines TNF-α, IL-2, IL-6, and IL-8, while up-regulating the protective cytokine IL-10 [3]。Some organic acids (citric acid, malic acid, etc.) can produce antibacterial activity against bacterial biofilms, such as rhamnolipids

have the ability to prevent and remove C. jejuni biofilms, and the anti-biofilm activity against C. jejuni is affected by concentration at a temperature of 25 ° C [2]。

Some of the natural active ingredients discovered so far may be candidates for novel or adjuvant therapies that can inhibit or kill specific pathogenic microorganisms, and the combination of natural active substances with other antimicrobials can also effectively avoid the emergence and spread of antibiotic resistance.

Keywords : *Campylobacter jejuni*; Multidrug-resistant; Natural active; Antibiotic replacement substances; Traditional Chinese medicines

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4763

Immunization of chickens with the enterobactin conjugate vaccine reduced *Campylobacter jejuni* colonization in the intestine

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Aim: *Campylobacter jejuni* is the leading bacterial cause of human enteritis in developed countries. Chicken is the major animal reservoir of *C. jejuni* and a powerful infection model for human campylobacteriosis. No commercial vaccine against *C. jejuni* is available to date. The high affinity iron acquisition mediated through enterobactin (Ent), a small siderophore, plays a critical role in the colonization of *C. jejuni* in the intestine. Recently, an innovative Ent conjugate vaccine has been demonstrated to induce high-level of Ent-specific antibodies in rabbits; the Ent-specific antibodies displayed potent binding ability to Ent and inhibited Ent-dependent growth of *C. jejuni*.

Methods: In this study, using specific-pathogen-free (SPF) chickens, we performed three trials to evaluate the immunogenicity of the Ent conjugate vaccine and its efficacy to control *C. jejuni* colonization in the intestine. The purified Ent was conjugated to the carrier keyhole limpet hemocyanin (KLH).

Results: Intramuscular immunization of chickens with the Ent-KLH conjugate for up to three times did not affect the body weight gain, the development of major immune organs, and the gut microbiota. In the first two trials, immunizations of chickens with different regimens (2 or 3 times of vaccination) consistently induced strong Ent-specific immune response when compared to control group. Consistent with the high-level of systemic anti-

Ent IgG, *C. jejuni* colonization was significantly reduced by 3-4 $\log_{10}$ units in the cecum in two independent vaccination trials. The third trial demonstrated that single Ent-KLH vaccination is sufficient to elicit high level of systemic Ent-specific antibodies, which could persist for up to 8 weeks in chickens.

Conclusion: The Ent-KLH conjugate vaccine could induce high-level of Ent-specific specific antibodies in chickens and confer host protection against *C. jejuni* colonization, which provides a novel strategy for *Campylobacter* control in poultry and humans.

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4796

Construction of a *S. cerevisiae* displaying tetra-virulence proteins of *Helicobacter pylori*

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Aim: *Helicobacter pylori* (*H. pylori*) is a Gram negative, spiral-shaped and micro-aerophilic bacterium infecting almost half of the world's population, and is classified as a class I carcinogen[1]. Vaccination against *H. pylori* is one of the most effective ways to control *H. pylori* infection, especially the oral vaccinas that induces an effective immune response at the site of infection. Oral vaccines based on *Saccharomyces cerevisiae* display technology can be rapidly engineered to express pathogenic antigens and fermented at low cost, and have emerged as a new strategy to enable rapid production of *H. pylori* vaccines. Various virulent factors contribute to the pathogenesis of *H. pylori* and the joint action of multiple antigens may be a breakthrough for the development of multivalent vaccines[2]. In this study, based on the a-agglutinin Aga1-Aga2 yeast surface display system, four main virulence proteins including UreB, FlaA, VacA and CagA were co-expressed on surface of ST1814G host strain to obtain a tetra-valent yeast strain, and the expression characteristic of the recombinant strain was also investigated.

Methods: The codon optimized UreB, FlaA, VacA and CagA genes were synthesized to preferred *S. cerevisiae* expression. The four complete transcription units of GPD-UreB/FlaA/VacA/CagA were constructed in vitro separately and were integrated into different chromosome of *S. cerevisiae* by homologous recombination to screen the co-expression recombinants. The co-expression of UreB&FlaA&VacA&CagA proteins in *S. cerevisiae* were further identified by Western Blot, flow cytometry assay and immunofluorescence analysis.

Results: The four transcription units were transferred together into the ST1814G host strain by lithium acetate method and the recombinant strains integrated four transcription unit were screened successfully by tryptophan and leucine dual nutrient-deficient plates. The expression characteristics of the recombinants were analyzed, UreB, FlaA, VacA and

CagA proteins were successfully co-expressed on the surface of the recombinant *S.cerevisiae* was confirmed.

Conclusion: In summary, we successfully constructed a S. cerevisiae recombinant strain surface-displaying *H. pylori* virulence proteins UreB, FlaA, VacA, CagA and FlaA simultaneously, which laid the foundation for future evaluation of the protective efficacy of oral yeast preparations.

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Funding: Construction of oral yeast strain surface displaying Helicobacter pylori virulence protein (2021GKF-0957)

4831

Validation of PCR methods for confirmation and species identification of thermotolerant *Campylobacter*

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Aim: As alternative to the phenotypic tests for confirmation and identification described in the reference method EN ISO 10272 – Microbiology of the food chain – Horizontal method for detection and enumeration of *Campylobacter*, molecular tests can be used, provided the alternative method performs at least equally well as the reference method. The study aimed to validate PCR methods which, if qualified, would be included in the first amendment to EN ISO 10272 and thus available to laboratories without further validation.

Methods: Three multiplex PCR methods, one conventional and two real-time PCR methods, were validated against the reference method for confirmation and identification described in both parts of EN ISO 10272:2017. The results of the PCR methods were compared against the reference method in a method comparison study and in an interlaboratory study, in large outlined according to ISO 16140-6:2019 – Microbiology of the food chain – Method validation Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.

Results: The performance, in terms of inclusivity and exclusivity, of each of the eight PCR targets were comparable to that of the reference method: close, equal, or better depending on the target. The PCR method identifying *C. upsaliensis* resulted in inclusivity deviations above the acceptability limit in the method comparison study. However, considering the true identity of the strains, it still performed equally well identifying *C. upsaliensis* as the reference method.

Conclusion: All three PCR methods were concluded to be suitable for molecular identification and/or confirmation of thermotolerant *Campylobacter* spp. *C. jejuni*, *C. coli* and *C. lari* isolated from the food chain.

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4957

Relative quantification of DNA uptake in *Campylobacter jejuni* and molecular analysis of outer and inner membrane DNA transport

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Aim: *C. jejuni* exhibit a diverse population structure, supported by extensive horizontal gene transfer. We addressed the question of how much DNA can be taken up by *C. jejuni* within a single transformation event and whether homologs of putative DNA uptake proteins are essential for this process.

Methods: Using a single cell assay and fluorescently labelled DNA we quantified DNA uptake in wild-type *C. jejuni* 81-176 in comparison to its relative *Helicobacter pylori* J99, which harbors a different DNA uptake machinery. Mutants lacking homologs of the PilQ (Cj1474c) outer membrane pore, the ComE (Cj0011c) periplasmic DNA-binding protein and the inner membrane channel ComEC (Cj1211) were tested for their DNA uptake capacity and for natural transformation using an antibiotic resistance marker.

Results: Uptake in *H. pylori* was fast and efficient. *C. jejuni* imported significantly less DNA per uptake location (focus) and cell than *H. pylori*. Maximal DNA amounts in distinct *C. jejuni* uptake foci seemed to be limited, since the distribution of DNA amount per location was nearly constant after 30 min of uptake within 2 h. The fraction of cells and the number of foci per cell increased 2-fold within 2h of contact with DNA. Furthermore, DNA uptake over the outer membrane was abolished in a Δ*pilQ* mutant, whereas a mutant lacking *comEC* was able to take up DNA into the periplasm but was transformation-deficient. A Δ*comE* mutant was marginally affected at the level of DNA transport but exhibited 1.5 log reduced transformation activity, suggesting that ComE might be involved

in downstream protection of incoming DNA, rather than in force generation for DNA import, as shown for other bacteria.

Conclusion: *C. jejuni* was highly active for uptake of free DNA. Knowledge about the mechanism of DNA import, hence, natural transformation is important for combatting the pathogens genetic diversity and adaptation.

Funding: This research was funded by the German Federal Ministry of Education and Research (BMBF), zoonoses research consortium PAC-CAMPY, project IP3/01KI1725B/01KI2007B.

4959

Short-term and long-term alterations of gastrointestinal microbiota with different H. pylori eradication regimens: a meta-analysis

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Background and Aims: The impacts of Helicobacter pylori (H. pylori) eradication on the gastrointestinal microbiota are controversial, and whether the short-term and long-term changes in the gastrointestinal microbiota following different eradication regimens are consistent remains inconclusive. This study aimed to examine the effects of various eradication regimens on the gastrointestinal microflora at follow-up evaluations within 7 days, at 1-3 months, and over 6 months changes in the gastrointestinal microbiota.
Materials and Methods: Studies reported on the PubMed, Embase, Cochrane Library, Web of Science, and ClinicalTrails.gov databases before March 2022 were collected. Data analysis and visualization were conducted using Review Manager 5.4.1. The tool of the Cochrane Collaboration to assess the risk of bias was suitable for randomized controlled trials with the Newcastle–Ottawa scale for nonrandomized controlled trials. In addition, the process was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.
Results: After a series of rigorous screenings, a total of 34 articles with 1,204 participants were included for this review analysis. The results showed changes in the gut microflora at the phylum level or the family and genus levels. After metronidazole-containing triple therapy, the number of *Enterobacteriaceae* increased at 1-3 months follow-up. After Metronidazole-free triple therapy, *Actinobacteria* decreased significantly, and this trend lasted for more than 6 months. Within 7 days after eradication treatment, the follow-up results showed a decrease in the number of *Lactobacillus.* After Bismuth-containing quadruple therapy, the changes in *Actinobacteria* fluctuated with the follow-up time. The changes in *Proteobacteria* showed a downward trend lasting for 1-3 months after eradication but returned to baseline levels over 6 months after eradication. Subgroup analyses indicated that host age could influence changes in the gut microbiota.
Conclusion: Different eradication regimens had varied effects on the short-term and long-term abundance of the gastrointestinal microbiota, but the decreasing trend of the microbiota diversity was the same for all regimens at the short-term follow-up. This study summarizes the changes of gut microbiota at different stages after different eradication regimens and hope to provide some references for supplementing probiotics, while further studies is needed to support these findings.

4964

Molecular epidemiology and drug resistance of Campylobacter jejuni /coli from patients with diarrhea in Beijing, China.

Purpose：To guide clinically appropriate antibiotics for Campylobacter infection and to provide an objective basis for the prevalence of Campylobacter infection in Beijing, China.

Methods：A total of 2062 stool samples from diarrhea patients 103 were collected continuously in Beijing Tongren Hospital, Beijing Puren Hospital and Beijing Miyun Hospital from April 2018 to March 2019. The real-time PCR detection and microaerobic bacteria isolation were used to detect Campylobacter . Antimicrobial resistance of Campylobacter isolates was determined using the Antibiotics MICs of Campylobacter detection kit by agar dilution method. The seven housekeeping genes aspA, glnA, gltA, glyA, pgm, tkt and uncA were detected by PCR and sequencing. The MLST results were analyzed by BioNumerics 7.6 software. Data were analyzed using IBM SPSS Statistics. 25.0. $\chi 2$ tests were used to analyze the epidemiological characteristics of positive and negative cases of Campylobacter and the drug resistance of C.jejuni and C.coli.

Results：The prevalence of PCR detection of Campylobacter jejuni and Campylobacter coli was 8.2% (168/2062, 144 C.jejuni and 24 C.coli) and at the culture detection was 5.0% (104/2062, 84 C.jejuni and 20 C.coli

strains). The diarrhea patients infected with C.jejuni/coli are more prone to have abdominal pain, fever and bloody stool, without other specific clinical 21 symptoms. Minimum inhibitory concentrations against ten different antibiotics (erythromycin, azithromycin, nalidixic acid, ciprofloxacin, gentamicin, streptomycin, chloramphenicol, tetracycline, telithromycin, and clindamycin) were analyzed. The strains showed resistance to all antibiotics, which were most frequently resistant to ciprofloxacin (95.2%), followed by: nalidixic acid (94.2%) and tetracycline (92.3%); gentamicin (15.4%); streptomycin (14.4%); erythromycin (11.5%) and clindamycin (11.5%). The resistance rate of C.coli is significantly higher than that of C.jejuni. The most prevalent sequence type (ST) complex of C.jejuni were ST21 complex (16.7%, 14/84), followed by ST354 complex (14.3%, 12/84), ST45 complex (8.3%, 7/84), ST464 complex (7.1%, 6/84)and ST574 complex (7.1%, 6/84), which of C.coli was ST828 complex (95%, 19/20). Conclusion：Campylobacter is an nonnegligible diarrhea pathogen in Beijing, which has a high resistance rate to antibiotics commonly used for intestinal infections. Our study highlights the need for continued surveillance of the epidemiology and antimicrobial sensitivities of Campylobacter species in Beijing to guide clinical treatment.

5514

Chickens, Chlorine and *Campylobacter*: A new role for sulfite in the potentiation of reactive chlorine species

Authors:
Aidan J Taylor & David J Kelly

Abstract Text:
Chlorination of chicken carcasses post-evisceration is a common practice in certain regions to reduce surface contamination by food borne pathogens, including *Campylobacter*. Likewise, during infection *Campylobacter* encounters reactive chlorine species (RCS), namely hypochlorite, generated by host neutrophils with myeloperoxidase activity, reaching local concentrations in the mM range.

Hypochlorite is a potent oxidiser with numerous targets that rapidly kills bacteria. Among protein targets, the sulfur containing amino acids methionine and cysteine are particularly susceptible to oxidation by hypochlorite, with sequential oxidation events leading to irreversible, deleterious modifications.

Some bacteria possess hypochlorite specific regulators which induce a defence response, however no such regulator is evident in *C. jejuni*. To understand the transcriptional response to RCS, we conducted a temporal RNAseq analysis on *C. jejuni* treated with hypochlorite in continuous culture. Here, we present some of our findings, with a focus on the role of the sulfite oxidase SorAB in defence against sulfite, which we show to potentiate the effects of hypochlorite both on cell viability and inactivation of susceptible enzymes.

5524

A sensor-less ModE maintains homeostasis of molybdenum, tungsten and selenium in the enteric pathogen *Campylobacter jejuni*

Authors:
Suraj Atram, Aidan J Taylor & David J Kelly

Abstract Text:

C. jejuni belongs to an unusual group of bacteria that encode high-affinity transporters for both molybdenum (Mo) and tungsten (W). Elemental Mo and W are highly similar with almost identical atomic radii, and thus somewhat interchangeable as pterin-bound cofactors for molybdo-pterin (MoCo) enzymes. However, previous work from our lab has shown that the MoCo enzymes of *C. jejuni* have a clear and often absolute functional preference for either Mo or W. How Mo vs. W insertion into apo-pterin during cofactor synthesis, and subsequent insertion into enzymes, is discriminated and regulated is unknown.

To address these questions, we have conducted an RNAseq analysis to define the regulon of the transcriptional regulator ModE, which is known to regulate Mo and W transporters in other bacteria. Our data shows *C. jejuni* ModE, in addition to the above, also regulates selenium uptake and selenocysteine synthesis, essential for the important MoCo enzyme formate dehydrogenase. We present our model for homeostasis of Mo, W and Se in *C. jejuni*. Furthermore, *C. jejuni* ModE lacks the sensory domain of canonical 2-domain ModE proteins. Here we explore how a sensor-less ModE may function.

5545

Genetic characteristics, antimicrobial resistance, and prevalence of *Arcobacter* spp. from various sources in Shenzhen, China

Running title: *Arcobacter* spp. in Shenzhen, China

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ABSTRACT

Worldwide, *Arcobacter* spp. is an emerging zoonotic and foodborne pathogen. However, we know little about its prevalence and antimicrobial resistance in China. To investigate the prevalence of *Arcobacter* spp. from different sources, 396 samples were collected from human feces; chicken cecum; and food specimens, including chicken meat, beef, pork, lettuce, and seafood. *Arcobacter* spp. was isolated using the membrane filtration method. The agar dilution method and next generation sequencing for 92 strains were used to investigate antimicrobial resistance and to obtain the whole-genome data respectively. The virulence factors database (VFDB) was queried to identify virulence genes. The ResFinder and Comprehensive Antibiotic Resistance Database (CARD) were used to predict resistance genes. Phylogenetic tree was constructed using the maximum likelihood (ML) method with core single nucleotide polymorphisms. We found that 27.5% of samples (n = 109) were positive for *Arcobacter* spp., comprising *A. butzleri* (53.0%), *A. cryaerophilus* (39.6%), and *A. skirrowii* (7.4%). The highest prevalence was observed in chicken meat (81.2%), followed by seafood (51.9%), pork (43.3%), beef (36.7%), lettuce (35.5%), chicken cecum (8%), and human feces samples (0%, 0/159). Antimicrobial susceptibility tests revealed that 51 *A. butzleri* and 40 *A. cryaerophilus* strains were resistant to streptomycin (98.1%, 70%), clindamycin (94.1%, 90%), tetracycline (64.7%, 52.5%) azithromycin (43.1%, 15%), nalidixic acid (33.4%, 35%), and ciprofloxacin (31.3%, 35%), but were susceptible to erythromycin, gentamicin, chloramphenicol, telithromycin, and clindamycin (≤10%). The virulence factors *tly*A, *mviN*, *cj1349*, *ciaB*, and *pldA*, were carried by all *Arcobacter* spp. strains, followed by *cadF* (95.7%), *iroE* (23.9%), *hecB (*2.2%*)*, *hecA* and *irgA* (1.1%). Only one *A. butzleri* strain (F061-2G) carried a macrolide resistance gene (*ereA*). One *A. butzleri* and one *A. cryaerophilus* harbored resistance island gene clusters, which were isolated from pork and chicken, respectively. Phylogenetic tree analysis revealed that *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* were obviously separated. To the best of my knowledge, this is the first report of the isolation of *Arcobacter* spp. from vegetables and seafood in China. The resistance island gene cluster found in pork and chicken meat .

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5546

Detection and drug resistance of *Campylobacter* in 408 samples from different sources in Shenzhen

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Abstract： The prevalence and drug resistance characteristics of *Campylobacter* from different sources in Shenzhen were analyzed, then were compared with those in previous years. *Campylobacter* was isolated by enhanced filtration method, and the drug sensitivity test was conducted by agar dilution method. Among 408 samples from different sources, the positive rate of *Campylobacter* in chicken cecum was the highest (72.0%, 36 / 50), followed by chicken (49.3%, 34 / 69). The isolation rate of *Campylobacter* from diarrhea patients was 10.7% (17 / 159). The isolation rate of *Campylobacter* from beef and pork was low. 1 *Campylobacter jejuni* strain was isolated from seafood. *Campylobacter* was not isolated from lettuce.The highest drug resistance rates of 53 strains of *Campylobacter jejuni* from diarrhea patients and chickens were nalidixic acid (94.3%) and ciprofloxacin (94.3%), followed by tetracycline (92.4%) and florfenicol (75.5%). The drug resistance rates of 32 strains of *Campylobacter coli* from diarrhea patients and chickens were the highest, which were nalidixic acid, ciprofloxacin and tetracycline (100%).73.6% (39 / 53) of *Campylobacter jejuni* and 100% (32 / 32) of *Campylobacter coli* had multiple drug resistance. *Campylobacter concisus* was isolated from diarrhea patients for the first time, and its pathogenic characteristics need to be further analyzed. The drug resistance of *Campylobacter jejuni* from diarrhea patients and chickens in Shenzhen is increasing, which should be paid attention to.

5575

Genomic insights into the emergence of campylobacteriosis caused by antimicrobial-resistant *Campylobacter coli*

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Abstract： *Campylobacter* is the leading bacterial cause of diarrheal illnesses worldwide. *Campylobacter jejuni* and *C. coli* are the most common species accounting for campylobacteriosis. Although the proportion of campylobacteriosis caused by *C. coli* is increasing rapidly in China, the underlying mechanisms of this emergence remain unclear. In this study, we analyzed the whole-genome sequences and associated environments of 1,195 *C. coli* isolates with human, poultry, or porcine origins from 1980 to 2021. *C. coli* isolates of human origin were closely related to those from poultry, suggesting that poultry was the main source of *C. coli* infection in humans. Analysis of antimicrobial resistance determinants indicated that the prevalence of multidrug-resistant *C. coli* increased dramatically since the 2010s, coinciding with the shift in abundance from *C. jejuni* to *C. coli* in Chinese poultry. Compared with *C. jejuni*, drug-resistant *C. coli* were better adapted and showed increased proliferation in the poultry production mode where multiple antimicrobial agents were frequently used. This study provides an empirical basis for the molecular mechanisms that have enabled *C. coli* to become the dominant *Campylobacter* species in poultry; we also emphasize the importance of poultry products as sources of campylobacteriosis by *C. coli* in human patients.

5577

DNA starvation/stationary phase protection protein of *Helicobacter pylori* as a potential immunodominant antigen for infection detection

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Abstract

Background: Application of chicken egg yolk immunoglobulin Y (IgY) for *Helicobacter pylori* (*H. pylori,* HP) has gained much interest in recent years. Comparing with for treatment, IgY may be more advantageous when used for *H. pylori* detection.

Methods: Nine strains of *H. pylori* with different genetic backgrounds were inactivated and used to immunize hens respectively for the preparation of polyclonal anti-*H. pylori* immunoglobulin Y (anti-HP IgY). The proteins of *H. pylori* with reactivity to anti-HP IgY were detected by Western Blot. The five protein bands that can be well recognized by anti-HP IgY of each group, and were prevalent in all nine strains were excised from SDS-PAGE gel, digested and identified by Nano-HPLC-MS/MS analysis. The potential of these identified proteins as antigen detection targets was then assessed by sequence analysis.

Results: Anti-HP IgY derived from each group of specific strain immunized hens can recognize self-strain and non-self-strain antigens well. Five universal immunodominant antigens were identified as chaperonin GroEL, flagellin A, urease subunit alpha, peroxiredoxin and DNA starvation/stationary phase protection protein. Sequences analysis showed that both peroxiredoxin and DNA starvation/stationary phase protection protein were present in all 1000 strains of *H. pylori* queried, and the amino acid sequences were highly conserved. The highest sequence consistency between the DNA starvation/stationary phase protection protein of *H. pylori* and non-Helicobacter organisms was 52.59 %, and the consistent sites were scattered and there was no continuous long fragment consensus sequence.

Conclusion: DNA starvation/stationary phase protection protein was identified as a universal immunodominant antigen of *H. pylori* and sequence analysis indicated that it could serve as a potential antigen target for the diagnosis of *H. pylori* infection.

5578

They look like *Campylobacter jejuni,* but are they?

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Aim: To characterize possible new *Campylobacter* species that masquerade as *C. jejuni* on conventional isolation media.

Methods: A collection of *Campylobacter* isolates were recovered from water and animals in New Zealand and Australia between 2008 and 2021 using isolation temperatures between 37 and 42°C and conventional media used for the isolation of *Campylobacter* (mCCDA or CAT). The isolates were negative for *C. jejuni* and *C. coli* by PCR. Whole genome sequencing (WGS) and analysis[1,2,3] was performed and the resulting genomes from these isolates and those of validly described *Campylobacter* species were compared using ANI[4], dDDH[5] and OrthoMCL[6,7]. The isolates were further characterized using biochemical tests, MALDI-ToF and 16S rRNA sequencing.

Results: The study isolates could not be assigned to a validly described *Campylobacter* species based on the WGS results. By ANI, dDDH and OrthoMCL analyses there are multiple potential new species and subspecies. Biochemical, MALDI-ToF and 16S rRNA sequencing results will be presented.

Conclusion: The isolates in this study were recovered using conventional plating media for *Campylobacter* suggesting that confirmation of species identification from media is necessary to prevent overestimation of well recognized species such as *C. jejuni*, especially from environmental and wild bird samples. Such overestimations may influence the

perceived public health risks associated with the samples. WGS is a powerful tool for confirming the identity of isolates, especially when biochemical identification is challenging as is the case for some *Campylobacter* species. The ability to assign an isolate to a species using WGS relies on a well curated database of genomes from validly described species. With the accessibility of WGS continuing to increase, it is tempting to apply genomic criteria to define new species, however phenotypic differences are still required to accurately describe new species. This can be challenging in relatively metabolically inert organisms of the genus *Campylobacter*.

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5582

Binding affinity prediction on a set of antigens from *Helicobacter pylori* and IgY from *Gallus gallus*

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Aim: Prediction of binding affinity between pathogenic protein and antigen is a very important aspect in researching on protein-protein interaction. It is a magnetic challenge that studying binding affinity of between antigens from *Helicobacter pylori* （*H. pylori*）and IgY from *Gallus gallus* (*G. gallus*).

Methods: Through searching for homology structure data of antigen and antibody combined with experimental binding affinity parameters, the predicted complex of antigen-antibody was assembled. Furthermore, the corresponding binding characteristics of complex were analyzed by free-energy perturbation and thermodynamic integration.

Results: A substantial number of interactions from antigens from *H. pylori* and IgY from *Gallus gallus* were calculated and assembled through the workflow. In predicted interaction, BabA in *H. pylori* and Ig Y in *G. gallus* could form a stabilized complex with high affinity.

Conclusion: The suitability of our approach should be demonstrated by verified interaction in experiment. The study would provide valuable references for researching on biotherapeutics, immunity and vaccines of *H. pylori*.

5589

Early emergence and transmission characteristics of *fexA*-positive *Campylobacter* during 2010-2019 in eastern China

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Aim: The recent emergence of florfenicol resistance gene *fexA* in *Campylobacter* threatens the utility of this class of antibiotic[1,2], but only scattered reports of epidemiological data on *fexA*-positive *Campylobacter* are available[3].

Methods: A total of 2330 *Campylobacter* isolates were obtained from different sources and were screened for the presence of *fexA* gene using PCR and sequencing. Antimicrobial susceptibility testing was used to analyze the resistance phenotype, and whole-genome sequencing was used to analyze the drug resistance genes, phylogenetic relatedness of *fexA*-positive isolates and genetic environment of *fexA* gene. Natural transformation was performed to verify whether the *fexA* gene carried in *fexA*-positive isolates could be transferred.

Results: The *fexA*-positive *Campylobacter* emerged as early as 2010, and a total of 69 (2.96%) *fexA*-positive *Campylobacter* were identified. In particular, the positive rate of *fexA* was higher for *Campylobacter coli* (*C. coli*) (5.51%) than that of *Campylobacter jejuni* (C. jejuni) (0.99%) ($p<0.05$). Subsequently, antimicrobial susceptibility testing

showed 69 *fexA*-positive *Campylobacter* exhibited a multidrug resistance (MDR) phenotype, with a co-resistance to florfenicol (FFC), ciprofloxacin (CIP), tetracycline (TET) and erythromycin (ERY), while the *fexA* gene was commonly associated with *tet(L)* gene and the mutation of gyrA T86I. Phylogenetic analysis indicated some of these isolates were closely related to other previously reported *fexA*-positive *Campylobacter* isolates. Interestingly, genetic environment analysis revealed the *fexA* was flanked by IS*1216E*, and we identified three circular intermediates resulted from the recombination of the two IS*1216E* copies. Moreover, natural transformation provided further evidence that IS*1216E* was highly associated with horizontal transfer of *fexA* in *Campylobacter*.

Conclusion: This study shows the transmission characteristics of *fexA*-positive *Campylobacter* in eastern China, which will help to develop accurate prevention and control strategies.

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5592

Single-Cell Identification, Drug Susceptibility Test, and Whole-genome Sequencing of *Helicobacter pylori* Directly from Gastric Biopsy by Clinical Antimicrobial Susceptibility Test Ramanometry

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AIM: The battle against *Helicobacter pylori* (*H. pylori*) infections demands fast, reliable, and sensitive methods for pathogen identification (ID), antimicrobial susceptibility tests (ASTs) based on metabolic response, and genome-wide mutation profiling that reveals resistance mechanisms. This research is aimed to devolop a process with a much shorter turn around time that accomplishes rapid ID, metabolism-inhibition- based AST, and high-quality WGS of cells with levofloxacin (Lev) or clarithromycin (Clr) resistance phenotypes, at precisely 1-cell resolution and directly from clinical biopsy samples.

METHODS: Here we introduce Clinical Antimicrobial Susceptibility Test Ramanometry for H. pylori (CAST- R-HP), and its validation with clinical samples. This method performs rapid ID, metabolism inhibition- based AST, and high-quality whole-genome sequencing for cells of targeted resistance phenotype, all at precisely 1-cell resolution and directly from biopsy samples.

RESULTS: In CAST-R-HP, automated acquisition and machine learning of single-cell Raman spectra (SCRS) enable distinguishing individual H. pylori cells directly from a biopsy sample, with 98.5 + 0.27% accuracy in ID. Moreover, by adding a 48- to72-h D2O feeding and drug exposure step prior to SCRS acquisition, CAST-R-HP reports AST for levofloxacin and clarithromycin with 100% accuracy, based on metabolic inhibition level. Furthermore, CAST-R-HP supports rapid sorting, low-bias DNA amplification, and full genome sequencing of single H. pylori cells with the SCRS defined, targeted drug-susceptibility phenotype, via Raman-activated gravity-driven cell

encapsulation and sequencing. The genome-wide mutation map (maximum 99.70% coverage), at precisely 1-cell resolution, not only elucidates the drug-susceptibility phenotypes but also unveils their underlying molecular mechanisms.

CONCLUSION: The culture independency, shorter turn around time, high resolution, and comprehensive information output suggest that CAST-R-HP is a powerful tool for diagnosing and treating *H. pylori* infections.

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Article

Genetic and Transcriptomic Variations for Amoxicillin Resistance in *Helicobacter pylori* under Cryopreservation

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Abstract: Some amoxicillin-resistant strains of *H. pylori* show a sharp decrease in amoxicillin resistance after freezing. In China, most clinical gastric mucosal specimens are frozen and transported for isolation and drug susceptibility testing for *H. pylori*, which may lead to an underestimation of the amoxicillin resistance. The objective of this study is to investigated reasons for the decreased amoxicillin resistance after cryopreservation. A high-level amoxicillin-resistant clone (NX24r) was obtained through amoxicillin pressure screening. After cryopreservation at −80 °C for 3 months, the minimum inhibitory concentration (MIC) of NX24r was reduced sharply. Mutations and changes of transcriptome were analyzed after amoxicillin screening and cryopreservation. Mutations in PBP1 (I370T, E428K, T556S) and HefC (M337K, L378F, D976V) were detected in NX24r, which may be the main reason for the induced amoxicillin resistance. No mutations were found in PBP1 or HefC after cryopreservation. However, transcriptome analysis showed that down-regulated genes in the cryopreserved clone were significantly enriched in plasma membrane (GO:0005886), including *lepB*, *secD*, *gluP*, *hp0871* and *hp1071*. These plasma membrane genes are involved in the biosynthesis and transport function of the membrane. The decreased amoxicillin resistance after cryopreservation may be related to the down-regulation of genes involved in membrane structure and transport function.

Keywords: *Helicobacter pylori*; *pbp1*; cryopreservation; amoxicillin resistance; transcriptome; plasma membrane

5597

Development of scFv proteins for detecting *Campylobacter* cell

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Rapid detection for Campylobacter is vital for monitoring the bacteria in food production chains and diagnosing patients, enabling effective intervention and treatments. Antibody-based quick detection methods, including LFD, demands sufficient production of highly specific antibodies with low cost. We immunized rabbit with whole cell of *C. jejuni* and constructed a scFv-displaying phage library. Panning the library using *C. jejuni* cell as the bait identified two scFv recombinant proteins with high specificity to *Campylobacter*. Characterizing their binding features to bacteria cells and testing their activity in fishing *Campylobacter* cells from liquid samples suggested that they recognize different targets on *Campylobacter* cells and have potential to be used in developing sensitive and rapid *Campylobacter* detection tools.

5603

Feeding Malic Acid to Chickens at Slaughter Age Benefits Microbial Safety in Regard to *Campylobacter*

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Aim: Chicken meat has become popular for consumption worldwide. However, chicken flocks suffer the *Campylobacter* infection during the rearing period. This study applied malic acid to chicken flocks and evaluated its potential benefits on the poultry production and microbial safety.

Methods: Malic acid was supplemented into the drinking water of the flocks that were found naturally *Campylobacter*-positive. The decontamination effect of malic acid-supplemented water and its influence on chicken performance were evaluated in AA broilers and partridge chicken.

Results: Chickens were provided with the malic acid-supplemented drinking water for three weeks. The contamination load of *Campylobacter* were decreased by 0.91 - 0.98 log after the first week of use ($P < 0.05$). However, this effect did not persist in time, significant decontamina-tion could not be found in the second and the third week of application. Thus, the malic acid-supplemented drinking water was used for five days to chickens at slaughter age, the *Campylobacter* carriage was found effectively decreased by 1.05 - 1.55 log ($P < 0.05$). Malic acid has no adverse effects on chicken body weight, weight gain, intestinal indices and microbiota. In addition, the quality of chicken meat was improved, which the moisture was increased by 5.12% - 5.92% ($P < 0.05$), and the fat was decreased by 1.60% ($P < 0.05$).

Conclusion: Our results provide an effective way to reduce the contamination of *Campylobacter* during chicken rearing period, which can be applied to promote safe development of the poultry farming and its products.

Prevalence of the phenicol resistance gene *fexA* in *Campylobacter* isolated from the poultry supply chain

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ABSTRACT

Florfenicol, an animal-specific broad-spectrum antibiotic, has been widely used in livestock and poultry breeding, which leads to the high antimicrobial resistance (AMR) of *Campylobacter* in food animals. Recently, a new florfenicol resistance gene, *fexA*, often located on various multidrug resistance genomic islands (MDRGIs) and confers resistance to various antimicrobial agents, was characterized in *Campylobacter*. However, the prevalence and genetic environments of *fexA* and its associated MDRGIs in *Campylobacter* in the poultry supply chain need further characterization. Here, a total of 111 (15.48%) *Campylobacter* isolates (63 *C. jejuni*, 40 *C. coli*, 8 *C. lari*) were obtained from 717 samples from farms, slaughterhouses, and supermarkets. Both phenotypic and genotypic analyses indicated that the AMR of *C. coli* was significantly higher than that of *C. jejuni*. PCR amplification and whole genome sequencing showed that the *fexA* gene was present in 26 out of 35 florfenicol-resistant *Campylobacter* isolates. This gene was located in the *tet*(L)-*fexA*-*tet*(O) MDRGI. The *fexA*-harboring isolates detected in the above sources could be clustered into the same branch, indicating that they may have the same ancestor. In addition, the *erm*(B) gene was identified in 17 *Campylobacter* isolates, and the A2075G point mutation in the 23S rRNA gene occurred in 26 isolates, emphasizing the high resistance of *Campylobacter* to macrolides. In summary, these results indicate that *fexA* within the MDRGI of *Campylobacter* can be transmitted through bacteria in the animal-based food supply chain, and it is necessary to strengthen the monitoring of the prevalence and spread of *fexA* in foodborne *Campylobacter* spp.

5620

Coexistence of *optrA* and *fexA* in *Campylobacter*

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ABSTRACT Previous studies indicated that *Campylobacter* has developed several mechanisms that confer resistance to florfenicol, which is used in food animal production. This study describes the coexistence of *optrA* and *fexA* in *Campylobacter jejuni* and *Campylobacter coli* isolates from pigs and poultry. Moreover, whole-genome sequencing data showed that the two genes are located in various multidrug resistance genomic islands within different regions of the *Campylobacter* genomes. The emergence of *optrA* and *fexA* may support the spread of florfenicol-resistant *Campylobacter* strains of animal origin.

IMPORTANCE Florfenicol is widely used for the treatment of respiratory infections and as a feed additive in food animal production. As a foodborne pathogen, *Campylobacter* is constantly exposed to florfenicol, and resistance to this antimicrobial agent has increased in recent years. Previous studies indicated that *Campylobacter* has developed several mechanisms that confer resistance to florfenicol. This study describes for the first time the coexistence of the florfenicol exporter FexA and the ribosomal protective protein OptrA in *Campylobacter jejuni* isolated from pigs. The two genes were located in various multidrug resistance genomic islands within different regions of the *Campylobacter* genomes. Although phenicols are not commonly used for the treatment of *Campylobacter* infections, the extensive use of florfenicol in food animals may play a role in the coselection of multidrug resistance genomic island (MDRGI)-carrying *Campylobacter* isolates which also exhibited resistance to critically important antimicrobial agents (macrolides, aminoglycosides, and tetracyclines) commonly used for the treatment of human campylobacteriosis.

KEYWORDS *Campylobacter*, *fexA*, *optrA*, multidrug resistance

5621

Emergence of *fexA* in Mediating Resistance to Florfenicols in *Campylobacter*

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Biao Tang and Yizhi Tang contributed equally to this study. Order was determined alphabetically by first name.

ABSTRACT Florfenicol belongs to a class of phenicol antimicrobials widely used as feed additives and for the treatment of respiratory infections. In recent years, increasing resistance to florfenicol has been reported in *Campylobacter* spp., the leading foodborne enteric pathogens causing diarrheal diseases worldwide. Here, we reported the identification of *fexA*, a novel mobile florfenicol resistance gene in *Campylobacter*. Of the 100 *Campylobacter jejuni* strains isolated from poultry in Zhejiang, China, 9 were shown to be *fexA* positive, and their whole-genome sequences were further determined by integration of Illumina short-read and MinION long-read sequencing. The *fexA* gene was found in the plasmid of one strain and chromosomes of eight strains, and its location was verified by S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) and Southern blotting. Based on comparative analysis, the *fexA* gene was located within a region with the *tet*(L)-*fexA*-*catA*-*tet*(O) gene arrangement, demonstrated to be successfully transferable among *C. jejuni* strains. Functional cloning indicated that acquisition of the single *fexA* gene significantly increased resistance to florfenicol, whereas its inactivation resulted in increased susceptibility to florfenicol in *Campylobacter*. Taken together, these results indicated that the emerging *fexA* resistance is horizontally transferable, which might greatly facilitate the adaptation of *Campylobacter* in food production environments where florfenicols are frequently used.

KEYWORDS *Campylobacter*, *fexA*, multidrug resistance, food safety