Water quality and the impact it has on people’s health is the primary focus of ESR’s drinking-water team. The team’s expertise helps clients ranging from government ministries, wanting a national overview of drinking water quality, to individual water suppliers who might be wrestling with a supply-specific problem. This team’s expertise is supported by the water information management system, WINZ. The team of data and water scientists has well over two decades of experience in organising, summarising and reporting on large water quality datasets. They also offer data analysis skills to ensure clients get maximum knowledge from the data they have collected. When detailed questions about drinking-water supplies in New Zealand are being asked, the WINZ team is there to help! Waterborne illness outbreaks cause a lot of media interest in drinking-water quality. ESR can, at very short notice, provide the Ministry of Health or regional and district councils with a wide range of information about specific or similar water supplies. This information will assist in determining what the most appropriate actions might be to ensure safe drinking water. WINZ is managed by ESR for the Ministry of Health. It is used extensively by public health units and water suppliers to help in assessing compliance with drinking-water standards. WINZ also provides a national overview of over 2,000 community drinking water supplies, which can be found at www.drinkingwater.esr.cri.nz. As well as providing ad hoc analysis of WINZ data, ESR carries out detailed data analysis for the Annual Report on Drinking-water Quality, and to support other scientific projects. The database supports the Ministry of Health, public health units and water suppliers in fulfilling their regulatory and statutory obligations.

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What are Campylobacter?

Campylobacter are bacteria and were first isolated from human faeces in the early 1970’s. Campylobacter are Gram negative, motile, curved or spiral in shape and importantly, they are micro-aerophilic, which means they only grow when there is essentially no oxygen present.

The organism is widespread in the animal kingdom as both a commensal (i.e., asymptomatic or benign carriage) and as a pathogen of farm and domestic animals, poultry and rodents. Chickens, sheep or cattle with Campylobacter in their gut are not usually unwell, but some of the Campylobacter carried by these animals can cause disease in humans.

The route of human infection by Campylobacter is by ingestion of contaminated water or foods, particularly foods of animal origin. When animal faeces comes into contact with food or water for human consumption, it is possible for these otherwise benign strains to cause human disease. The incubation period (the time from ingestion to symptoms first appearing) is usually two to five days. Initial symptoms may be severe; fever, abdominal pain suggesting appendicitis and diarrhoea (sometimes bloody).

Campylobacter species are known to be a major cause of gastroenteritis (tummy bug) in the developed world and are the most common identifiable bacterial cause of diarrhoea in New Zealand. There are quite a few species of Campylobacter (currently 24), but in reality C. jejuni accounts for about 90% of reported human infections, with most of the remaining cases caused by C. coli and C. lari. These three Campylobacter species are thermophilic, i.e., they will grow at about 37-42°C, but not at 25°C or below.

Finding the source of water pollution

The inability to detect the source of faecal contamination using only FIB has led to the development of new tests that can identify various chemical and microbial markers. These new tests, faecal source tracking (FST), help to discriminate between human and non-human faecal sources and also between different animal species. FST can be used in conjunction with FIB to increase information leading to identification of actual sources of faecal pollution.

The chemical markers for FST can be divided into the chemicals which are inherently detected in faeces such as sterols, and those which are strongly associated with faecal waste, such as fluorescent whitening agents, caffeine and pharmaceuticals. The last three chemical indicators only identify human sources. Fluorescent whitening agents are added to laundry detergents to whiten and brighten clothes so they are consequently discharged into the sewer network with the laundry water. In comparison, faecal sterols (such as cholesterol) are identified in the faeces of all mammals and birds but are present in different concentrations, so we can generate a specific sterol fingerprint. This sterol fingerprint can discriminate between humans, ruminants and birds.

The microbial markers for FST are based on the bacteria living in the gut of each animal and bird species, and are specific to that particular host species. This inherent specificity is due to differences in diet and digestive systems between the host species. At ESR we have developed a tool box of DNA-based microbial markers, which can discriminate a range of animal and bird faecal sources from human sources. So far we have developed markers that can identify faecal inputs from humans, cattle, sheep, horses, dogs, possums, ducks and wildfowl in general.

Our research unit at ESR is continually testing and refining our toolbox of FST markers to deliver optimal performance for the identification and remediation of faecal contamination in the rural and urban environments of New Zealand.

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In human hosts, diarrhoea caused by Campylobacter is usually brief and secondary symptoms are uncommon. However, Campylobacter species infection may occasionally become invasive, and occasionally infection may produce conditions such as Guillain-Barre Syndrome, Miller-Fisher Syndrome, reactive arthritis, bursitis, endocarditis and neonatal sepsis.

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When used during the Darfield waterborne outbreak of Campylobacter in 2012, Campylobacter MBiT produced results two days earlier than Pulsed Field Gel Electrophoresis which was the gold standard at the time and demonstrated that the majority of the cases had a single MBiT type, meaning a single source of infection. In addition, Campylobacter MBiT provided data that suggested additional cases, originally thought to be geographically distinct, were actually part of the outbreak. By comparing the Campylobacter MBiT types observed in an outbreak, with types observed from historical samples from a range of animals, it is also possible to hypothesise on the source of C. jejuni/coli implicated in outbreaks of campylobacteriosis.

To be able to identify the source of an outbreak requires a library of isolates from known sources such as ruminants, poultry, avian, canine etc. Isolates of unknown origin are compared to this library to determine their source. Isolates which are genetically similar containing the same combination of these 18 genes, and cluster together when displayed. This allows for isolates from different sources to be displayed within the same circle or pie, if they are genetically similar. This can be seen in figure 1 and 2. This allows for comparisons and similarities to be drawn such as Campylobacter isolated from deer are indistinguishable from those isolated from sheep and cattle. It also allows for clustering of clinical outbreak cases with a suspect host for example poultry. The diversity of types isolated in the environment from a number of sources can be seen as well as the lack of cross-over of types isolated in avian such as Pukeko in human clinical cases.

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Campylobacter genotyping

While identification of disease causing organisms is useful, it is often insufficient to understand disease transmission, sources of infection and appropriate control measures.

It is important to further characterise isolates to identify those which may be more closely related and therefore share a common source. Genotyping of Campylobacter in conjunction with epidemiological investigations is one the most conclusive ways of determining the source of infection and therefore appropriate interventions to stop the spread of disease. While some methods exploit phenotypic characteristics, a growing number of methods involve looking at the DNA of pathogens. Two methods actively in use at ESR are MBiT1 genotyping and Whole Genome Sequencing.

**CAMPYLOBACTER MBiT**

ESR has developed a multiplex ligation- dependent probe amplification-binary typing (MBiT) assay for subtyping Campylobacter jejuni and Campylobacter coli. MBiT uses a DNA (genomic) amplification process to rapidly (one to two days) characterise bacteria based on the presence or absence of 18 genes. These genes were chosen because they have variable carriage in C. jejuni/coli and have been associated with pathogenicity or survival. A binary code is generated from the presence/absence of each gene and this is converted to a six digit code that can easily be shared between laboratories.

- MBiT is scalable from analysis of just a few isolates, to analysis of 90 or more.
- Cost of analysis is a fraction of the price of most other genotyping methods.
- While a standard turnaround time is 10 working days, in outbreak situation results can be obtained within 24 hours of receipt of a sample.
- MBiT analysis only requires a small amount of an isolate, which doesn’t have to be viable (it can be dead). So an isolate on a plate which can no longer be cultured can be used for MBiT analysis.
- Isolates with the same MBiT profile may have a common source, while those with different MBiT profiles are less likely to have a common source.

![FIGURE 1: All isolates of Campylobacter currently in the library. This eBurst diagram shows the diversity of Campylobacter isolates typed with MBiT and the source of them identified by colour. The size of a circle indicates the number of isolates with each MBiT profile, with each circle split into segments to represent each isolate. The further apart an isolate circle is, the greater the difference between them. Those within the same circle are indistinguishable with the MBiT method.](image-url)

1 MBiT – multiplex ligation-dependent probe amplification-binary typing, a rapid pathogen genetic subtyping technology for Campylobacter.
There are many organisations that have a responsibility to make sure people can trust the supply and quality of the water they drink, play in and use. ESR works in partnership with key stakeholders, including government, industry, the community and Māori, to improve the safety of freshwater and groundwater resources for human use and the safer use of bio-wastes. We support and help health authorities, local and central government, industry and communities by supplying scientific advice and expertise on the management of drinking-water, groundwater, recreational and wastewater. We report on drinking-water quality, provide scientific advice on health and environment-related public policy, conduct research on quality issues related to drinking water and recreational water and undertake information systems management.

WHOLE GENOME SEQUENCING (WGS)

Bacterial genomes are typically between 1.6 (Campylobacter) and 5 (E. coli) million base pairs in size. DNA has one of four nucleobases – either cytosine (C), guanine (G), adenine (A), or thymine (T). Ten years ago sequencing a bacterial genome took months and cost tens of thousands of dollars. Technological advances have reduced that cost dramatically to the point it is now equivalent and sometimes lower than traditional typing methods. Worldwide, laboratories are now in the process of transitioning to the use of WGS to characterise bacteria in public health and research contexts.

The process: DNA is extracted and randomly cleaved into small pieces. The fragments are then sequenced in a so called “massively parallel sequencing” process. For each isolate millions of pieces of DNA may be sequenced. Computers are then used to assemble these fragments into a whole genome sequence. Typically there are gaps in the sequence, so for most sequenced genomes, they are actually in 10-50 pieces.

Comparisons of full genome sequences are computationally complex, particularly as the number of genomes to be compared increases. Therefore for most public health purposes, determining the relatedness of isolates using WGS utilises methods such as wgMLST (whole genome Multi Locus Sequence Typing). In wgMLST genes are identified in each bacteria, and gene sequences compared. It is possible to compare just genes as in traditional (7 gene) MLST, 50 ribosomal genes, up to 1500 core genes (genes in every individual in a species) or pan genome MLST (all genes in population – ~5,000 of more). Comparison performed with large number of alleles can have a very high level of discrimination. It is also possible to extract genes associated with virulence, antimicrobial resistance and clinical or physiological relevance. Efforts are currently underway to standardise wgMLST databases.

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