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CULTURE-INDEPENDENT DETECTION OF CAMPYLOBACTER BY METAGENOMIC SEQUENCING OF FAECAL SAMPLES

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Campylobacter is one of the main causes of foodborne infections worldwide. Detection and characterisation of Campylobacter in surveillance is done using cultivation methods, which can be laborious and time consuming. In addition, these methods introduce a bias towards the cultivable sub-populations, and typically only one Campylobacter isolate per sample is characterised. A metagenomic approach using next generation sequencing and bioinformatics provide new opportunities to rapidly obtain more information directly from uncultured samples.

To test this approach, Campylobacter negative faecal samples from humans (n=8) and chickens (n=6) were spiked with C. jejuni in 10-fold dilutions from 10² to 10⁶ CFU/mL, and human samples also with 10⁷ and 5×10⁷ CFU/mL. Negative controls were included. DNA was extracted from human samples with QIAamp Stool kit (Qiagen) and from chicken samples with QIAamp Fast Stool kit (Qiagen). Libraries for all samples were prepared by Nextera (Illumina) and all samples were paired-end sequenced on Illumina MiSeq with read lengths of 150 bp. For data analysis IDBA-Hybrid, Burrows-Wheeler Aligner, Basic Local Alignment Search Tool and Kraken were used.

For chicken samples the Kraken results revealed that all spiked samples had more hits to Campylobacter than the negative control. Campylobacter could with a high degree of certainty be detected in the sample spiked with 10⁶ CFU/mL, and with less degree of certainty also in samples spiked with 10⁴ and 10⁵ CFU/mL. A Ranked Biased Overlap analysis showed approximately 90% [84-99%] similarity in diversity among the five spiked samples and the negative control. A Principal Component Analysis showed that the main variation was due to variations in the dominating genera Enterococcus, Klebsiella, Lactobacillus, Escherichia and Bacteroides.

This study is a proof of concept of detection of Campylobacter in uncultured spiked faecal samples. The method for data analysis may be transferred to all kinds of metagenomic next generation sequencing data independent of sampling material and contribute to aid the resolution of infection dynamics.