

Investigations of genetic plasticity in *Campylobacter jejuni* strains of serovar O:2

F. Summary

In the present experimental work the genomic diversity of 13 epidemiologically unrelated strains of *C. jejuni* belonging to serovars O:2 (12) and O:6 (1) was investigated by using the highly discriminatory PFGE technique in combination with mapping of virulence-associated markers to the obtained restriction fragments as well as Fla-PCR-RFLP typing. Although restriction analyses with *Bss*HIII, *Eag*I and *Sun*I revealed low levels of overall genetic similarity for the investigated strains, human isolates NCTC11168 and 5042 proved indistinguishable by each of the restriction endonucleases used. Besides isolates BgVV1766 and 1187-I were shown to share an identical *Eag*I- and *Sun*I-genotype with these strains and differed only by two resp. three band in *Bss*HIII restriction analysis. As with PFGE the genetic diversity of the entire genome can be analyzed, which may have arisen as a result of mutation and accumulates as time passes and organisms spread, these minor changes probably reflect a single genetic event. Such minor variations point towards high clonal relationship, leading to the assumption that these four strains belong to the same clone. Interestingly, the indistinguishable macrorestriction patterns from these strains correlate with identical Fla-profiles generated with restriction endonuclease *Dde*I. Only strain BgVV1766 showed a different Fla-type which does, however, not exclude an otherwise identical genotype. The usability of Fla-PCR-RFLP typing for long-term epidemiological studies is assumed to be limited because of the sensitivity of the flagellin gene locus to spontaneous genetic change due to selection. As Fla-typing is a single-locus genotyping tool, it is furthermore less discriminatory than PFGE, and the data obtained in this work confirm this fact. However, the combination of both typing methods is useful and provides the information that some degree of genomic conservation exists even within certain PCR-Fla-types. In this study, identical Fla-types were mostly consistent with identical or highly similar macrorestriction patterns but were also seen among strains which displayed unique PFGE profiles.

According to these results it can be assumed that the genotypes of certain *C. jejuni* strains derived from different hosts and different geographic regions in Germany remained stable over many years, in this case for a time period of at least fifteen years. Moreover, reference strain NCTC11168 (isolated in Worcester/UK in 1978) showed a highly similar or even identical genotypic profile indicating the existence of such stable genotypes over considerable geographic distances. While stable clones of *C. jejuni* are often correlated with serotypes such

as O:19, O:41 and O:55, only limited knowledge exists for clonal complexes in O:2 or other serotypes. As shown by this study, stable clonal lineages are also found in this serotype and one might suggest that genomic plasticity is not as essential for *C. jejuni* adaptation and survival as previously thought. The recent finding that other serotypes like O:6 also contain such stable genotypes makes it rather likely, that clonal lineages exist in all serotypes and that this species in principle has a clonal framework with different subgroups that are again more panmictic within the subgroup. Contributing factors for the outcome and persistence of clonal lineages could be the lack of natural competence for DNA uptake or the adaptation to niches. The presumption of a clonal frame for the *C. jejuni* genome is nicely displayed by the stability of certain genetic markers, as confirmed by the identical distribution of the virulence-associated genes *cadF*, *cdtB*, *cheY*, *flaA*, *fur*, and *porA* on the *EagI* resp. *SunI* fragments in eight resp. nine of the twelve strains tested. Considering the loss of a restriction site for 1187-I, this strain and strains NCTC11168, 5042 and BgVV1766 again were shown to be genetically highly related as they revealed identical mapping results even within fragments generated with the most discriminatory enzyme, *Bss*HII.

In order to investigate the genotypic variations at the nucleotide level, a representational difference analysis (RDA) for strains NCTC11828 (O:6), BgVV1766 (O:2) and 1187-I (O:2) was performed, giving the possibility to detect serotype- as well as strain-specific DNA sequences. For these strains a total of 28 different RDA fragments was generated from which 15 of them showed high (>70%), six only low (<70%) and a further seven showed no similarity compared to the NCTC11168 genome sequence. Six of the fragments isolated from strains NCTC11828 and K14 revealed similarity to genes involved in synthesis and modification of surface structures in NCTC11168. These data generally confirm considerable levels of genetic variability in the LOS, KPS and FM gene loci not only in strains displaying different but also in strains of the same serotype. Besides detection of smaller sequence polymorphisms in these genes there was evidence for genetic rearrangements in these regions, too. For example, strain K14 was shown to harbour a modification within an enzyme which is involved in KPS biosynthesis in NCTC11168. The KPS gene locus determines serotype specificity and is assumed to be highly conserved within strains of the same serotype. Furthermore DNA sequences associated with ABC transport systems showing no or only low similarity to those found in NCTC11168 have been identified in all three strains tested. These findings point towards a possibly serotype- or strain-specific distribution of these systems which are involved in the transport of a variety of substrates. RDA fragments 11828-E7, 11828-G8, 1187-I-A11, K14-B12, and K14-F6 with no homology to the NCTC11168 genome

sequence revealed similarity to various hypothetical proteins from different bacterial species. Currently the function of these proteins is far away from being well defined and their possible role in *C. jejuni* pathogenicity needs further investigation. The identification of a HP1-related sequence in RDA fragment 1187-I-A6 provides rare evidence for integration of phage DNA into the *C. jejuni* chromosome. Possibly, contribution of these mobile genetic elements to the agents genetic diversity is more relevant than previously thought. Summarizing these results, RDA is a very sensitive and useful tool for identifying not only new genes and sequence polymorphisms but it can also be used to detect genetic rearrangements and differences in gene order in *C. jejuni*.