Phenotypic diversity of campylobacter isolates from sporadic cases of human enteritis in Northern Ireland

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Thermophilic campylobacters, particularly *Campylobacter jejuni*, *C. coli* and *C. lari* remain the most common cause of acute bacterial enteritis in the Republic of Ireland and Northern Ireland, resulting in approximately 3000 positive results per annum and a trend that continues to rise. Most recent data for the year 2000 records 1001 positive results in Northern Ireland,[1] which approximates to 59 cases per 100,000 individuals. This compares with a rate of 106 and 127 cases per 100,000 individuals for England & Wales and Scotland, respectively. A recent UK epidemiological study,[2] however, estimates that the true prevalence of this infection is approximately 10-fold higher, due to patient under-reporting.

Campylobacter spp. occupy a natural reservoir in the intestine of a wide range of feral and domesticated animal and birds, and enter the human food chain on raw animal products such as poultry, red meat and offal.[3] Non-pasteurised milk has been the source of several large outbreaks and campylobacters can be found in inland and coastal waters as a result of faecal contamination by animals and sewage discharge.

Human infections with *Campylobacter* spp. arise from direct contact with animals or with naturally contaminated raw or undercooked food products. Fortunately, large outbreaks of disease are rare and the majority of infections are sporadic. However, the vehicle of infection in most cases remains unidentified.

The epidemiology of *C. jejuni* enteritis is hampered by a lack of a standardised identification and typing scheme. Few laboratories in the UK identify isolates to the species level and fewer still utilise any of the recognised typing schemes. The consequence of inadequate identification is scanty information about the frequency and distribution of the strain types that cause human infection and where they are to be found in the food chain.

Differences in the attack rate between England & Wales and Northern Ireland merit further examination, as there is approximately 50% fewer laboratory-reported cases of campylobacteriosis per 100,000 population in Northern Ireland compared to Britain. Clarification of the reasons for these differences may permit the introduction of effective intervention controls to reduce the incidence of the disease within the British Isles. Hence, it may be postulated that such differences are attributed to social behaviour and food

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Table 1. Comparison of Preston biotypes of campylobacters isolated from faecal specimens from symptomatic patients from England & Wales and Northern Ireland

	Biotype – number of isolates; percentage	
	England & Wales	**Northern Ireland**
Period	December 1990-June 1991	December 1991-June 1992
Total isolates examined	831	118
Species distribution	754 (90.7%) *C. jejuni*	100 (84.7%) *C. jejuni*
	74 (8.9%) *C. coli*	17 (14.4%) *C.coli*
	3 (0.4%) *C. lari*	1 (0.9%) *C. lari*
***C. jejuni* biotypes**	6000 (89; 10.7%)	6010 (24; 20.3%)
	6010 (87; 10.5%)	6000 (22; 18.6%)
	6004 (67; 8.1%)	6004 (17; 14.4%)
	6014 (63; 7.6%)	6014 (9; 7.6%0
	6114 (56; 6.7%)	6002 (4; 3.4%0
	Others (392; 47.2%)	15 other biotypes (24; 20.3%)
***C. coli* biotypes**	2250 (13; 1.6%)	2450 (4; 3.4%)
	2440 (9; 1.1%)	2440 (4; 3.4%)
	2640 (7; <1.0%)	2610 (3; 2.5%)
	2650 (6; <1.0%)	2410 (2; 1.7%)
	2450 (6; <1.0%)	4 other biotypes (4; 3.4%)
	Others (33; 4.0%)	
***C. lari* biotypes**	0073 (2; <1.0%)	0031 (1; 1.0%)
	0033 (1; <1.0%)	

Table 2. Comparison of Preston phage types of campylobacters isolated from faecal specimens from symptomatic patients from England & Wales and Northern Ireland

	Phage type – number of isolates; percentage	
	England & Wales*	**Northern Ireland**
Period	December 1990-June 1991	December 1991-June 1992
Total isolates examined	831	117
Species distribution	754 (90.7%) *C. jejuni*	100 (85.5%) *C. jejuni*
	74 (8.9%) *C. coli*	16 (13.7%) *C. coli*
	3 (0.4%) *C. lari*	1 (0.8%) *C. lari*
***C. jejuni* phage types**	69 (96; 12.7%)	52 (22; 18.8%)
	90 (89; 11.8%)	90 (9; 7.7%)
	52 (87; 11.5%)	55 (9; 7.7%)
	121 [78; 10.3%]	69 (8; 6.8%)
	115 & 91 (27; 3.6%)	91 (7; 6.0%)
	Others (217; 28.9%)	15 other phage types (28; 24.0%)
	Non-typable (133; 17.6%)	Non-typable (17; 14.5%)
***C. coli* phage types**	UD	85 (6; 5.1%)
		90 (4; 3.4%)
		125 (4; 3.4%)
		Non-typable (2; 1.7%)
***C. lari* phage types**	Non-typable (3)	Non-typable (1; 0.9%)

* expressed as a percentage total of *C. jejuni* isolates

UD undetermined

hygiene practices in the two populations and/or the occurrence of less virulent campylobacter strains and subspecies in the Northern Ireland populations. To date, however, there have been no reports in the literature of studies comparing subtypes of human clinical campylobacters from Northern Ireland and England & Wales.

Therefore, the aim of this study is to employ phenotypic typing techniques (a combination of biotyping and phage typing) to examine the similarity of local campylobacter isolates with those from England and Wales, as previously described.

Given the emerging clinical significance of campylobacters in acute bacterial gastroenteritis in Northern Ireland, in 1991 an archive of significant clinical campylobacter isolates was established at the Northern Ireland Public Health Laboratory, Belfast City Hospital, whereby all primary isolates from across Northern Ireland were deposited in the collection. A retrospective examination of campylobacter isolates from Northern Ireland during the initial phase of collection was undertaken and compared with similar data published previously from England and Wales. A total of 118 campylobacter isolates were collected from symptomatic human sources isolated from faecal samples originating from patients with enteritis submitted by general practitioners in the community, as well as from hospital in-patients in Northern Ireland during the period December 1991 to June 1992, representing approximately 28.6% of human clinical isolates from across Northern Ireland for this period. Isolates were received from all nine clinical microbiology laboratories in Northern Ireland hospitals and were characterised by techniques described previously.

The Preston Biotyping scheme[4] is based on phenotypic differences with hippurate hydrolysis, temperature tolerance tests and resistotyping tests to cephazolin, arsenite, TTC, pyronin, metronidazole, 5-fluorouracil, cycloserine and nalidixic acid, as well as γ-glutamylalkaline phosphatase activity and DNase activity. The results were organised in accordance with Bolton *et al.*,[4] with the 12 tests arranged in four groups of three. Each positive reaction was given a numerical value and a four-digit code was obtained for each strain. The Preston phage-typing scheme[5] consists of a set of 16 bacteriophages isolated from clinical, veterinary and environmental sources. Phages were propagated and titrated to determine the routine test dilution (RTD) using conventional techniques. Test campylobacter cultures were subcultured onto blood agar (No. 2) plates that contained 5% (v/v) defibrinated sheep blood and incubated microaerobically (5% O_2, 10% CO_2, 85% N_2) at 42°C for 24 h. Growth was harvested into brain-heart infusion broth (Difco) to give suspensions with an absorbance of 2.0 at 420 nm (approximately 10^8 cfu/mL) in a Perkin Elmer 6/20 spectrophotometer. The suspensions were flooded onto the dried surface of Brucella agar plates (Oxoid CM691) and the excess fluid discarded.

The plates were allowed to dry at room temperature and then inoculated with 10 μL amounts of the 16 phage suspensions at RTD, using a Lidwell phage applicator. Plates were incubated microaerobically as above for 18-24 h and examined for confluent lysis or plaques. Lysis was recorded as semi-confluent to confluent (+++), 50-100 plaques (++), 20-49 plaques (+) and 1-19 plaques (±). Only +++ or ++ phage reactions were recorded as positive. Phage patterns were recorded and assigned to one of eight previously designated phage groups, defined from different studies of more than 1000 sporadic isolates from different parts of the UK. Phage types with similar lytic patterns (i.e., two or less differences) were clustered to form groups.

The Preston biotyping scheme proved highly discriminatory and produced 20 biotypes for *C. jejuni* and eight biotypes for *C. coli* (Table 1). The phage-typing technique gave equal discrimination as 20 phage types were recorded for *C. jejuni* and three for *C. coli* (Table 2); however, it did produce a significant number of untypable isolates (20/117 [17.1%]). This may have been because the reference phages were too specific, possibly reflecting the fact that a limited pool of campylobacters were studied. Consequently, in order for phage typing to be considered an acceptable typing technique, additional phages need to be isolated from other sources in order to reduce this level of non-typability. Reference phages used in the scheme were isolated from a wide range of clinical and non-clinical sources.

Overall, this comparison of a limited number of Northern Ireland isolates with a larger group of isolates from England and Wales showed no marked difference in phenotype frequency, using either biotyping or phage typing as epidemiological markers. This would suggest that phenotypic differences do not account for the difference in attack rates between the groups and that Northern Ireland shares a similar pool of phenotypes with England and Wales.

Previously, LaFong and Bamford[6] suggested that differences in attack rates between Britain and Northern Ireland might be due to (a) relatively low consumption of unpasteurised milk in Northern Ireland, (b) a higher ratio of red meat to white meat consumption in Northern Ireland, (c) climatic factors and (d) a social likeness for ‘well done’ food in Northern Ireland.

The data presented here would support the hypothesis that such differences are probably due to social aspects of human behaviour (e.g., food preparation) rather than to any differences in isolate type.

We await the results of the current UK Campylobacter Sentinel Surveillance Scheme with interest, as this will permit a comprehensive and current comparison of Northern Ireland types with those from Britain and should help elucidate additional risk factors for human campylobacteriosis in Northern Ireland. ☐

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Multilocus enzyme electrophoresis typing of clinical *Helicobacter pylori*

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Multilocus enzyme electrophoresis (MLEE) has been used extensively for many years in eukaryotic cell genetics and has become a standard method in evolutionary biology.[1-3] Allelic variation in a structural gene can be detected using MLEE by assessing the net electrostatic charge of the polypeptide encoded. The rate of migration of a protein during electrophoresis is determined by the amino acid sequence of the peptide (i.e. the net electrostatic charge). Hence, the mobility variants (electromorphs or allozymes) of an enzyme can be equated directly with alleles at the corresponding structural gene locus.

As different structural genes are represented by different enzymes, choice of gene product permits the visualisation of specific bands following electrophoresis of cell lysates. Consequently, the data provided by this method not only allows consistent identification of strains for epidemiological purposes but also a measurement of genetic distances among strains.

Previously, only one study performed in the mid-1990s has used MLEE to type *Helicobacter pylori*.[4] Therefore, the present study aims to optimise laboratory parameters (including phase and basal growth medium) for the cultivation of *H. pylori* for MLEE analysis. In addition, optimised conditions are employed in a small preliminary study to examine genetic relatedness, based on cluster analysis of differences at nine enzyme loci in isolates associated with different gastric pathologies.

Details of the clinical isolates of *H. pylori* (n=12) used in the study are presented in Table 1. The identity of each was

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