Epidemiol. Infect. (1997), **118**, 81–89

Genotype analysis of human blood isolates of *Campylobacter jejuni* in England and Wales

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(*Accepted 19 November 1996*)

SUMMARY

Genomic profiles were obtained for 76 strains of *Campylobacter jejuni* isolated from bacteraemic patients in England and Wales over the period 1981–94. Genotyping was performed by restriction fragment length polymorphism (RFLP) analysis using a random cloned DNA probe, and by ribotyping with a PCR-generated *C. jejuni* 16S ribosomal DNA probe. Phenotypic characterization was achieved by heat-stable (HS) and heat-labile (HL) serogrouping, and Preston phagetyping and biotyping. The blood isolates were genomically heterogenous, with 24 RFLP/16S profiles occurring within the 76 strains. Forty-four percent of isolates belonged to one of three RFLP/16S genotypes, reflecting the patterns seen in faecal isolates, except that genotypes usually associated with the HS 1 antigen were uncommon. The two most prevalent genotypes, characteristic of HS 2 and HS 4 strains, showed similarity by cluster analysis. Further evidence was seen of associations between phenotypic and genotypic characters within some HS serogroups. Chromosomal profiling by RFLP analysis does not indicate that particular genotypes have a predisposition to invade the bloodstream.

INTRODUCTION

Reported episodes of extraintestinal infection due to *Campylobacter* spp. are infrequent, with a total of 394 cases of campylobacter bacteraemia documented by the Public Health Laboratory Service (PHLS) Communicable Disease Surveillance Centre (CDSC) in England and Wales over the decade 1981–91 [1]. This represents an incidence of 0·15 per 100 faecal isolates, compared with 1·0–1·5 per 100 for non-typhoidal salmonella bacteraemias [2, 3]. A recent study of campylobacter septicaemia in Denmark [4] showed an even lower incidence of 0·008 bloodstream infections per 100 faecal isolates. In both of these studies, the size of the sampled patient group relative to the total number of individuals with *C. jejuni* infection is relatively small. Campylobacter gastroenteritis does not generally result in hospitalization, and blood cultures are taken from hospitalized patients only where there is a clinical suspicion of septicaemia. Sampling in this manner may underestimate the true incidence of campylobacter blood stream invasion. During a 15-year (1969–83) retrospective study [5] of systemic non-typhoidal salmonellas, blood cultures were taken from all patients with acute diarrhoea admitted to a regional infectious diseases unit. The isolation rate from blood was 8 per 100 faecal isolates, more than six times that of a later study recording data from routine hospital admissions [3].

Although the true incidence of bacteraemia may be higher than reported, extraintestinal campylobacter infections in developed nations are usually associated with very low morbidity and mortality [1]. This is in contrast with countries which experience high rates of childhood malnutrition and gastrointestinal disease,

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Table 1. *RFLP and 16S ribotype profiles for 76 blood culture isolates of* C. jejuni

RFLP type	16S ribotype	Serogroup*		Preston		Strain number
		HS	HL	Biotype	Phage-group	
1	1	1	2	6131	1	C/6978
		1	2	6615	146	C/7543
		1	nd	nd	nd	C/22363
2	1	2	4	6004	52	C/4537
		2	4	6004	nt	C/4533
		2	4	6020	52	B/23383
		2	1	6014	52	B/23386
		2	nt	6611	52	C/5679
		2	nt	6010	1	B/23391
		2	nd	6014	83	B/9973
		2	nd	6004	69	B/14329
		2	nd	6011	128	B/23573
		2	nd	6014	69	B/24154
		nt	5	6010	52	C/4535
		nt	2	6004	52	B/7729
		nd	nd	6114	nt	C/23381
3	15	2	nt	6070	56	C/2840
4	1	4	1	6315	69	B/23384
		4	1	6014	97	B/23389
		4	1	6315	69	B/7728
		4	1	6315	69	B/16320
		4	1	6111	69	B/19007
		4	1	6010	97	B/18350
		4	1	6014	97	B/2027
		4	1	6010	97	B/23529
		4	2	6314	nt	B/12160
		4	nt	6004	nt	B/7731
		4	nd	6034	44	B/14360
		4	nd	6110	106	B/23530
		4	nd	6016	97	B/627
		4	nd	6014	97	B/14328
		4	nd	6214	97	B/13825
		43	nt	6004	69	B/14443
		43	nd	6010	nt	B/4759
		nt	nt	6034	44	B/7730
5	3	15	nt	6006	nt	C/4422
		nt	nt	6012	69	B/2839
		nd	nt	6112	90	C/23108
5	16	21	nd	6102	91	C/22802
		42	nt	6102	69	B/1915
5	23	1	nd	nd	nd	C/24167
		5	nd	6110	90	B/15493
		40	nt	6311	90	B/2026
		53	nd	6110	90	C/21965
7	1	11	nt	6014	52	C/5820
8	4	4	7	6010	44	C/8817
		4	7	6010	58	B/8794
		4	7	6000	44	B/1338
		4	7	6000	58	B/2841
		4	nt	6000	44	B/7725
		4	nt	6010	58	B/1451
		4	nd	6000	44	B/16023

Table 1 (*cont.*)

RFLP type	16S ribotype	Serogroup*		Preston		Strain number
		HS	HL	Biotype	Phage-group	
10	20	1	nt	6014	nt	C/4536
13	10	17	nt	6000	nt	C/15730
20	2	46	nd	6012	85	B/12008
24	2	9	nd	6030	1/11	B/14359
28	8	53	11	6114	nt	B/1301
		nt	11	6000	nt	B/23390
29	25	4	nd	6010	nt	B/4758
30	3	4	nt	6024	69	B/2567
31	2	18	nt	6110	69	B/2181
		18	nt	6110	nt	B/8733
		18	nd	6130	76	B/14357
		18	nd	6115	nt	B/23330
32	26	11	nd	6012	90	C/8734
		19	7	6012	90	C/4539
		19	17	6002	90	B/2035
33	27	41	9	6010	146	C/20627
34	1	10	nd	6010	1	B/11209
35	2	3	nt	6010	69	B/23387
		5	11	6014	125	C/5821
		5	nd	6111	nt	C/21705
35	8	6	nd	6110	90	C/22067
36	2	18	nd	6010	90	C/4818
		23	nt	6135	76	B/1916
37	2	18	nt	6010	90	B/4537

* nt, not typable; nd, not done.

where campylobacter septicaemia in infants represents a common and life-threatening complication of *C. jejuni* enteritis [6]. Other patient groups in which systemic campylobacteriosis poses a significant clinical problem are those with acquired or congenital immune diseases. The diagnosis and treatment of extraintestinal campylobacter infections are of increasing relevance in the management of individuals with the acquired immune deficiency syndrome (AIDS) [7, 8].

In the present study, RFLP profiling was carried out on 64 strains from the original retrospective analysis [1], plus 12 *C. jejuni* blood isolates from 1992–4. The aims of our study were to investigate whether genotypes of systemic *C. jejuni* isolates differed from those of faecal isolates, and to discover if there were any genetic markers which specifically characterized the bacteraemia strains. We also aimed to show whether systemic strains sharing the same phenotype markers could be subtyped using RFLP methods.

MATERIALS AND METHODS

Bacterial strains

Systemic isolates of *Campylobacter* spp. submitted to Manchester Public Health Laboratory (PHL) for HS/HL serogrouping were stored at −70 °C in Brain–Heart Infusion broth (Oxoid, Unipath, Basingstoke). Of the 124 isolates of *C. jejuni*/*C. coli* serogrouped over the study period (1981–94), 76 *C. jejuni* strains were successfully retrieved from storage.

Strain characterization–phenotypic

Serogrouping for HS and HL antigens and biotyping and Preston phagetyping were performed as described previously [9]. A panel of 46 Penner antisera and a restricted set of 12 Lior antisera were used to identify the HS and HL antigens respectively.

Strain characterization–genotypic

Genomic DNA was digested with restriction enzyme *Hae* III, immobilized by Southern transfer, and probed using a random *C. jejuni* chromosomal probe and a PCR-generated 16S ribosomal DNA probe [9]. The numbering of the RFLP and ribotype patterns followed the scheme used previously [9].

Plasmid extraction

Plasmid isolation was attempted from 15 strains selected at random (HS serogroups 2, 4, 9, 10, 18, 27 and 46), using a modified alkaline lysis method [10]. Two *E. coli* hosts (V517 and 39R861) harbouring ten plasmids in the range 1·4–42 kb were extracted for use as size markers, along with a laboratory strain of *C. upsaliensis* containing two plasmids, which served as a control.

Comparison of RFLP and ribotype profiles

Band patterns generated by RFLP and ribotype analysis were digitized using the 'MolMatch' system (UV Products; Genetic Research Instrumentation Ltd, Dunmow, Essex, UK), which uses a simple matching (Dice) coefficient [11] to compute pattern similarities. The resulting matrix of pairwise similarities was analysed using SPSS statistics software (SPSS Inc., Chicago, Illinois) to construct a hierarchical cluster analysis, using a method of average linkage.

RESULTS

Genotype analysis of the 76 systemic *C. jejuni* isolates

Twenty-one RFLP and 13 16S ribotype patterns were identified amongst the 76 strains, giving a combined total of 24 distinct RFLP/ribotype combinations (Table 1). In the previous genotypic analysis of HS/HL serogroup reference strains and clinical and environmental isolates of *C. jejuni* [9], ten RFLP types (types 28–34 inclusive) and two 16S ribotypes (types 26 and 27) were exclusive to blood isolates. Conversely, several RFLP types (6, 9, 11, 12, 14–19, 21–23, 25–27) and 16S ribotypes (5–7, 9, 11–14, 17–19, 21, 22, 24) identified in the same study were not present amongst genotypes of the systemic isolates. The majority of strains (34 of 76) were represented by three genotype combinations, RFLP/ribotypes 1/1, 2/1 and 4/1, which also predominated amongst human faecal isolates. The two genotypes associated with HS 4 strains, RFLP/ribotypes 4/1 and 8/4, were both present in blood isolates in the approximate proportions in which they were found in faecal strains.

None of the 15 systemic isolates examined at random contained plasmid DNA.

Phenotype and genotype associations

Evidence of linkage between certain phenotype and genotype markers has been reported for faecal strains of *C. jejuni* [9], and similar conservation, particularly between genotype and HS/HL serogroups, was apparent in the blood isolates. However, several strains showed serogroup/genotype associations which appeared atypical. Isolate B/1916 was serogroup HS 23 but genotypically belonged with the HS 18 strain complex (RFLP/ribotype 36/2). Two strains of serogroup HS 4 (B/4758 and B/2567) had RFLP/ribotype profiles 29/25 and 30/3 respectively, quite different from either of the predominant HS 4 genotypes, 4/1 and 8/4. Genotypes of blood isolates within the HS 1 serogroup were diverse, with three different RFLP/ribotype patterns identified amongst the five strains in this group (RFLP/ribotypes 1/1, 5/23 and 10/20). One RFLP type (RFLP 5) comprised seven different HS serogroups and three ribotypes. Genotyping therefore differentiated between strains of the same HS serogroup (particularly HS 1), but most discrimination was achieved by combined use of both phenotype and genotype markers.

In certain cases, strains from the Penner HS serostrain collection showed genotypes dissimilar from systemic isolates which shared the same HS marker. The Penner HS 18 serogroup strain was genotype RFLP/ribotype 11/2 [9]. The systemic *C. jejuni* HS 18 isolates shared the same 16S ribotype as the serostrain, but had dissimilar RFLPs: types 31, 36, 37. Similarly, the Penner HS 19 serogroup strain was RFLP/ribotype 3/2, whilst the two systemic HS 19 isolates were types 32/26.

Three of the isolates which could not be HS serogrouped (HS 'nt') demonstrated genotype profiles typical of the most common faecal isolates. Strain B/7730 was RFLP/ribotype 4/1, and strains C/4535 and B/7729 respectively were genotype 2/1. This suggests that HS 'nt' reactions may have resulted from the loss or masking of the HS antigens, rather than representing novel HS types for which antisera are not available.

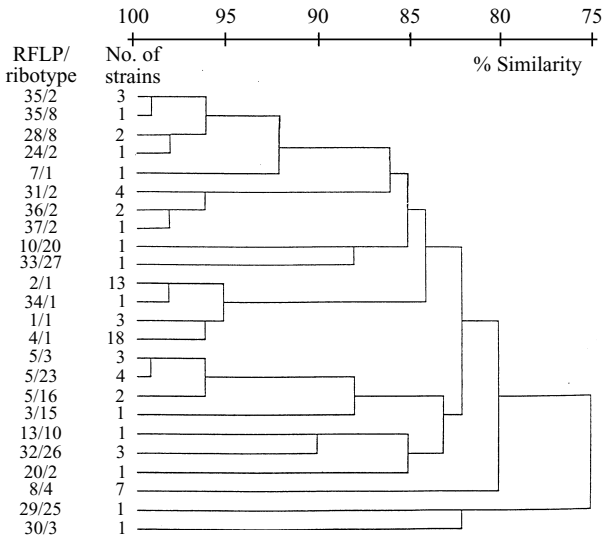

Fig. 1. Dendrogram from a cluster analysis based on similarities between combined *Hae* III RFLP and 16S ribotype patterns for 76 systemic isolates of *C jejuni*. A simple matching (Dice) coefficient was used to determine percentage similarities (horizontal axis).

Pattern comparisons

Clusters analysis using the combined *Hae* III RFLP/ribotype patterns (Fig 2a/b; Table 2) was used to construct the dendrogram in Figure 1. One central grouping contained 46% (35/76) of the total isolates at 95% similarity. These were predominantly strains of serogroups HS 1, HS 2 and HS 4 complex. A defined, stable cell line within HS 4 complex strains, previously characterized in faecal isolates [9], was represented by the group of seven strains having the RFLP/ribotype profile 8/4. These isolates clustered separately from the majority of HS 4 strains, which had RFLP/ribotype genotypes 4/1, further confirming their separate identity.

Strains associated with the HS 18 antigen also formed a related grouping at the 95% level, separate from the main serogroup HS 1, 2 and 4 cluster. All six HS 18 isolates gave an identical ribotype (2), and the associated RFLP patterns 31, 36 and 37 showed polymorphism in only a single, low molecular weight band. The close relation of these RFLP profiles and sharing of the HS 18 serogroup and 16S ribotype markers may delineate a cell lineage of common ancestry, or putative 'clone complex', although the phagetype and biotype data of these strains are not congruent.

HS 4 and HS 18 serogroups have been identified as being potentially more invasive or serum resistant than other HS serogroups [1]. The separate clustering, at 84% similarity, of the genotypes associated with these serogroups (Fig. 1) implies that they have not diverged from a common 'invasive' ancestor. By comparison, RFLP/ribotypes of the predominant HS 1, HS 2 and HS 4 serogroup strains clustered at 95% similarity, indicating some degree of chromosomal identity between these isolates.

Table 2. *Band sizes of 21 E3CJC2 RFLP and 13 16S ribotype patterns from 76* C. jejuni *blood isolates*

	Band size (kilobase pairs)
RFLP type	
1	5·10, 3·75, 2·60, 1·60, 1·30, 1·27, 1·20
2	8·75, 2·60, 1·60, 1·30, 1·27, 1·20
3	8·75, 2·80, 2·60, 1·60, 1·33, 1·27
4	6·00, 2·60, 1·80, 1·60, 1·30, 1·27, 1·20
5	8·75, 4·90, 2·60, 1·27, 1·08
7	8·75, 3·30, 2·60, 2·23, 1·33, 0·80
8	6·00, 2·60, 2·42, 1·80, 1·66, 1·39, 1·27
10	8·75, 4·10, 2·60, 1·33, 1·27, 1·20
13	4·90, 2·80, 2·60, 2·23, 1·33, 1·08
20	4·90, 2·60, 2·42, 2·23, 1·60, 1·44, 1·42, 1·27, 1·20
24	6·00, 4·10, 2·60, 2·23, 1·33, 1·20
28	8·75, 4·10, 2·60, 1·33, 1·20
29	25·00, 4·90, 3·75, 2·80, 2·23, 1·27, 1·20
30	4·90, 3·75, 3·10, 2·80, 1·25, 1·23, 1·16
31	6·00, 2·60, 1·80, 1·44, 1·35, 1·33, 1·25, 1·20
32	4·90, 2·90, 2·60, 2·23, 1·44, 1·33, 1·20
33	6·00, 4·10, 2·60, 1·80, 1·33, 1·20
34	8·75, 2·60, 1·66, 1·60, 1·33, 1·27, 1·20
35	8·75, 5·40, 2·60, 1·33, 0·80
36	6·00, 2·60, 2·23, 1·66, 1·35, 1·33, 1·25, 1·20
37	6·00, 2·60, 1·80, 1·66, 1·35, 1·33, 1·25, 1·20
16S ribotype	
1	6·00, 2·90, 2·60
2	7·50, 4·90, 2·60
3	12·5, 8·75, 2·23
4	12·5, 8·00, 2·60
8	4·90, 2·90, 2·60
10	8·75, 4·90
15	8·75, 4·10, 2·60
16	12·50, 3·30, 2·30
20	7·00, 1·27
23	12·50, 8·75, 2·60
25	8·75, 4·10, 2·30
26	12·50, 1·20
27	10·00, 8·75, 2·80

Comparison of specific HS serogroups in blood and faecal isolates

The data from the original retrospective analysis have

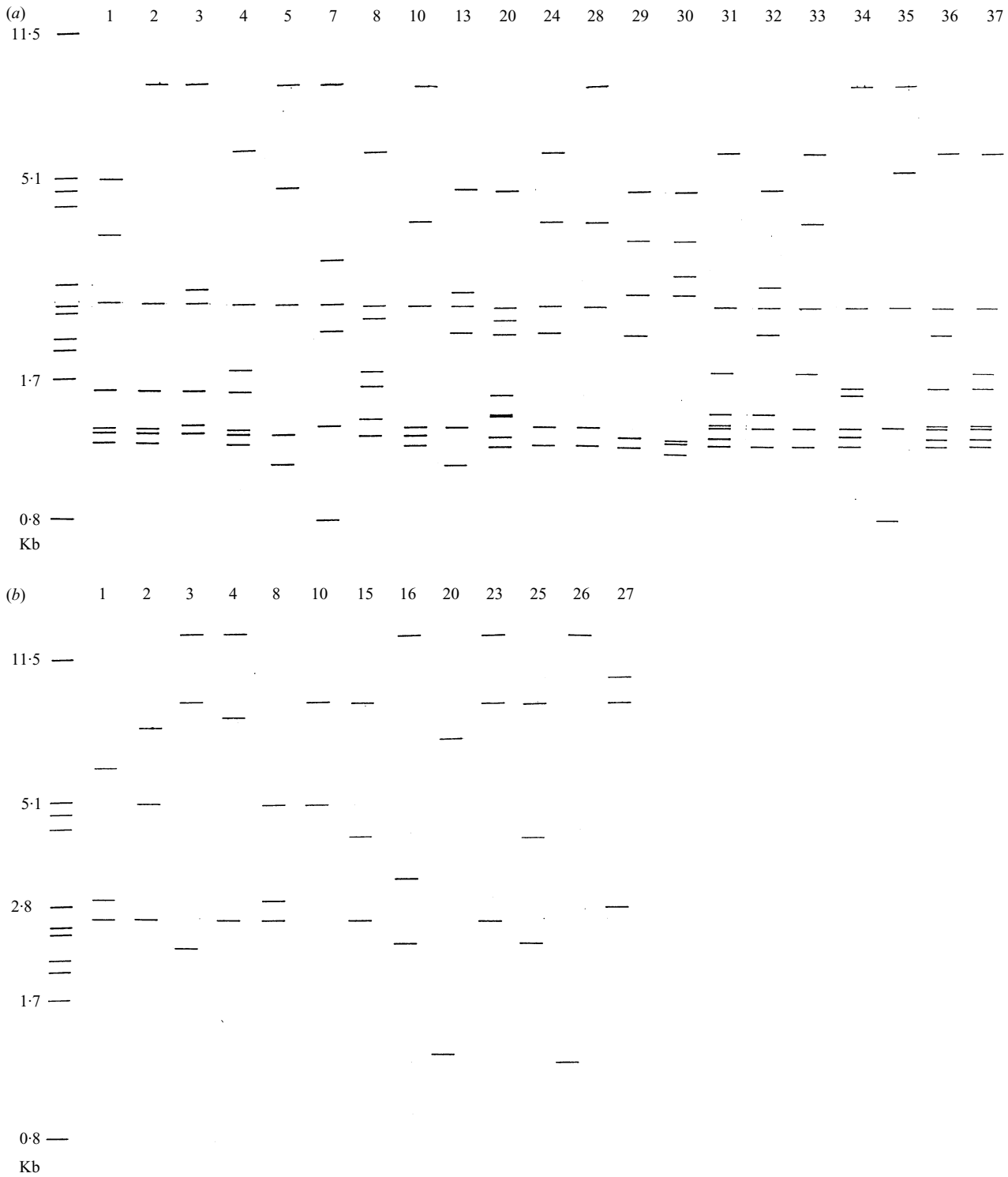

Fig. 2. Autoradiographs of (*a*) the RFLP and (*b*) 16S ribotype patterns [9] shown by the 76 *C. jejuni* systemic isolates were digitized using the 'Molmatch' gel documentation system. Scanned profiles representing each pattern are shown above, and the corresponding band sizes are listed in Table 2.

been updated to include 19 systemic strains serogrouped in the period 1992–4. Two isolates originally characterized as serogroup HS 1 showed strong cross-reactions with HS 43 antisera. These were re-analysed, and the HS 43 reaction appeared immunodominant. On the basis of this reaction, and from the genotype profiles, these isolates (B/7725 and B/7731) were reclassified as HS 4 complex strains (strains possessing

Table 3. Campylobacter jejuni *strains from blood, England and Wales, 1981–94*

HS serogroup	Total no. isolates (blood and faeces)	Blood isolates	Blood/faeces (%)	CI 95
1	1160	7	0·6	±0·44
2	1490	13	0·87	±0·47
3	155	2	1·3	±1·77
4, 13, 16, 43, 50	2183	42	1·9	±0·55
5	495	3	0·6	±0·68
6	400	1	0·25	±0·49
9	418	7	1·67	±1·23
10	179	2	1·11	±1·53
11	570	2	0·35	±0·48
15	181	2	1·1	±1·52
17	142	1	0·7	±1·37
18	201	8	4·0	±2·71
19	243	4	1·65	±1·60
21	209	3	1·43	±1·61
23	170	1	0·58	±1·14
40	55	1	1·8	±3·51
41	58	1	1·72	±3·34
42	10	1	10	±18·6
46	32	1	3·1	±6·0
53	229	2	0·87	±1·2
ND	950	3	0·31	±0·35
NT	1634	17	1·04	±0·49
Total	11164	124		

any of the cross-reacting HS antigens 4, 13, 16, 43 or 50).

The data in Table 3 show blood isolates as a percentage of faecal isolates of *C. jejuni* for different HS serogroups. The very low numbers of systemic ($n = 124$) relative to faecal isolates ($n = 11164$) produced high 95% confidence intervals (CI 95) for most HS serogroups, and analysis of a larger number of invasive strains would be required to increase the significance of these values. Strains of serogroup HS 1 showed the lowest invasive index (0·6/100 faecal isolates) of the three most common HS groups (HS 1, 2 and 4). The relatively high percentage of HS 18 isolates in blood relative to faeces (4·0%) was qualified by a CI 95 value of ±2·7. Similarly, the apparent prevalence of serogroup HS 4 in blood isolates [1] appeared less pronounced using this analysis (1·9% CI±0·55), and approximated to that seen for the two most common serovars of salmonella: *S. enterica* serovar. *enteritidis* (1·4% CI±0·086) and *S. enterica* serovar. *typhimurium* (1·1% CI±0·08), contrasting with the values for invasive serovars of *S. enterica*, such as *cholera-suis* (74·1% CI±33·9) and *dublin* (25·5% CI±4·75) [3].

DISCUSSION

Campylobacter jejuni is rarely isolated from blood cultures, despite a recorded incidence of enteric infection as high as 44000 cases/annum (CDSC, Colindale, England). Although predisposing factors such as immune deficiency and increasing age enhance the risk of campylobacter septicaemia, blood stream invasion with this organism is recognized as being essentially a transient complication of campylobacter enteritis [1]. The results presented here support this observation, with a majority of the systemic isolates reflecting the RFLP types found in faecal strains. Several of the blood isolates had RFLP or 16S ribotypes not yet identified from faecal or serogroup reference strains. However, genotype analysis of significant numbers of faecal isolates of serogroups HS 18 and HS 19 has not yet been carried out, so a direct comparison with systemic strains of these two serogroups is not possible. Some of the other unique RFLP profiles occurred in single blood isolates with uncommon HS serogroups, and similar patterns may be identified once a larger and more varied sample of faecal strains has been analysed. Therefore it seems unlikely that genotypes so far found only in the blood

isolates are representative of a more invasive subset of strains.

Several 'atypical' associations between phenotype/genotype markers were seen, and these may have resulted from genes encoding HS and HL serogroup antigens transferring horizontally to generate new serovar combinations [12, 13]. Lateral translocation of the HS 4 antigen could have accounted for the association of this serogroup with the genotypically unique isolates B/4758 and B/2567. Similarly, C/8734 was found to be genetically and phenotypically related to the HS 19 strains but expressed a HS 11 antigen. A further consideration is that individual HS serogroup antigens, such as HS 23 and HS 36, can be highly related at the biochemical and presumably genetic level [14], although very few have been well characterized. Therefore it is important to define the biochemistry of differing HS antigens from strains of similar genotypic backgrounds, to confirm that they do not represent merely minor variants of the same LPS molecule.

Data from the isolates examined here and elsewhere [15, 16] indicate that *C. jejuni* shows a high level of chromosomal diversity, with 24 different genotypes found amongst the 76 systemic isolates. Other species within the family Campylobacteraceae also show extensive genomic polymorphism when examined by a variety of different methods [17, 18]. It has been proposed that in *H. pylori*, this variation results at least in part from rapid genomic rearrangements and microevolution of the chromosome [19]. Using a polyphasic approach, examining both phenotype and genotype characters, we have previously identified epidemiologically defined cell lines within the variable *C. jejuni* strain population [9; manuscript in preparation], a finding similar to that for *C. coli* [20]. Although many different combinations of chromosomal and phenotypic markers are seen amongst the blood isolates, the same stable associations identified in the faecal strains are evident. The biogenesis of these conserved cell lines is unknown, but modern livestock practices, particularly the intensive rearing of broiler chickens [21], may provide ideal conditions for the proliferation and dissemination of isogenic campylobacter strains into the human food chain.

Recent reports have demonstrated molecular mimicry between the LPS of Penner serogroups HS 1, HS 4 and HS 19, and human GM1 and GD1a gangliosides [22, 23]. Molecular mimicry has been implicated as a mechanism for increasing serum resistance by evasion of the host immune response for some pathogenic microorganisms. The similarity of the genotypes of blood culture and faecal isolates, coupled with the relative infrequency of systemic infection with these and other HS serogroups, indicate that *C. jejuni* which are recovered from blood are unlikely to be especially invasive or serum resistant.

ACKNOWLEDGEMENTS

E. coli strains V517 and 39R861 were gifts from the Central Public Health Laboratory, Colindale, London. Our thanks go to the many clinicians who over the years have submitted systemic campylobacter isolates for typing, and in so doing have made this study possible.

This work was supported by the Public Health Laboratory Service of England and Wales.

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