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Phage typing of *Campylobacter jejuni* and *Campylobacter coli* and its use as an adjunct to serotyping

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SUMMARY

Campylobacter is the most commonly reported cause of gastro-intestinal infection in England and Wales, with over 50000 reported cases in 1997. The majority of human campylobacter isolates in England and Wales are *C. jejuni* (*c.* 90%) with most of the remainder being *C. coli*. We describe the use of phage typing as an extension to serotyping for more detailed characterization within these two species. The scheme was piloted during a study of 2407 *C. jejuni* and 182 *C. coli* strains isolated in Wales between April 1996 and March 1997. Fifty-seven *C. jejuni* phage types were identified, with the ten most prevalent phage types accounting for 60% of isolates tested; 16% of isolates were untypable. The most common phage type was PT 1 which represented *c.* 20% of isolates. A further 7% of isolates reacted with the phages but did not conform to a designated type (RDNC). Only 12 phage types were identified among *C. coli*, with the two most common types, PT 2 and PT 7 accounting for 75·2% of isolates. When used in conjunction with serotyping, the ability of phage typing to identify between 6 and 29 subtypes within each of the predominant HS types has enabled a further level of discrimination to be achieved that enhances the epidemiological typing of *C. jejuni* and *C. coli*.

INTRODUCTION

Since 1981, campylobacters have been the most commonly reported cause of acute bacterial enteritis in England and Wales with 50247 reported infections in 1997 [1]. Several studies have compared different methods for subspecies typing within *C. jejuni* and *C. coli*, and both Patton and colleagues in the United States [2] and Owen and Gibson in the United Kingdom [3] concluded that for large-scale surveillance, serotyping was the most practical solution for a reference laboratory to adopt. The most widely used approach to serotyping is the scheme developed in Canada by Penner and Hennessy [4], which identifies soluble heat-stable antigens by passive haemagglutination. More recently, an adaptation of this scheme has been described, based on the detection of heat-stable antigens by absorbed antisera utilizing whole cell agglutination, that further improves discrimination within both *C. jejuni* and *C. coli* [5]. However, there remains a need to further subtype within the predominant serotypes.

Campylobacter phage typing schemes have been described by Grajewski and colleagues (1985) in the United States [6], Salama and colleagues (1990) in the United Kingdom [7] and Khakhria and Lior (1992) in Canada [8]. All three schemes share some common phages. The original scheme developed in the United States used 14 virulent bacteriophages isolated from poultry faeces. The scheme developed in the United Kingdom combined six phages from the original USA scheme with 10 virulent phages isolated from various sources in the United Kingdom, including pig and poultry manure and sewage effluent. The United States scheme was subsequently extended to form the

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Canadian scheme in which the original 14 phages were supplemented with an additional five phages isolated from chicken litter in Canada.

In this paper we describe the application of the UK phage typing scheme (16 phages) to the characterization of strains of *C. jejuni* and *C. coli* isolated in Wales between April 1996 and March 1997 from cases of human enteritis. In the present study defined phage types have been recognized and designated reference strains documented. The increased discrimination afforded by combining serotyping and phage typing, as is the current practice for reference typing in England and Wales, is demonstrated by use of the scheme to subtype within the predominant serotypes.

MATERIALS AND METHODS

Source of strains

A total of 2407 *C. jejuni* and 183 *C. coli* clinical isolates were examined for this study. These were isolated during the course of a pilot study for the PHLS Campylobacter Reference Unit (CRU) carried out between April 1996 and March 1997 in conjunction with Public Health Laboratory Service and NHS hospital laboratories in Wales. After typing, all isolates were archived at −80 °C in cryovials (Microbank; ProLab Diagnostics, Ontario, Canada). When required, these were recovered by serial sub-culture on Columbia blood agar plates (Oxoid CM331, Unipath, Basingstoke, UK) containing 5% horse blood, incubated at 37 °C in a Variable Atmosphere Incubator (VAIN) (Don Whitley Scientific Ltd, Shipley, West Yorkshire, UK) under microaerobic conditions (5% CO_2, 5% O_2, 3% H_2, 87% N_2).

Culture, identification and serotyping

Campylobacter isolates were referred to the Campylobacter Reference Unit in Amies Transport Medium (Oxoid CM425) as swabs or stabs. Prior to testing, these were subcultured onto Columbia blood agar with 5% horse blood, at 37 °C in a VAIN under microaerobic conditions (as above). Identification was confirmed using tests for microaerobic growth at 25 and 42 °C, oxidase and catalase production, and indoxyl acetate hydrolysis. *C. jejuni* and *C. coli* were differentiated on the basis of hippurate hydrolysis as described by Bolton and colleagues [9]. All isolates were serotyped using the direct agglutination method for heat stable antigens described by Frost and colleagues [5].

Source of phages and propagating strains

The phages and propagating strains for the UK phage typing scheme were obtained from the National Collection of Type Cultures (NCTC, PHLS, UK) (Table 1). Phages 4 and 12, which are not currently available from NCTC, were kindly supplied by Preston PHL (Public Health Laboratory, Royal Preston Hospital, PO Box 202, Preston, UK).

Phage propagation

The propagation method was a modification of the soft-agar overlay technique described by Sambrook and colleagues [10]. The propagating strains were subcultured onto Columbia blood agar for 18 h at 37 °C under microaerobic conditions and harvested into 5 ml volumes of Brain Heart Infusion broth supplemented with 10 mmol $MgSO_4$ and 1 mmol $CaCl_2$ (CBHI). Bacterial suspensions were adjusted to a cell density equivalent to MacFarland No. 1 (*c.* 10^8 c.f.u./ml) and incubated for 4 h at 37 °C. Equal 2·5 ml volumes of stock phage suspension, titrated to 10^5, 10^6 and 10^7 p.f.u./ml in sterile SM buffer (0·05 M Tris–Cl, pH 7·5, supplemented with NaCl (5·8 g/l), $MgSO_4 . 7H_2O$ (2·0 g/l) and gelatin (5 ml/l of 2% w/v solution, Sigma G-1393) [10], and the corresponding propagating strain suspension (Table 1) were mixed and incubated for 15 min at 37 °C to allow the phages to adsorb.

One ml aliquots of each adsorped suspension were added to 3 ml of NZCYM overlay agar [NZCYM (Sigma N-3643) broth with 0·7% Select Agar (Sigma A-5054)], tempered to 50 °C in a water bath and applied to the surface of NZCYM plates containing 1·2% Select Agar, as above. Overlays were allowed to set on a level surface and incubated for 15–18 h at 37 °C under microaerobic conditions. After incubation the propagation plates were examined and the degree of lysis recorded. Five ml of sterile SM buffer were added to the surface of each plate and the phages eluted on a gyratory shaker at 60 cycles/min for 15 h at 4 °C. The phage and cell suspensions were harvested and filtered through 0·2 μm membrane filters (Sartorius AG, Göttingen, Germany). Phage stocks were stored in SM buffer with the addition of 0·2% v/v chloroform (Sigma C-5312) at 4 °C.

Phage titration and preparation of Routine Test Dilutions (RTD)

Titration of phage suspensions was performed on agar overlay plates, prepared using Nutrient Broth

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Table 1. *Campylobacter typing phages and corresponding propagating strains*

Bacteriophages: reference numbers			Propagating strains: reference numbers/identities		
No.	NCTC	ATCC	No.	Species	NCTC
ϕ1	12673	35925-B2	PS 1	*C. jejuni*	12661
ϕ2	12674	35922-B2	PS 1	*C. jejuni*	12661
ϕ3	12682		PS 3	*C. coli*	12667
ϕ4	12676*	35920-B1	PS 4	*C. jejuni*	12663
ϕ5	12678		PS 5	*C. jejuni*	12664
ϕ6	12680	35924-B1	PS 6	*C. jejuni*	12665
ϕ7	12671		PS 7	*C. jejuni*	12660
ϕ8	12681		PS 8	*C. coli*	12666
ϕ9	12669		PS 9	*C. jejuni*	12658
ϕ10	12683		PS 10	*C. coli*	12668
ϕ11	12679	35922-B3	PS 5	*C. jejuni*	12664
ϕ12	12677*	35920-B2	PS 4	*C. jejuni*	12663
ϕ13	12672		PS 7	*C. jejuni*	12660
ϕ14	12675		PS 14	*C. jejuni*	12662
ϕ15	12684		PS 10	*C. coli*	12668
ϕ16	12670		PS 16	*C. jejuni*	12659

NCTC, National Collection of Type Cultures; ATCC, American Type Culture Collection.

Phages 4 and 12 are currently not available from NCTC. Preparations of these phages were obtained from Preston PHL – see text under 'Source of phages and propagating strains'.

(Oxoid CM 67) supplemented with 10 mmol $MgSO_4$, 1 mmol $CaCl_2$ and 0·7% agar (Oxoid L11) as the basal medium (CPTA), and CBHI containing 0·7% agar (Oxoid L11) as the overlay. Prior to inoculation, 6 ml volumes of overlay agar were melted in a steamer, and tempered in a 50 °C water bath.

For phage titration, 450 μl volumes of suspension of the appropriate propagating strain in CBHI at a cell density equivalent to MacFarland No. 1 were added to 6 ml of molten overlay agar, mixed and poured onto plates containing 25 ml CPTA on a level surface. After allowing to set, the inoculated plates were dried for 30 min at 37 °C. Serial dilutions from 10^{-1} to 10^{-12} of 20 μl of each undiluted phage suspension were prepared in 180 μl volumes of SM buffer in a microtitre plate, spotted onto the surface of the matching pre-dried CPTA overlay plate, allowed to dry and incubated for 18 h at 37 °C under microaerobic conditions. Plates were read and the number of plaque-forming units per ml (p.f.u./ml) of undiluted phage suspension calculated. The Routine Test Dilution (RTD) was determined as that dilution which produced just less than confluent lysis (< CL) of the propagating strain under these conditions. For each phage in the typing set, the RTD was equivalent to a phage concentration within the range $1{\cdot}0 \times 10^6$ to $1{\cdot}0 \times 10^7$ p.f.u./ml.

Phage typing

Test strains and a panel of control strains subcultured onto Columbia blood agar for 18 h at 37 °C under microaerobic conditions were inoculated into 5 ml volumes of CBHI to a cell density equivalent to MacFarland No. 1, and incubated for 4 h at 37 °C. Overlays of these suspensions were prepared as described above. The 16 typing phages at RTD were dispensed into the wells of a phage typing block in a 4×4 configuration and spotted onto the inoculated CPTA plates using the Lidwell phage typing apparatus [11]. Phage inocula were allowed to dry for *c.* 15 min at ambient temperature, before incubating the plates for 18 h at 37 °C under microaerobic conditions. The phage reactions were examined against a dark background using a 10× hand lens where necessary, and recorded as degrees of lysis using standard nomenclature, as follows: CL or OL (con-

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Table 2. *Phage typing scheme for* Campylobacter jejuni *and* C. coli*

Phage type (PT)	Reference strain number	ϕ1	ϕ2	ϕ3	ϕ4	ϕ5	ϕ6	ϕ7	ϕ8	ϕ9	ϕ10	ϕ11	ϕ12	ϕ13	ϕ14	ϕ15	ϕ16
1	C605	—	—	—	< OL	—	—	—	—	—	—	—	< OL	—	—	—	—
2	C682	—	—	SCL	OL	—	—	—	SCL	—	SCL	—	< OL	—	SCL	SCL	—
3	C4376	< CL	SCL	—	< OL	< CL	< CL	< CL	—	< CL	+ + +	< CL	< OL	< CL	+ +	SCL	< CL
4	C7566	< CL	< CL	+ +	< OL	< CL	< CL	< CL	—	< CL	—	< CL	SOL	< CL	±	—	CL
5	C856	—	—	—	SOL	SCL	—	SCL	—	SCL	—	—	< OL	SCL	—	—	—
6	C594	< CL	< CL	—	OL	CL	CL	—	—	—	—	CL	SOL	< CL	±	—	—
7	C10719	—	—	—	—	—	—	—	< CL	—	< CL	—	—	—	—	< CL	—
8	C10838	—	—	+ +	—	—	—	—	—	—	—	—	—	—	+ +	—	—
9	C7851	SCL	SCL	—	SOL	< SCL	SCL	< SCL	—	—	—	SCL	< OL	< SCL	±	—	—
10	C824	—	—	—	SOL	< CL	SCL	—	—	—	—	< CL	SOL	SCL	±	—	—
11	C8839	< CL	SCL	< SCL	SOL	< CL	SCL	< SCL	SCL	—	SCL	—	SOL	SCL	< SCL	< CL	< SCL
12	C7300	—	—	SCL	SOL	—	—	—	+ +	—	SCL	< SCL	—	SOL +	< SCL	SCL	—
13	C9120	SCL	SCL	+ + +	< OL	< CL	SCL	SCL	—	< CL	SCL	< CL	SOL	< CL	+ +	< CL	< CL
14	C10054	< CL	< CL	+ + +	SOL	< CL	< CL	CL	+ + +	SCL	< SCL	CL	SOL	< CL	< SCL	< SCL	CL
15	C10018	SCL	SCL	—	SOL	< CL	SCL	—	—	SCL	—	—	SOL	< SCL	—	—	—
16	C2939	< CL	< CL	+ +	< OL	< CL	< CL	SCL	—	< CL	—	—	SOL	< CL	—	—	< CL
17	C8859	—	—	< SCL	< OL	—	—	—	—	—	< SCL	—	SOL	—	< SCL	SCL	—
18	C9348	—	—	< SCL	SOL	—	—	—	< SCL	—	—	—	SOL	—	+ +	+	—
19	C11288	—	—	SCL	SOL	< CL	SCL	< CL	SCL	SCL	SCL	< CL	SOL	SCL	< SCL	< CL	CL
20	C10075	SCL	SCL	—	SOL	SCL	SCL	—	—	—	—	—	SOL	SCL	—	—	—
21	C978	—	—	—	SOL	—	—	—	—	—	—	SCL	SOL	—	—	—	—
22	C2822	—	—	—	SOL	+ +	—	+ +	—	SCL	—	—	SOL	±	—	—	—
23	C1873	—	—	—	SOL	< CL	SCL	—	—	SCL	—	—	SOL	—	—	—	—
24	C8652	—	—	+ + +	—	—	—	—	—	—	—	—	—	—	SCL	+ +	—
25	C10126	< CL	< CL	+ +	< OL	< CL	< CL	< CL	+ + +	< CL	—	< CL	SOL	< CL	+ +	—	< CL
26	C681	SCL	SCL	+ +	SOL	SCL	SCL	—	—	SCL	—	—	SOL	SCL	—	—	SCL
27	C10211	< SCL	< SCL	+ +	SOL	+ +	+ +	—	—	+ +	+ +	—	SOL	SCL	—	< SCL	SCL
28	C2971	< CL	< CL	+ +	< SOL	< SCL	< CL	SCL	+ +	SCL	< SCL	< CL	< SOL	< CL	+ +	+ +	—
29	C11759	—	—	—	SOL	—	—	—	—	—	—	—	SOL	—	—	+	—
30	C16121	+ +	+ +	+ + +	SOL	< SCL	SCL	+ +	SCL	—	+ + +	SCL	SOL	+	±	±	—
31	C12434	< CL	< CL	—	< OL	< CL	< CL	< CL	—	SCL	—	< CL	< OL	< CL	+ +	—	SOL + +
32	C8820	—	—	+ +	—	—	—	—	SCL	—	< CL	—	—	—	—	CL	—
33	C1312	< CL	< CL	—	< OL	CL	< CL	< CL	—	< CL	—	< CL	< OL	CL	—	—	SCL
34	C13503	—	—	—	SOL	SCL	—	SCL	—	SCL	—	—	SOL	SCL	—	—	< CL
35	C13553	< CL	< CL	—	SOL	SCL	< CL	< CL	—	SCL	—	—	SOL	< CL	—	—	SCL
36	C1758	< CL	< CL	—	SOL	SCL	< CL	SCL	—	SCL	—	—	SOL	CL	—	—	—
37	C10567	SCL	SCL	+ + +	SOL	< CL	< CL	—	SCL	< CL	< SCL	—	SOL	SCL	+ +	< SCL	< CL
38	C1865	SCL	SCL	—	< OL	SCL	< CL	—	—	SCL	—	—	< OL	SCL	—	—	SCL

PT	Ref. no.	ϕ1	ϕ2	ϕ3	ϕ4	ϕ5	ϕ6	ϕ7	ϕ8	ϕ9	ϕ10	ϕ11	ϕ12	ϕ13	ϕ14	ϕ15	ϕ16
39	C1657	—	—	SCL	SOL	—	—	—	SCL	—	—	—	SOL	—	SCL	—	—
40	C9085	< CL	< CL	—	SOL	++	SCL	SCL	—	++	—	< CL	< SOL	< CL	±	—	—
41	C2055	—	—	—	SOL	< CL	SCL	—	—	SCL	—	—	< OL	SCL	—	—	—
42	C9759	—	—	—	< SOL	—	—	—	—	++	—	—	< SOL	—	—	—	SCL
43	C7849	—	—	—	< SOL	+	—	—	—	SCL	—	—	< SOL	< SCL	—	—	—
44	C10131	—	—	—	< OL	—	—	—	—	—	SCL	—	< OL	—	—	SCL	—
45	C2062	—	—	—	SOL	—	—	—	—	—	—	—	—	—	—	—	< CL
46	C5357	—	—	< SCL	SOL	< CL	SCL	SCL	< SCL	SCL	< SCL	< CL	SOL	< SCL	< SCL	< SCL	—
47	C2321	< CL	< CL	SCL	< OL	—	++	—	SCL	< SCL	< CL	SCL	—	< SCL	< CL	< CL	SCL
48	C10855	< CL	< CL	—	SOL	++	SCL	—	—	SCL	—	< CL	< SOL	< CL	±	—	—
49	C8076	—	—	—	SOL	—	—	—	—	< SCL	—	—	SOL	< SCL	—	—	—
50	C12610	—	—	—	< SOL	< SCL	—	SCL	—	< SCL	—	SCL	< SOL	< SCL	±	—	—
51	C9939	—	—	—	SOL	+	+	—	—	—	—	—	SOL	< SCL	—	—	—
52	C10055	—	—	—	< OL	< CL	< SCL	—	—	< SCL	—	—	SOL	SOL++	—	—	< CL
53	C2364	—	—	SCL	SOL	SCL	SCL	SCL	SCL	SCL	SCL	—	SOL	SCL	SCL	SCL	SCL
54	C2914	< CL	< CL	—	< OL	< CL	< CL	—	—	< CL	SCL	—	< OL	SCL	—	SCL	< CL
55	C2796	< SCL	SCL	—	< OL	< CL	SCL	—	+	SCL	++	< CL	SOL	SCL	—	++	—
56	C16044	+	++	SCL	< OL	< SCL	< SCL	++	< CL	—	SCL	< CL	< OL	+	+	++	—
57	C7376	—	—	—	SOL	—	—	—	—	—	—	—	SOL	< SCL	—	—	—
58	C16928	—	—	—	< SOL	+	—	—	—	SCL	—	—	SOL	< SCL	—	—	SCL
59	C9918	—	—	—	< SOL	—	—	—	—	< SCL	—	—	SOL	—	—	—	—
60	C9734	—	—	—	—	+++	—	—	—	—	—	—	SOL	—	—	—	—
61	C7593	—	—	—	< SOL	SCL	—	< CL	—	++	—	< CL	SOL	< SCL	±	—	< CL
62	C5181	—	—	—	SOL	< CL	CL	CL	—	CL	—	CL	< OL	SCL	±	—	CL
63	C5720	—	—	—	< OL	CL	SCL	SCL	—	SCL	—	—	< OL	++	—	—	—
64	C6779	—	—	—	SOL	SCL	SCL	SCL	—	SCL	—	SCL	< OL	++	—	—	—
65	C16965	< CL	SCL	< SCL	SOL	< CL	< CL	SCL	< CL	SCL	SCL	—	SOL	< CL	SCL	< CL	< CL
66	C15739	+++	+++	—	OL	< CL	SCL	< CL	—	< CL	SCL	—	SOL	< CL	—	SCL	SCL
67	C5395	—	—	—	SOL	++	++	—	—	—	—	—	SOL	—	—	—	—
68	C4841	—	—	SCL	SOL	< SCL	—	SCL	SCL	SCL	SCL	—	< OL	SCL	SCL	SCL	< CL
69	C5121	—	—	—	SOL	—	—	—	—	—	—	++	—	—	—	—	+++
70	C5196	SCL	SCL	—	SOL	< CL	SCL	—	—	SCL	+++	—	< OL	SCL	—	< SCL	—
71	C4197	SCL	SCL	—	SOL	SCL	< SCL	< SCL	—	SCL	< SCL	SCL	< SOL	SCL	—	< SCL	< SCL
72	C6769	< CL	< CL	++	< OL	< CL	< CL	—	++	—	—	< CL	OL	< CL	±	—	—
73	C16941	—	—	—	SOL	< CL	SCL	—	—	—	—	SCL	SOL	—	—	—	—
74	C14417	—	—	—	SOL	++	—	—	—	—	—	—	SOL	—	—	—	< CL
75	C15499	—	—	—	SOL	< CL	< SCL	SCL	—	SCL	—	—	< OL	SCL	—	—	< SCL
76	C17042	—	—	++	SOL	< CL	SCL	< CL	—	SCL	< SCL	< CL	SOL	< SCL	< SCL	< SCL	< CL

* Typing phages at routine test dilution (RTD) showing lytic reaction patterns against type strains. For key to abbreviations see text under 'Phage typing'.

Table 3. *Phage type distribution among* C. jejuni *strains isolated in Wales, April 1996–March 1997*

Phage type	No.	%	Phage type	No.	%	Phage type	No.	%
PT 1	471	19·6	PT 38	31	1·3	PT 50	7	0·3
PT 2	216	9·0	PT 49	29	1·2	PT 54	7	0·3
PT 33	179	7·4	PT 44	27	1·1	PT 62	7	0·3
PT 5	152	6·3	PT 20	24	1·0	PT 58	6	0·2
PT 34	108	4·5	PT 18	23	1·0	PT 21	5	0·2
PT 7	98	4·1	PT 14	21	0·9	PT 41	5	0·2
PT 35	63	2·6	PT 36	20	0·8	PT 61	5	0·2
PT 6	59	2·5	PT 4	19	0·8	PT 3	4	0·2
PT 8	45	1·9	PT 43	15	0·6	PT 9	4	0·2
PT 19	38	1·6	PT 17	13	0·5	PT 28	4	0·2
			PT 15	12	0·5	PT 51	4	0·2
			PT 24	12	0·5	PT 55	4	0·2
Top 10	1429	59·4	PT 48	12	0·5	PT 56	4	0·2
RDNC*	160	7·0	PT 37	11	0·5	PT 59	4	0·2
UT†	371	15·4	PT 39	11	0·5	PT 60	4	0·2
			PT 45	11	0·5	PT 16	3	0·1
			PT 46	10	0·4	PT 52	3	0·1
			PT 40	10	0·4	PT 22	2	
			PT 10	9	0·4	PT 47	2	
			PT 53	8	0·3	PT 63	2	
			PT 57	8	0·3	PT 26	1	
			PT 23	7	0·3	PT 29	1	
			PT 32	7	0·3	PT 64	1	
			PT 42	7	0·3			
						Total	2407	

* Reacts with phages but does not conform to a designated phage type; † Untypable.

fluent clear or opaque lysis), < CL or < OL (less than confluent), SCL or SOL (semi-confluent), < SCL or < SOL (less than SCL, but plaques not discrete). For lower readings exhibiting discrete plaque reactions the estimated number of plaque-forming units (p.f.u.) was recorded as +++ (> 100 discrete plaques), ++ (50–99 discrete plaques), + (20–49 discrete plaques), ± (1–19 discrete plaques). The absence of lysis was recorded as negative (−). Readings of + or above were regarded as positive.

RESULTS

Phage type definition

A phage type was defined as two or more epidemiologically unrelated isolates giving the same phage reaction pattern, and by this criterion 76 phage types have been defined to date (Table 2). Reference strains for these types are maintained in the CRU culture collection. While most reactions were reproducible on repeat testing, including after storage at −80 °C, repeat testing of pairs of strains differing only by reactions with phages 4 and/or 12 indicated that the reactions exhibited by these two phages were difficult to reproduce, and notably sensitive to variation when

Table 4. *Phage type distribution among* C. coli *strains isolated in Wales, April 1996–March 1997*

Phage type	No.	%	Phage type	No.	%
PT 2	94	51·6	PT 5	1	0·5
PT 7	43	23·6	PT 8	1	0·5
PT 44	9	4·9	PT 14	1	0·5
PT 17	6	3·2	PT 32	1	0·5
PT 1	5	2·7	PT 33	1	0·5
PT 39	4	2·2	PT 64	1	0·5
			RDNC*	5	2·7
			UT†	11	6·0
			Total	183	

* Reacts with phages but does not conform to a designated phage type.
† Untypable.

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Table 5. *Relationship between serotype and phage type for the top 20* C. jejuni *HS types*

			Phage types (% of total isolates in each of the top 10 phage types, other PTs, RDNCs and UT)												
Serotype	No. isolates	Total no. PTs	PT1	PT2	PT33	PT5	PT34	PT7	PT35	PT6	PT8	PT19	Other PTs	RDNC*	UT†
HS 50	245	18	10		< 1	28	27		2	7			20	2	4
HS 2	271	29	21		20	< 1	< 1	< 1	10	< 1			29	3	13
HS 44	183	28	18	5	15	< 1		3	7	1		5	23	8	14
HS 11	161	13	12	16	< 1	< 1		18	< 1		5		16	2	30
HS 16	98	21	11	7	3	14	8			3	1	1	20	7	24
HS 13	95	23	14		13	4	3	5	2	6		5	22	8	17
HS 6	82	8	28	37							1		23	2	9
HS 37	81	7	40	4				2					15	26	14
HS 21	71	8	6	10				1			34		14		35
HS 5	70	13	23	1	4		1	30	3	1			12	7	17
HS 1	61	17	10		20		2	3	2			13	36	13	2
HS 14	54	12	17	7	13	17	4						17	9	17
HS 8	46	12	24		18				2			9	38	7	2
HS 18	46	12	12	23	2		5	7			2		28	12	9
HS 12	41	5	7	73				7					7		5
HS 9	40	7	10	25							3	3	10	20	30
HS 35	37	12	34	3	9	11	3		3			3	14	3	17
HS 59	34	7	3			35	18			24			9	3	9
HS 15	33	6	42	3		3		6					6		39
HS 29	31	6	58		13				6			6	3		13
Rough	41	9	33		8			5	5	5			8	13	23
UT†	467	37	22	11	7	7	3	4	1	4	< 1	2	17	7	15

* Reacts with phages but does not conform to a designated phage type.
† Untypable.

subjected to slight changes in growth conditions. This was taken into account in the definition of phage types by allowing variation in readings with either or both of these two phages within a type definition.

A unique phage reaction pattern or one seen only in closely related isolates, was designated RDNC (reacts with the phages but does not conform to a designated phage type). A proportion of isolates did not react with any of the phages, and these were designated untypable (UT).

Distribution of phage types among *C. jejuni* and *C. coli* isolated in Wales between April 1996 and March 1997

Fifty-seven phage types were identified among the 2407 *C. jejuni* typed (Table 3). Three hundred and seventy-one isolates (15·4%) were untypable and a further 160 (7·0%) were designated RDNC. Most strains in this last category had unique phage reaction patterns. One thousand four hundred and twenty-nine isolates were assigned to the top 10 phage types (59·4%) and of the 57 phage types identified, only 15 each accounted for 1% or more of the total isolates.

The most common phage type for *C. jejuni* was PT 1 (471 isolates, 19·6% of the total), a type defined on the basis of reactions to phages 4 and 12 (see 'Phage type definition', above). Only 12 phage types were identified in *C. coli* (Table 4) and two of these, PT 2 and PT 7 – together accounted for 75·2% of isolates tested. Six phage types were each represented by a single isolate. Eleven *C. coli* (6·0%) were untypable and 5 (2·7%) RDNC.

Relationship between serotype and phage type for predominant *C. jejuni* serotypes

Phage typing can be used to subdivide the predominant serotypes so that use of the two schemes together provides a more discriminatory typing strategy than serotyping alone. A total of 336 different serotype/phage type combinations were identified among the 2407 *C. jejuni* isolates in the pilot study [5]. Phage typing of isolates belonging to the 20 most common serotypes in the study showed that each HS type could be divided into between 6 and 29 phage types (Table 5).

Within some serotypes one or two phage types predominated and these groupings may indicate a closer epidemiological relationship. For example, very little phage type variation was found among strains of serotype HS 12. Of the 41 *C. jejuni* HS 12 isolates identified in this study, 73% were phage type PT 2. In contrast, the two most common serotypes could each be divided into 29 (HS 2) and 18 (HS 50) phage types.

The majority (85%) of isolates that were untypable by serotyping reacted with the phages and were widely distributed between 37 different phage types (Table 5). Only 69 (2·9%) *C. jejuni* isolates were untypable by both serotyping and phage typing.

Contribution of phage typing to outbreak investigation

In February 1997, the Environmental Health Department (EHD) in Cardiff was alerted to a possible campylobacter outbreak associated with a restaurant [12]. All five of the culture-positive cases were infected with *C. jejuni* HS 50, PT 49 and follow-up of patients infected with the same sero/phage type identified a further patient who had eaten at the same restaurant 2 days previously. Statistical analysis showed a significant association between illness and consumption of unmarinated stir-fried chicken [12].

Since March 1997, subtyping of *C. jejuni* isolates has been used in the investigation of over 20 outbreaks or local clusters of campylobacter infection and details of these epidemiological studies will be published separately.

DISCUSSION

All of the published campylobacter phage typing schemes are closely related and based on all or part of the original phage set of Grajewski and colleagues [6]. The phages that comprise the Salama scheme [7] are a combination of 6 of those from the Grajewski scheme [6] and 10 phages isolated in the UK. In this study we describe the application of this phage typing scheme, together with serotyping, to a sample of 2590 isolates of *C. jejuni* and *C. coli* referred by laboratories in Wales over a 12-month period. The scheme has subsequently been used for routine typing in the Reference Unit and a total of 76 phage types, with reference strains, has now been defined (Table 2). The phages are available in the UK National Collection of Type Cultures and type strains can be made available, enabling standardization of the method across laboratories.

The predominant phage type in *C. jejuni* was PT1, a type defined on the basis of sensitivity only to phages 4 and 12. Reactions with these two phages are difficult to reproduce and the phages themselves have atypical morphological features [13]. While for ease of

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use it would beV tempting to remove these phages from the scheme, this would increase the proportion of untypable isolates to 35% thus reducing the value of the scheme. Further extension of the scheme by addition of new phages would improve discrimination and potentially address the problems associated with phages 4 and 12.

For *C. jejuni* and *C. coli*, the primary reference typing method is serotyping [5] and in the present study the top 10 serotypes accounted for 53% of isolates. Phage typing enabled the subdivision of each of these serotypes into at least six subtypes, the number of types reflecting variation within serotypes. A number of phage types was identified in both *C. jejuni* and *C. coli*, although the frequency distributions differed in the two species. The predominant serotype in *C. jejuni*, HS 50, was subdivided into 18 phage types and the epidemiological value of this further subtyping has been demonstrated in outbreak investigations one of which is cited here [12]. Phage typing of those isolates which are at present untypable by serotyping demonstrated a wide range of variation with 37 different subtypes identified by phage type.

Given that there are to date 66 serotypes and 76 defined phage types in the two schemes, a theoretical total of 5016 different sero/phage type combinations would be possible if the two characteristics were unrelated and there were no relationships among the isolates tested. In this study, 336 sero/phage type combinations were identified among a sample of only 2407 *C. jejuni* isolates. This hierarchical approach to subtyping gives a level of fine discrimination for epidemiological studies and reinforces the observation that *C. jejuni* and *C. coli* demonstrate a wide range of variation although some prevalent subtypes can be clearly identified. When these two typing methods are used in combination 97% of *C. jejuni* can be assigned to a recognized type.

A combination of phenotypic and/or genotypic typing methods is advised for epidemiological studies [2] and the choice of methods must be appropriate for the scale of the problem being investigated. For campylobacter, with over 50000 infections reported annually [1], the use of phage typing in conjunction with serotyping facilitates the screening of large numbers of isolates and has been demonstrated to produce both a high level of discrimination and epidemiologically valid data. The use of internationally agreed typing schemes would facilitate a greater understanding of the global epidemiology of campylobacter infections.

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