Extended phage-typing scheme for *Campylobacter jejuni* and *Campylobacter coli*

R. KHAKHRIA AND H. LIOR

National Reference Service for Campylobacters, National Laboratory for Enteric Pathogens, Laboratory Centre for Disease Control, Ottawa, Ontario KIA 0L2, Canada

SUMMARY

The extended phage-typing scheme described for *Campylobacter jejuni* and *Campylobacter coli* has established 46 different phage types using 19 typing phages. Altogether 754 campylobacter isolates, 672 *C. jejuni* and 82 *C. coli*, isolated from human and non-human sources received from 17 different countries were phage-typed. Overall, 80·6% of the total isolates were typable. Among typable strains, 9 phage types (3, 5, 10, 11, 18, 19, 23, 26 and 44) represented 57·0% of the strains, 21·3% of the strains belonged to another 37 phage types and the remaining 2·3% of isolates were designated atypical. The most common phage type 11 (140/754) was frequently observed among *C. jejuni* isolates from human (113/561) and non-human sources (18/111), whereas type 44 was frequent among *C. coli* isolates from human (22/59) and from non-human sources (8/23). A study of the animal host-associations of common phage types showed that contaminated cattle and poultry appear to be the most common sources of human infection. The greatest variety of phage types was observed in Canada (24 phage types), followed by Portugal (17 types) and the UK (14 types), reflecting the larger sample sizes from these countries. Phage type 11 was encountered in 12 different countries and prevalence of other phage types varied from one country to another. The number of isolates typable with the scheme varied from 93·2% (261/280) in Canada to 61% (47/77) in Thailand. However, the number and diversity of phage types makes phage typing the method of choice in epidemiological studies of campylobacter infections.

INTRODUCTION

Campylobacter jejuni and *Campylobacter coli* have now been well established as frequent causes of human enteritis [1, 2]. Animal sources have been the main reservoirs for strains infecting humans [2]. This has emphasized the need for differentiation for epidemiological purposes of outbreak isolates and determination of the possible role that non-human sources play in human infections. Serotyping [3] and biotyping schemes [4] are now well established and used by various investigators to study the epidemiology of campylobacter enteritis [5–7]. Grajewski and colleagues [8] reported a phage-typing scheme designed to provide epidemiological markers for campylobacter strains. Another phage-typing scheme was developed in the UK using six of the Grajewski phages together with an

additional ten phages [9, 10]. Grajewski and colleagues' phage-typing scheme was evaluated in our laboratory [11, 12] and 24 provisional phage types were reported after studying a total of 301 *C. jejuni* isolates from human and non-human sources.

The purpose of this study was to extend the phage-typing scheme by incorporating new phages isolated in Canada and to determine the typability of *C. jejuni* and *C. coli* strains from various countries.

MATERIALS AND METHODS

Bacterial cultures

A total of 754 campylobacter cultures, 672 *C. jejuni* and 82 *C. coli*, received from 17 different countries were phage-typed (Table 5). Of these, 620, 561 *C. jejuni* and 59 *C. coli*, were isolated from human sources and the remaining 134 strains, 111 *C. jejuni* and 23 *C. coli*, were isolated from non-human sources (Tables 3 and 4). In addition 66 campylobacter isolates belonging to other species, *C. fetus* subsp. *fetus*; 'C. lari'; *C. upsaliensis*; 'Arcobacter cryaerophilus'. 'A. butzleri', were also investigated with the phage-typing scheme (Table 2).

Growth conditions

All strains were cultured on Mueller-Hinton Agar (Oxoid Ltd. London. UK) containing 5% sheep blood. The *C. jejuni, C. coli* and *C. lari* strains were incubated for 24 h at 43 °C, under a gas mixture of 5% O_2, 10% CO_2, 85% N_2 in anaerobic jars without catalyst, whereas the remaining *Campylobacter* species were incubated for 24–48 h at 37 °C under the same microaerophilic condition.

Media

Brucella broth (Gibco, USA) dispensed in 4·5 ml volumes was used for preparation of bacterial or phage suspensions. The base agar plates were prepared by adding 1·3% phage agar (Difco Laboratories, USA) to Brucella broth. The 4 ml soft agar overlay contained 0·6% phage agar in Brucella broth. All media contained 0·01 M-$MgSO_4$, 0·001 M-$CaCl_2$ and 0·005 M-$MgCl_2$ and the final pH was adjusted to 6·8.

Phages

Fourteen phages and their propagating strains were received from B. Grajewski (NIOSH. Cincinnati, OH, 45226, USA) and J. Bryner (USDA Ames. IA. 50010. USA). Phages 2–6 were stabilized by using a suitable intermediate host for propagation according to the method of Callow [13].

Isolation of phages

A total of 141 samples of faecal materials (chickens, 97: sheep, 7: monkey, 37) was screened with 65 *C. jejuni* strains for phage isolation using the Grajewski method [8]. Altogether 27 phages were isolated from chicken faeces. Of these, five phages were selected on the basis of host-range specificity and reproducibility and others were discarded because of instability or our inability to obtain adequate titres. The selected five phages were then grown in bulk by the standard agar layer

technique on their specific propagating strains [14], filtered by using low protein binding 0·22 μm cellulose acetate filters (Gelman, USA), titrated and stored at 4 °C. The selected phages were numbered (15, 16, 17, 18 and 19) and added to the existing 14 phages (Table 1).

Phage-typing technique

The technique used was basically the same as described by Grajewski and colleagues [8] with slight modifications as follows: a freshly grown 24 h culture was inoculated into 4·5 ml of Brucella broth and incubated for 5–6 h at 43 °C under microaerophilic conditions. 0·75 ml of broth culture (McFarland Standard 1) was added to 4 ml of melted Brucella soft agar and poured over a basal agar plate and allowed to dry for 15 min. The phages at their Routine Test Dilution (RTD) were spotted onto the agar surface by use of a multi-syringe phage applicator [15]. The plate was incubated in a moist chamber for 18–24 h at 43 °C in duplicate. The combined positive lytic reactions were recorded and the extended scheme was set up according to the established method of recording degrees of lysis on phage-typing plates [16]. Strains showing a pattern that did not conform to any recognized phage types were considered atypical (AT). Strains which were not lysed by any of the typing phages were considered untypable (UT).

RESULTS

The extended phage-typing scheme for *C. jejuni* and *C. coli* showed 46 different phage types established using 19 typing phages (Table 1) Designated reference type strains were representative of nine different countries and were lysed by one or more of the typing phages. The typing phages in the scheme were specific for strains of *C. jejuni* in the range of 80·8% (543/672) and 79·3% (65/82) for *C. coli* isolates (Table 2) investigated to date. Strains of the other *Campylobacter* species did not show any lysis with the phages.

Frequency of phage types of C. jejuni *and* C. coli *from all sources*

A total of 754 isolates, 672 *C. jejuni* and 82 *C. coli*, from all sources were phage-typed using the extended phage-typing scheme, as shown in Tables 3 and 4. Overall, 80·6% (608/754) of the isolates were typable and 19·4% (146/754) of the strains remained UT. Among typable strains, 9 phage types (3, 5, 10, 11, 18, 19, 23, 26 and 44), each comprising 20 or more isolates, were encountered frequently and represented 57% (430 isolates) of the total strains. 21·3% (161 strains) belonged to another 37 phage types in different frequencies. The remaining 2·3% (17 isolates) of the typable strains from all sources gave AT patterns. A phage-type designation would be assigned if these AT strains were to give a consistently reproducible and clearly recognizable lytic pattern, and/or became epidemiologically significant.

Among human isolates, 44 different phage types were identified among 561 *C. jejuni* strains and 12 phage types among 59 *C. coli* strains (Table 3). Phage type 11 (113/561) is common among *C. jejuni* strains and type 44 (22/59) among *C. coli* strains.

A total of 15 different phage types were identified among 111 *C. jejuni* and 23 *C. coli* isolated from non-human sources (Tables 3 and 4). Phage type 11 (18/111)

Table 1. *Extended phage-typing scheme for* Campylobacter jejuni *and* Campylobacter coli *strains*

Phage type	1	2	3	4	5	6	7	8	9	10	Country of origin of type strains
				Typing pages in routine test dilution showing lytic patterns with type strains							
1	SCL	≪OL	<OL	CL	+++	<OL	CL	CL	CL	SCL	UK
2	SCL	–	–	–	–	–	+++	+++	–	SCL	USA
3	–	<OL	OL	OL	≪OL	OL	CL	CL	OL	CL	Canada
4	–	<OL	<OL	SCL	++	SCL	SCL	–	SCL	+++	UK
5	–	<OL	<OL	SCL	<OL	OL	SCL	+++	CL	SCL	Canada
6	–	<OL	<OL	<OL	<OL	<OL	+++	+++	–	–	Canada
7	–	SCL	SCL	SCL	SCL	<OL	CL	CL	–	–	UK
8	–	SCL	<OL	<OL	SCL	<OL	SCL	SCL	–	–	UK
9	–	<OL	++	<OL	≪OL	<OL	–	–	SCL	SCL	UK
10	–	++	<OL	OL	+++	<OL	SCL	SCL	<OL	SCL	UK
11	–	≪OL	OL	OL	≪OL	OL	–	–	–	–	Canada
12	–	≪OL	<OL	<OL	+++	OL	SCL	SCL	–	–	Peru
13	–	++	≪OL	<OL	++	≪OL	SCL	+++	–	–	USA
14	–	–	+++	<OL	+++	<OL	–	+++	+++	+++	Canada
15	–	–	++	++	–	<OL	–	–	SCL	+	UK
16	–	–	–	<OL	–	<OL	–	<OL	–	–	Canada
17	–	–	–	–	+++	OL	–	–	–	–	UK
18	–	–	–	–	–	OL	–	–	–	–	UK
19	–	–	–	–	–	–	CL	+++	+++	SCL	Chile
20	–	–	++	<OL	–	<OL	SCL	SCL	<OL	<OL	Canada
21	–	–	–	–	–	–	SCL	SCL	+++	SCL	UK
22	–	–	–	–	–	–	SCL	SCL	SCL	–	Canada
23	–	–	–	–	–	–	–	–	<SCL	–	Canada
24	–	–	–	–	–	–	–	–	–	–	Canada
25	–	<OL	OL	OL	≪OL	OL	CL	SCL	CL	CL	Canada
26	–	≪OL	OL	OL	≪OL	OL	–	–	–	CL	China
27	–	–	+++	<OL	–	SCL	–	–	–	–	Canada

Table 1. (*cont.*)

Phage type	Typing pages in routine test dilution showing lytic patterns with type strains									Country of origin of type strains
	11	12	13	14	15	16	17	18	19	
1	SCL	CL	CL	CL	—	CL	—	CL	—	UK
2	SCL	SCL	SCL	SCL	—	< SCL	—	CL	—	USA
3	SCL	CL	CL	CL	CL	SCL	CL	OL	—	Canada
4	SCL	SCL	SCL	SCL	—	SCL	—	SCL	—	UK
5	SCL	SCL	SCL	SCL	—	+ + + / +	—	CL	—	Canada
6	—	+ + / +	—	—	—	+	—	—	—	Canada
7	—	—	SCL	SCL	—	CL	—	—	—	UK
8	—	+ + / +	—	+ + +	—	SCL	—	—	—	UK
9	SCL	SCL	SCL	SCL	CL	—	—	≪ OL	—	UK
10	SCL	SCL	—	—	—	SCL	SCL	< SCL	—	UK
11	—	—	—	—	—	—	—	—	—	Canada
12	—	—	SCL	SCL	SCL	—	SCL	—	—	Peru
13	+ + / +	+ + / +	+ + / +	—	—	+ + + / +	≪ SCL	—	—	USA
14	+ + / +	+ + / +	SCL	SCL	—	SCL	—	CL	—	Canada
15	+ + / +	+ + / +	+ + / +	+ + + / +	—	SCL	—	SCL	—	UK
16	—	SCL	—	—	—	—	—	—	—	Canada
17	—	—	—	—	—	—	—	—	—	UK
18	—	—	—	—	—	—	—	—	—	UK
19	SCL	SCL	SCL	SCL	—	CL	—	< SCL	—	Chile
20	SCL	SCL	+ + / +	+ + + / +	—	CL	—	CL	—	Canada
21	SCL	SCL	—	—	—	—	—	≪ OL	—	UK
22	+ + / +	SCL / +	SCL	SCL	—	SCL	—	SCL	—	Canada
23	+ + / +	+ + +	SCL	SCL	—	+ +	—	OL	—	Canada
24	—	—	—	—	—	—	—	—	—	Canada
25	SCL	CL	CL	CL	CL	OL	—	OL	—	Canada
26	—	—	—	—	—	—	CL	—	—	China
27	—	—	—	—	—	—	—	—	—	Canada

Table 1. (cont.)

Typing pages in routine test dilution showing lytic patterns with type strains

Phage type	1	2	3	4	5	6	7	8	9	10	Country of origin of type strains
28	–	–	–	–	–	–	–	–	+++	SCL	China
29	–	–	–	–	–	–	–	–	–	–	Portugal
30	–	–	–	–	–	–	«OL	«OL	–	–	Portugal
31	–	–	«OL	«OL	«OL	–	–	–	–	–	China
32	–	–	–	–	–	–	SCL	–	–	–	China
33	–	«OL	<OL	<OL	«OL	<OL	–	–	–	«OL	China
34	–	–	–	–	–	–	–	–	<SCL	OL	Guatemala
35	–	OL	OL	OL	OL	OL	–	–	–	OL	Guatemala
36	–	–	+	+	–	–	–	–	–	–	Guatemala
37	–	–	–	–	–	–	–	–	–	–	Guatemala
38	–	–	–	–	–	–	–	–	–	–	Thailand
39	–	–	–	–	–	–	–	–	–	–	China
40	–	–	–	–	–	–	–	–	–	–	Portugal
41	–	–	–	–	–	–	–	–	–	–	USA
42	–	–	–	–	–	–	–	–	–	–	Portugal
43	–	–	–	–	–	–	–	–	–	–	Peru
44	–	–	–	–	–	–	–	–	–	–	Thailand
45	–	–	–	–	–	–	–	–	–	–	Portugal
46	–	–	–	–	–	–	–	–	–	–	Canada
UT*	–	–	–	–	–	–	–	–	–	–	Thailand

* Untypable.

Degree of lysis: CL or OL, confluent or opaque lysis; SCL, semiconfluent lysis; ++ to +++, increasing numbers of discrete plaques (40 plaques onwards); –, no reaction to less than 40 plaques; SCL, a possible range of reactions between semi-confluent lysis to few plaques.

Table 1. (*cont.*)

Phage type	Typing pages in routine test dilution showing lytic patterns with type strains									Country of origin of type strains
	11	12	13	14	15	16	17	18	19	
28	SCL	SCL	—	—	—	—	—	≪OL	—	China
29	—	—	OL	OL	—	OL	—	—	—	Portugal
30	—	—	—	—	—	—	—	—	—	Portugal
31	—	—	—	—	—	—	—	—	—	China
32	—	—	—	—	—	—	—	—	—	China
33	—	—	—	—	—	—	—	—	—	China
34	OL	OL	OL	OL	—	—	—	CL	—	Guatemala
35	—	—	—	—	SCL	SCL	SCL	—	—	Guatemala
36	CL	CL	CL	CL	CL	—	—	CL	—	Guatemala
37	—	—	—	—	CL	—	—	—	—	Thailand
38	—	—	—	—	—	CL	—	—	—	China
39	—	—	—	—	—	—	CL	—	—	Portugal
40	—	—	—	—	—	—	—	CL	—	USA
41	—	—	—	—	—	—	—	—	OL	Portugal
42	—	—	—	—	+++	+++	CL	—	—	Peru
43	—	—	—	—	CL	—	CL	CL	—	Thailand
44	—	—	—	—	CL	—	CL	—	—	Portugal
45	—	—	—	—	CL	—	—	CL	—	Canada
46	—	—	—	—	—	OL	—	OL	—	Thailand
UT*	—	—	—	—	—	—	—	—	—	

* Untypable.

Degree of lysis: CL or OL, confluent or opaque lysis; SCL, semiconfluent lysis; ++ to +++, increasing numbers of discrete plaques (40 plaques onwards); —, no reaction to less than 40 plaques; SCL, a possible range of reactions between semi-confluent lysis to few plaques.

Table 2. *Typability of* Campylobacter *species with the extended phage-typing scheme for* C. jejuni *and* C. coli

Species	No. of isolates	No. of typable cultures	No. of untypable cultures
C. jejuni	672	543	129
C. coli	82	65	17
C. lari	10	—*	—
C. fetus subsp. *fetus*	5	—	—
'*A. butzleri*'	22	—	—
C. upsaliensis	25	—	—
'*A. cryaerophilus*'	4	—	—

* No lysis.

was also common among *C. jejuni* strains and type 44 (8/23) among *C. coli* isolates from non-human sources.

Of the 62 bovine isolates studied, 59 *C. jejuni* and 3 *C. coli*, 82·2% (51) were typable and belonged to 7 different phage types (Table 4). The phage types 11 and 23 were common. Of 46 poultry isolates, 38 *C. jejuni* and 8 *C. coli*, 76% (35) were typable and belonged to 8 different types; phage type 18 was the most common, whereas phage type 11 and 31 were common among 8 isolates of *C. coli* isolated from porcine (Table 4).

Phage types of C. jejuni *and* C. coli *from various countries*

The geographical distribution of *C. jejuni* and *C. coli* phage types is presented in Table 5. The greatest variety of phage types was observed in Canada (24 phage types), followed by Portugal (17 types) and UK (14 types), reflecting the larger sample size from these countries. The predominant phage type 11 was encountered in 12 different countries.

DISCUSSION

Grajewski and colleagues [8], on isolating the phages, standardized the phage-typing scheme and used it as an epidemiological marker for campylobacter infection caused by *C. jejuni* or *C. coli* strains. The scheme is based on converting the lytic patterns to the mnemonic system of Farmer [15] and reported 77 phage patterns obtained with 14 typing phages. The scheme was used to phage-type 95·8% of the *C. jejuni* and 60·0% of the *C. coli* strains from Illinois (USA). However, on studying an international set of 51 isolates, the scheme phage-typed 88·1% of *C. jejuni* isolates and 44·4% of *C. coli* isolates, thus indicating a need for the addition of phages from a wider geographical area to improve the typability of *C. jejuni* and *C. coli* isolates from different countries.

In our present study, the addition of five phages, 15 to 19, to Grajewski's 14 typing set allowed us to add 10 new phage types, 36 to 46, and subdivide the two most common phage types, 3 into 3 and 25, and 11 into 11 and 26 (Table 1). Furthermore, 61 isolates, 27 *C. jejuni* and 34 *C. coli*, belonged to the new types (Table 3) and were isolated in 7 countries (Table 5). Thus, the extended phage-typing scheme has improved performance for *C. jejuni* and *C. coli* isolates by increasing the typability from 75·8% (516/672) to 80·8% (543/672) for *C. jejuni* strains and from 37·8% (31/82) to 79·3% (65/82) for *C. coli* isolates. It has also

Table 3. *Frequency of phage types of* C. jejuni *and* C. coli *strains isolated from human and non-human sources*

Phage type	No. C. jejuni isolates		No. C. coli isolates		Total no. isolates
	H.	N.H.	H.	N.H.	
1	1	—	—	—	1
2	2	—	—	—	2
3	45	11	2	—	58
4	—	1	—	—	1
5	32	8	1	—	41
6	1	—	—	—	1
7	2	1	—	—	3
8	1	—	—	—	1
9	10	6	—	—	16
10	36	—	3	—	39
11	113	18	4	5	140
12	5	—	—	—	5
13	5	—	1	—	6
14	3	—	—	—	3
15	8	—	—	—	8
16	3	—	—	—	3
17	9	—	—	—	9
18	21	14	—	—	35
19	19	1	—	—	20
20	9	—	—	—	9
21	5	1	1	—	7
22	1	—	—	—	1
23	22	14	—	—	36
24	3	—	—	—	3
25	15	—	—	—	15
26	14	1	3	2	20
27	10	—	—	—	10
28	7	—	—	—	7
29	3	—	—	—	3
30	—	2	—	—	2
31	5	—	2	4	11
32	2	—	—	—	2
33	3	—	—	—	3
34	6	—	—	—	6
35	1	—	1	—	2
36	1	—	—	—	1
37	1	—	2	—	3
38	2	—	—	—	2
39	2	—	1	—	3
40	3	2	—	—	5
41	2	—	1	—	3
42	1	—	—	—	1
43	1	—	—	—	1
44	10	1	22	8	41
45	1	—	—	—	1
46	1	—	—	—	1
Atypical	13	2	2	—	17
Untypable	101	28	13	4	146
Total	561	111	59	23	754

Table 4. *Phage types associated with non-human sources*

Source	No. of isolates	*C. jejuni* phage type (numbers)	No. of isolates	*C. coli* phage type (numbers)
Bovine	59	3(9); 4(1); 5(8); 9(3); 11(14); 19(1); 23(14); UT(9)	3	11(1); UT(2)
Poultry	38	7(1); 9(3); 11(4); 18(14); 26(1); 30(2); 44(1); AT(2); UT(10)	8	11(1); 31(1); 44(5); UT(1)
Porcine	0		8	11(3); 26(1); 31(3); UT(1)
Others	14	3(2)*; 21(1)*; 40(2)†; UT(9)‡	4	26(1)§; 44(3)*
Total	111		23	

* Unspecified sources. † Avian. ‡ Water. 7; avian. 1; monkey. 1. § Pet dog.

Table 5. *Phage types of* C. jejuni *and* C. coli *identified in various countries*

Country	No. of C. jejuni isolates	No. of C. coli isolates	Total no. of strains	No. of designated phage types	Phage types	No. of untypable strains
Bangui	4	1	5	2	10.11	2
Canada	266	13	280	24	2.3.4.6.9.10.11.12.13. 14.15.16.17.19.20.21. 22.23.24.26.36.37.44. 45.AT	19
Chile	9	2	11	4	5.11.19.20.AT	2
China	40	—	40	4	3.9.11.12.AT	8
Denmark	1	—	1	1	28	—
France	1	8	9	2	39.44	3
Germany (West)	—	1	1	1	11	—
Guatemala	37	4	41	13	3.5.9.11.12.23.26.31. 34.35.36.37.39	4
India	—	3	3	2	26.44	1
Israel	—	1	1	1	11	—
Pakistan	—	3	3	0	AT	1
Peru	4	1	5	3	11.12.42.AT	2
Portugal	85	40	125	17	3.9.10.11.13.15.17.19. 21.28.31.34.35.36.38. 41.44	42
Rwanda	1	—	1	1	20	—
Thailand	74	3	77	10	5.9.10.11.17.21.23.26. 37.46.AT	30
UK	120	—	120	14	1.3.4.5.7.8.9.10. 11.15.17.18.21.23.AT	28
USA	30	1	31	6	3.5.11.22.23.26	4
Total	672	82	754			146

proved valuable in epidemiological investigations of campylobacter outbreaks studied from various countries (17–19).

The extended phage-typing scheme provided strain discrimination of *C. jejuni* and *C. coli* isolates from human and from animal and environmental strains (Tables 3 and 4). The nine common phage types (3, 5, 10, 11, 18, 19, 23, 6 and 44) observed among humans were also observed among non-human isolates. The most common phage type 11 was found among humans, bovines, poultry and swine, whereas phage type 23, seen in humans, was found only among bovine isolates.

Phage type 14 was found among poultry isolates and was also observed among human isolates. All of these findings suggest the potential implication of animals as a contaminating source for human infections. Other studies [2, 3] have shown animal sources to be a major reservoir for strains infecting humans. So far 30 other phage types were exclusively observed in human infections (Tables 3 and 4), further emphasizing the necessity of examining a sufficiently large number of campylobacter strains from non-human sources.

In the UK, Salama and colleagues [9, 10] used 10 newly isolated phages together with 6 of Grajewski's phages and set up a new phage-typing scheme for *C. jejuni* and *C. coli* strains which they applied to 5 campylobacter outbreaks. Their scheme is based on recording phage patterns obtained with 16 typing phages and assigning to one of the previously designated phage groups. Altogether 140 phage groups were recognized (unpublished data). The scheme [9] has not yet been evaluated in studies using isolates from other countries. In our present scheme we have adopted the internationally established procedure of recording degrees of lysis with the typing phages as recommended by Anderson and Williams [16] for enteric pathogens (Table 1) and report 46 different phage types identified from human and non-human sources in 17 different countries (Tables 3, 4, and 5).

In our present investigation, we have employed our extended scheme to type isolates from 17 different countries (Table 5) and have shown the prevalence of different phage types. Because of sample size, it is difficult to assess the distribution of phage types in various countries. However, when compared with 20 or more isolates from each country, the scheme provides typability from 93·2% (261/280) for Canadian isolates to 61·2% (47/77) for isolates from Thailand. The common phage type 11 was observed in 12 countries, followed by phage type 3 in 6 countries, indicating comparable distribution of these phage types. Phage types 1, 7, 8 and 18 identified among isolates from the UK have not been observed in any other country, whereas types 28, 38 and 41 were exclusively observed among isolates from Portugal, etc. (Table 5).

When phage-typing is combined, as recommended in our previous study, with serotyping and biotyping, it offers additional strain discrimination for epidemiological studies [12]. This combination was investigated by various workers [9, 17, 19], thus suggesting the complementary use of phage typing in conjunction with other markers for epidemiological analysis of campylobacter infections.

REFERENCES

1. Butzler JP, Skirrow MB. *Campylobacter enteritis.* Clin Gastroenterol 1979: **8**: 737–65.

2. Blaser MJ, Taylor MJ, Feldman RA. Epidemiology of *Campylobacter jejuni* infections. Epidemiol Rev 1983; **5**: 157–76.

3. Lior H, Woodward DL, Edgar JA, Laroche LJ, Gill P. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. J Clin Microbiol 1982; **15**: 761–8.

4. Lior H. Extended biotyping scheme for *Campylobacter jejuni*. J Clin Microbiol 1984; **20**: 636–40.

5. Figueroa G, Troncoso M, Galeno H, Soto V, Toledo MS. Biotypes, serogroups and antibiotic susceptibility of *Campylobacter jejuni* and *Campylobacter coli* in Chile. J Infect 1990; **20**: 123–7.

6. Hirschl AM, Lior H, Wolf D, et al. Occurrence, serotypes and biotypes of thermophilic *Campylobacter* isolated in Vienna. Zbl Bakt Hyg 1987; **A 266**: 94–103.

7. Bar W, Fricke G, Goosens H. Distribution of serotypes and biotypes of thermophilic *Campylobacters* in the Federal Republic of Germany: a comparison with other countries. Zbl Bakt 1989; **271**: 127–34.

8. Grajewski BA, Kusek JW, Gelfand HM. Development of a bacteriophage typing system for *Campylobacter jejuni* and *Campylobacter coli*. J. Clin Microbiol 1985; **22**: 13–8.

9. Salama SM, Bolton FJ, Hutchinson DN. Application of a new phage-typing scheme to *campylobacters* isolated during outbreaks. Epidemiol Infect 1990; **104**: 405–11.

10. Salama SM, Bolton FJ, Hutchinson DN. Improved method for the isolation of *Campylobacter jejuni* and *Campylobacter coli* bacteriophages. Lett Appl Microbiol 1989; **8**: 5–7.

11. Khakhria R, Lior H. Phagetyping of *Campylobacter jejuni*. In: Kaijser B, Falsen E, eds. Campylobacter IV. Goterna, Kungalu, Sweden, 1988: 134.

12. Lior H, Woodward D, Khakhria R. Characterization of *Campylobacter jejuni* outbreaks by 3 epidemiological typing markers. In: Kaijser B, Falsen E, eds. Campylobacter IV. Goterna, Kungalu, Sweden, 1988: 103–4.

13. Callow BR. A new phage typing scheme for *Salmonella typhimurium*. J Hyg 1959; **57**: 346–59.

14. Adams MH. Bacteriophages. New York: Interscience Publishers Inc., 1959.

15. Farmer JJ III, Hickman FW, Sikes JV. Automation of *Salmonella typhi* phage typing. Lancet 1975; ii: 787-90.

16. Anderson ES, Williams REO. Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. J Clin Pathol 1956; **9**: 94–114.

17. Lior H. Les *Campylobacter* Marqueurs Epidémiologiques. Méd Malad Infect 1989; **19**: special Mars 18–24.

18. Patton CM, Wachsmuth IK, Evins GM, et al. Evaluation of 10 methods to distinguish epidemic-associated *Campylobacter* strains. J Clin Microbiol 1991; **29**: 680–8.

19. Cabrita J. Contribuição para o estudo da bioecologia do Campylobacter Enteropatogénico. [Thesis] Lisbon, Portugal: Universidade de Lisboa, 1989: 138–50.