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Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the Basis of Thermostable Antigens

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Hippurate hydrolysis tests performed on the serotype reference strains of the serotyping scheme based on thermostable antigens under development for *Campylobacter jejuni* showed that 42 strains were *Campylobacter jejuni* and 17 were *Campylobacter coli*. Moreover, only four (0.2 %) of 2025 hippurate positive *Campylobacter jejuni* isolates reacted in *Campylobacter coli* antisera and 12 (4.3 %) of the 282 *Campylobacter coli* reacted in *Campylobacter jejuni* antisera. Evidently each species has its own array of antigenic specificities. Separate schemes for serotyping *Campylobacter jejuni* and *Campylobacter coli* are advocated.

In an earlier study, it was demonstrated that bacteria, then classified as *Campylobacter fetus* subspecies *jejuni*, could be serotyped on the basis of their thermostable antigens (1). Sheep erythrocytes were readily sensitized with preparations of extracted thermostable antigens and rabbit antisera could be titrated against the antigen-coated erythrocytes using the passive (indirect) hemagglutination technique. Isolates could be differentiated by the reactions of their extracted thermostable antigens in antisera produced against strains of different serotypes. Essentially similar procedures for serotyping these bacteria have been reported by other investigators (2).

It was later shown that two species, *Campylobacter jejuni* and *Campylobacter coli*, occurred among this group of bacteria (3). In light of this finding a re-examination of the results of serotyping was undertaken to determine if relationships existed between the serotypes and the newly-defined species. In this report, the extension of the earlier serotyping scheme is described and its division into separate schemes for each species is proposed.

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Materials and Methods

Bacteria. Strains of *Campylobacter jejuni* and *Campylobacter coli* that were used as serotype reference strains were obtained from several laboratories. The contributors and corresponding first letters of their strain designations (in parenthesis) are as follows: M. Karmali (MAK), P. C. Fleming (PC), H. Lior (C), P. McMyne (Van), S. Lauwers (B, Ca, D, S, Z), J. Wells (A), M. Fuksa (St. J), A. Pearson (RN), M. Rogol (RO), G. Whiteley (DPH). Strain J21 was obtained from R. Sakazaki. Toronto General Hospital strains are designated TGH. A collection of 2025 *Campylobacter jejuni* and 282 *Campylobacter coli* isolates included contributions from the above mentioned investigators and from at least ten others who sent isolates for serotyping.

Serotyping and biotyping. Serotyping was performed on the basis of saline extracted thermostable antigens using the passive hemagglutination technique as described previously (1). The test for hippurate hydrolysis was performed according to Hwang and Ederer (4).

Results

Separate Schemes for Serotyping Campylobacter jejuni *and* Campylobacter coli

The serotyping scheme based on soluble extracted thermostable antigens as previously described (1) was expanded by including new serotypes as they were identified among untypable

isolates. The antisera produced against the new types were titrated against antigen from each serotyping reference strain and included in the scheme if the titration results established that the antisera identified new specificities. In most cases, the newly-included antisera had no cross-reactions or only low-titered cross-reactions with other serotype reference strains. In a few cases, cross-reacting antisera (such as antiserum 6 which cross-reacted with serotype reference strain 7) were also included after it had been demonstrated by cross-absorption and cross-titration experiments that they identified new thermostable antigenic specificities. The newly included antisera were numbered consecutively.

In earlier reports isolates were classified as *Campylobacter fetus* subsp. *jejuni* but later two species, *Campylobacter jejuni* and *Campylobacter coli*, were recognized (3). The test for hippurate hydrolysis distinguished between the two species and with the application of the test to the serotype reference strains, 42 were found to be *Campylobacter jejuni* (hippurate positive) and 17 were *Campylobacter coli* (hippurate negative). Some isolates of both species reacted in *Campylobacter jejuni* antiserum 5 but apart from this one case, isolates of *Campylobacter jejuni* generally reacted in antisera against *Campylobacter jejuni* serotype reference strains. Likewise, the *Campylobacter coli* isolates most often reacted only in *Campylobacter coli* antisera. Except for the one shared antigenic specificity it was therefore clear that each species was characterized by its own distinct set of antigenic specificities. The establishment of a separate serotyping scheme for each species was indicated. An antiserum was produced against a *Campylobacter coli* strain (PC330) that reacted in *Campylobacter jejuni* antiserum 5 to provide a *Campylobacter coli* antiserum for this specificity. To avoid confusion the numerical designations of the serotypes and the corresponding antisera were not changed. Serotyping schemes on the basis of 42 *Campylobacter jejuni* antisera and 18 *Campylobacter coli* antisera are shown in Tables 1 and 2.

Hippurate hydrolysis tests were performed on the isolates and the efficacy of the applicability of the separate schemes was evaluated. It was observed that of the 2025 *Campylobacter jejuni* isolates in our collection, 1962 (96.9 %) reacted in *Campylobacter jejuni* antisera,

Table 1: Serotyping scheme for *Campylobacter jejuni*.

Serotype	Strain	Cross-reactions
1	MK5 (S7360)	44
2	PC72	
3	TGH9011	
4	MK7	13, 16
5	C88	
6	C6	7
7	DPH-1	6
8	C-142	
9	PC69	
10	MK1	
11	MK155	
12	S5	
13	MK16	4, 16, 50
15	TGH10043	
16	TGH4936	4, 13, 50
17	MK15	
18	MK52	
19	MK104	
21	MK172	
22	TGH8551	
23	MK198	36
27	PC37	
29	B-1	
31	MK192	
32	PC267	
33	MK262	
35	PC264	
36	MK290	23
37	PC342	
38	MK27	
40	PC353	
41	PC312	
42	St.J.15	
43	VANA5	
44	D67	1
45	VANE8	
50	MK307	
52	A1616	
53	VAND10	
55	A2412	
57	J21	
58	RN16	

Table 2: Serotyping scheme for *Campylobacter coli*.

Serotype	Strain	Serotype	Strain
5	PC330	39	PC285
14	Z2	46	VANH13
20	MK100	47	Ca72
24	PC66	48	Ca77
25	PC70	49	A1618
26	PC67	51	PC228
28	PC262	54	PC354
30	MK219	56	RO268
34	PC347	59	PC349

4 (0.2 %) in *Campylobacter coli* antisera and 59 (2.9 %) were untypable in antisera of both species. Of the 282 *Campylobacter coli* isolates, 260 (92 %) were typed with *Campylobacter coli* antisera, 12 (4.3 %) with *Campylobacter jejuni* antisera and 10 (3.5 %) were untypable in antisera from both schemes.

Isolates to be serotyped are now first tested for their hippurate reaction and then tested in antisera of the same species. Only untypable isolates are tested in antisera of both species.

Serotypes of Campylobacter jejuni

Forty-two antisera were used for serotyping *Campylobacter jejuni*. Serotypes of isolates were of two kinds. The majority (78 %) were identified by a single reaction in one or another of the 39 antisera shown in Table 3. Others were identified by reactions in two or more antisera. These multiply reacting isolates comprised 18.6 % of the *Campylobacter jejuni* (Table 4).

Cross-reacting antisera such as those for serotypes 1 and 44, 6 and 7 and 23 and 36 identified isolates either by a single reaction in one or the other of the pair or by reactions in both antisera. Some multiply reacting isolates, such as those of serotypes 5/31, 8/17 and 23/53 were identified by pairs of antisera for which cross-reactions were not known. Three antisera (13, 16 and 50) were always associated with multiply reacting isolates. The isolates that typed with these three antisera along with antisera 4 and 43 defined a closely related group. The antigenic specificities contributing to the interrelationships among such complex multiply reacting isolates remain undefined.

For routine serotyping unabsorbed antisera were used. On this basis, the most frequently-occurring serotypes were 1, 2, 3, 4 and 5 and they constituted 50 % of the collection. Less common serotypes that formed 2 % to 3 % of the total were 6/7, 8, 23/36, 11, 13/16/50, 18 and 1/44. In addition to the multiply reacting isolates shown in Table 4, the existence of 93 others are indicated. Included among them are some with different serotypes, some that have weak reactions and others that have not yet been tested in the most recently included antisera.

Table 3: Distribution of serotypes among 1586 isolates of *Campylobacter jejuni.*

Serotype	No. of isolates	Percentage
1	209	10.3
2	292	14.4
3	141	7.0
4	291	14.3
5	81	4.0
6	7	0.35
7	2	0.1
8	58	2.9
9	3	0.15
10	27	1.3
11	49	2.4
12	7	0.35
15	17	0.84
17	1	0.05
18	42	2.1
19	32	1.6
21	38	1.9
22	4	0.2
23	35	1.7
27	9	0.4
29	7	0.35
31	34	1.7
32	2	0.1
33	12	0.6
35	11	0.5
36	6	0.3
37	37	1.8
38	6	0.3
40	7	0.35
41	15	0.7
42	27	1.3
43	3	0.15
44	14	0.7
45	10	0.5
52	1	0.05
53	4	0.2
55	14	0.7
57	4	0.2
58	8	0.4

Table 4: Multiply reacting isolates of *Campylobacter jejuni.*

Serotypes	No. of isolates	Percent
1/44	40	2.0
4/13/16/50	10	0.5
13/16/50	46	2.3
13/16/43/50	15	0.7
13/16/43	5	0.2
5/31	9	0.4
6/7	65	3.2
8/17	19	0.9
23/36	56	2.8
23/53	18	1.4
others	93	4.6

The application of cross-absorbed antisera was tested and found useful for further subclassification of the largest group of multiply reacting isolates. Isolates that reacted in both unabsorbed antiserum 6 and 7 were retested in antiserum 6 (absorbed with strain 7) and antiserum 7 (absorbed with strain 6). Isolates reacted in one or the other. If the cross-reacting antigen is provisionally designated "a", the serotypes for these may be written as 6/a and 7/a to distinguish them from serotypes 6 and 7 assigned to isolates that gave only single reactions in one of the unabsorbed antisera. Preliminary studies on serotype 23/36 isolates indicated similar subclassification may also be performed for that group.

Antisera have been prepared against five of the 57 untypable isolates. Results of cross-titrations with all other serotype reference strains have shown that each defines a new serotype. However, they have not typed any of the other 52 untypable isolates. They will be given numerical designations and be included in the serotyping scheme when they are found to identify the new specificity among other isolates.

Serotypes of Campylobacter coli

None of the antisera against the 18 *Campylobacter coli* serotype reference strains showed cross-reactions with other *Campylobacter coli* reference strains. The number of isolates that were serotyped by a reaction in only one of the antisera outnumbered, as for *Campylobacter jejuni*, the number of multiply reacting isolates (Table 5). The most frequently occurring serotypes in order of their frequency, were serotypes 48, 34, 46, 49, 30, 51 and 39 making up 53 % of this small collection of 282 isolates.

Table 5: Serotyping of *Campylobacter coli* isolates.

Serotypes	No. of isolates	Percentage
5	9	(3.2)
5/24	5	(1.8)
5/28	1	(0.4)
5/30	10	(3.5)
5/48	1	(0.4)
14	8	(2.8)
20	4	(1.4)
20/48	1	(0.4)
24	14	(5.0)
24/54	2	(0.7)
25	4	(1.4)
25/48	1	(0.4)
26	4	(1.4)
26/30/34/40	2	(0.7)
26/34	2	(0.7)
28	12	(4.3)
30	18	(6.4)
30/49	1	(0.4)
34	24	(8.5)
34/48	1	(0.4)
39	16	(5.7)
39/47	5	(1.8)
46	19	(6.7)
46/47	11	(3.9)
47	9	(3.2)
48	37	(13.1)
49	19	(6.7)
51	17	(6.0)
54	1	(0.4)
56	1	(0.4)
59	1	(0.4)
not typable	10	(3.5)

Discussion

In this study it has been shown that the specificities of the thermostable antigens of *Campylobacter jejuni* in almost all cases, are distinct from those of *Campylobacter coli*. This is additional evidence in support of the two-species classification scheme and attests to the reliability of the test for hippurate hydrolysis as a means of differentiating the species. The possibility exists that the 12 hippurate negative isolates that reacted in *Campylobacter jejuni* antisera are, in fact *Campylobacter jejuni* that have lost their ability to hydrolyse hippurate. These isolates and the four hippurate positive isolates that reacted in *Campylobacter coli* antisera are under further study.

The procedure adopted for identifying the antigenic specificites involved the use of extracted antigens and the passive hemagglutination technique for titrating the antisera. These procedures were selected instead of the methods used in classical serology because preliminary studies had shown that the use of heated bacterial cell suspensions (the "0 antigens" (5) in classical serology) resulted in numerous non-specific reactions (1). The serotyping scheme was developed and extended by preferential selection of serotyping reference strains that

did not cross-react or had only minor cross-reactions with other reference strains of the scheme. This was to meet the first objective of assembling a set of antisera for serotyping for epidemiological investigations. By excluding cross-reacting antisera that immediate necessity for using absorbed antisera was avoided. As expected, most of the isolates reacted in only one or another of the antisera (Table 3, Table 5). In the second phase of the study, cross-reacting serotypes were included if they possessed antigenic specificities that were not defined by the antisera already in the scheme or if they were found to separate closely related isolates. Cross-absorbed antisera for further differentiation have been prepared in some cases and it is expected that further development of the scheme will include a greater number to provide more precise differentiation.

The characteristics of the thermostable antigens resemble the somatic (0) lipopolysaccharide antigens of the outer membrane of gram-negative bacteria in several respects. Like the 0 antigens of *Enterobacteriaceae*, they occur in a wide range of specificities and are useful markers for differentiating among isolates of the same species. They can be extracted by methods that are well-known for 0 antigens. These include extraction with ethylenediaminetetraacetate, with hot phenol water and with heat treatment of saline suspensions of the bacteria (1, 6, 7). Moreover, phenol water extracted antigens have the chemical composition of 0 lipopolysaccharides with 2-keto-3-deoxy-octonate (KDO), a constituent characteristic of the 0 antigen (7). Like typical 0 antigens, the extracted thermostable antigen sensitizes mammalian erythrocytes making them specifically agglutinable in antisera.

Comparisons of the serotype distribution among isolates in the collection described in this study with those in previously reported studies (8, 9) should take into account the adjustments continually being made in serotyping as increasingly greater numbers of antisera are used. Isolates, particularly those of the multiply reacting kind, may be found to have additional specificities identified by the newer antisera. The manner in which the isolates were collected should also be considered. In our collection, isolates were obtained from a wide range of sources and some were sent blind, without clinical data, for serotyping and included duplicates of outbreak strains. Furthermore, most of the isolates from two previously reported studies (8, 9) are included in our collection. In these earlier studies some of the reactions produced by isolates in the newer antisera are not shown. It is important, therefore, that the serotype designations be regarded as provisional while the serotyping scheme is under development. Nevertheless, the serotyping scheme has been demonstrated to be effective in differentiating isolates (10). Its applicability to clinical studies (11, 12) and to investigations of family and waterborne outbreaks (9, 13) has been demonstrated and it promises continued usefulness in gaining insight into the epidemiology of infections caused by these two *Campylobacter* species.

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References

1. **Penner, J. L., Hennessy, J. N.:** Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. Journal of Clinical Microbiology 1980, 12: 732–737.
2. **Lauwers, S., Vlaes, L., Butzler, J. P.:** *Campylobacter* serotyping and epidemiology. Lancet 1981, i: 158–159.
3. **Harvey, S. M.:** Hippurate hydrolysis by *Campylobacter fetus*. Journal of Clinical Microbiology 1980, 11: 435–437.
4. **Hwang, M.-N., Ederer, G. M.:** Rapid hippurate hydrolysis method for presumptive identification of Group B streptococci. Journal of Clinical Microbiology 1975, 1: 114–115.
5. **Berg, R. L., Jutila, J. W., Firehammer, B. D.:** A revised classification of *Vibrio fetus*. American Journal of Veterinary Research 1971, 32: 11–22.
6. **Leive, L., Shovlin, V. K., Mergenhagen, S. E.:** Physical, chemical, and immunological properties of lipopolysaccharides released from *Escherichia coli* by ethylenediamineterteraacetate. Journal of Biological Chemistry 1968, 243: 6384–6391.
7. **Naess, V., Hofstad, T.:** Isolation and chemical composition of lipopolysaccharide from *Campylobacter jejuni*. Acta Pathologica, Microbiologica et Immunologica Scandinavica (B) 1982, 90: 135–139.

8. **Karmali, M. A., Penner, J. L., Fleming, P. C., Williams, A., Hennessy, J. N.:** The serotype and biotype distribution of clinical isolates of *Campylobacter jejuni-coli* over a three year period. Journal of Infectious Diseases 1983, 147: 243–246.
9. **McMyne, P. M. S., Penner, J. L., Mathias, R. G., Black, W. A., Hennessy, J. N.:** Serotyping of *Campylobacter jejuni* isolates from sporadic cases and outbreaks in British Columbia. Journal of Clinical Microbiology 1982, 16: 281–285.
10. **Bradbury, W. C., Marko, M. A., Hennessy, J. N., Penner, J. L.:** The occurrence of plasmid DNA in serologically defined strains of *Campylobacter jejuni* and *Campylobacter coli*. Infection and Immunity 1983, 40: 460–463.
11. **Ahnen, P. J., Brown, W. R.:** *Campylobacter* enteritis in immune-deficient patients. Annals of Internal Medicine 1982, 96: 187.
12. **Karmali, M. A., Kosoy, M., Newman, A., Tischler, M., Penner, J. L.:** Reinfection with *Campylobacter jejuni*. Lancet 1981, ii: 1104.
13. **Blaser, M. J., Penner, J. L., Wells, J. G.:** Diversity of serotypes in outbreaks of enteritis due to *Campylobacter jejuni*. Journal of Infectious Diseases 1982, 146: 826.