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# Serotyping Scheme for *Campylobacter jejuni* and *Campylobacter coli* Based on Direct Agglutination of Heat-Stable Antigens

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**Campylobacter is now the most frequently reported cause of gastrointestinal disease in England and Wales, yet few isolates are characterized beyond the genus level. The majority of isolates are *Campylobacter jejuni* (90%), with most of the remainder being *Campylobacter coli*. We describe an adaptation of the Penner serotyping scheme in which passive hemagglutination has been replaced by detection of heat-stable antigens by direct bacterial agglutination; absorbed antisera are used where appropriate. This scheme has been used to type 2,407 *C. jejuni* samples and 182 *C. coli* samples isolated in Wales between April 1996 and March 1997. Forty-seven *C. jejuni* serotypes were identified, with the 10 most prevalent serotypes accounting for 53% of the isolates tested; 19% of the isolates were untypeable. Only fifteen *C. coli* serotypes were identified, with three serotypes accounting for 69% of the isolates. This scheme provides a baseline for epidemiological studies of *C. jejuni* and *C. coli*.**

The role of campylobacter as a cause of enteric disease in humans was not fully recognized until the development of isolation methods and selective media during the 1970s (10). Subsequently, the number of human campylobacter infections reported in England and Wales has increased annually, and since 1981 campylobacter has been the most commonly reported cause of acute bacterial enteritis in England and Wales (reports to the Public Health Laboratory Service [PHLS] Communicable Disease Surveillance Centre). Although the organisms in the majority of the 43,240 reports in 1996 were identified simply as "campylobacter", the available data suggest that circa 90% are *Campylobacter jejuni*, 10% are *Campylobacter coli*, and less than 1% are *Campylobacter lari* (10).

A national case-control study in England and Wales during 1990 and 1991 and the interim report of the Department of Health Advisory Committee on the Microbiological Safety of Food (2, 3) recognized that reference subtyping was needed in order to reach a better understanding of both sources of infection and routes of transmission.

There have been a number of studies comparing different methods for subspecies typing within *C. jejuni* and *C. coli*, and both Patton et al. (22) in the United States and Owen and Gibson (18) in the United Kingdom concluded that, for surveillance on a broad scale, serotyping is the most practical solution. Two serotyping schemes for campylobacter developed in Canada in the 1980s have been widely used, either separately or together (23). The Penner scheme (24) is based on soluble heat-stable antigens, while the Lior scheme (14) detected variation in heat-labile antigens. The Penner scheme has been more widely used in the United Kingdom (13, 27) and was therefore used as the basis for further development by the Laboratory of Enteric Pathogens (LEP).

Two main drawbacks of the Penner scheme have been identified (18). Passive hemagglutination (PHA) was introduced as the detection system in an attempt to eliminate nonspecific agglutination reactions. However, reproducibility problems can occur as a result of variation in the source, age, concentration, and condition of the erythrocytes (8, 18). Also, the Penner scheme used unabsorbed antisera and a significant proportion of isolates agglutinated more than one antiserum, particularly serogroups 4, 13, 16, and 50 (13) or 4, 13, 16, 43, and 50 (21).

The present paper describes a modified serotyping scheme for *C. jejuni* and *C. coli* based on the use of absorbed antisera to heat-stable antigens and utilizing whole-cell agglutination to replace PHA.

## MATERIALS AND METHODS

**Bacterial isolates.** Sixty-six type strains for the Penner campylobacter serotyping scheme, 47 *C. jejuni* strains and 19 *C. coli* strains, were obtained from the National Collection of Type Cultures (NCTC; PHLS, London, United Kingdom). Antisera raised against these strains have been used to type 2,407 *C. jejuni* samples and 182 *C. coli* samples isolated as part of a pilot study for the PHLS Campylobacter Reference facility carried out between April 1996 and March 1997 in conjunction with the PHLS and National Health Service hospital laboratories in Wales. All isolates are stored at −80°C in cryovials (Microbank; Pro Lab Diagnostics, Richmond Hill, Ontario, Canada).

**Culture, identification, and determination of species.** Campylobacter isolates were cultured on Columbia blood agar (Oxoid CM331; Unipath, Basingstoke, United Kingdom) with 5% horse blood at 37°C in a variable-atmosphere incubator (VAIN; Don Whitley Scientific Ltd., Shipley, West Yorkshire, United Kingdom) under microaerobic conditions (5% $CO_2$, 5% $O_2$, 3% $H_2$, and 87% $N_2$). Identification was confirmed by testing 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 et al. (6).

**Antiserum production.** Antisera were prepared in New Zealand White rabbits against the strains shown in Table 1. Bacterial suspensions in saline were standardized ($10^9$ cells/ml) and heated at 100°C for 30 min. Preimmune antisera were prepared from blood samples taken via the marginal ear vein before immunization. The rabbits received 0.5 ml of bacterial suspension intravenously on day 1 followed by 1 ml on days 5 and 10 and 2 ml on days 15 and 20. Antibody levels were assessed in antiserum samples from approximately 5 ml of blood from a marginal ear vein taken on day 25. If the titer was 320 or greater, the rabbits were bled and antisera were separated and stored at −20°C. Where antibody levels were inadequate, two further injections of 2 ml each were given at 5-day intervals and the rabbits were bled on day 40.

**Absorption of typing antisera.** All antisera were titrated against the complete set of type strains. Where a current clinical isolate agglutinated with more than one antiserum, absorptions were performed, essentially as described by Abbott et al. (1). For every milliliter of antiserum, one 9-cm-diameter plate of confluent growth of the cross-reacting type strain was suspended in phosphate-buffered saline (PBS), heated to 100°C for 30 min, and added to the antiserum. After being mixed the mixture was incubated for 2.5 h at 50°C before centrifugation to

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remove cells from the absorbed antiserum. This absorbed antiserum was then tested against all type strains which reacted with the unabsorbed antiserum. Absorptions were repeated until cross-reactions no longer occurred. All resulting monospecific antisera were diluted to 1:40 for routine use.

**Serotyping by direct agglutination.** Growth harvested from a 24-h plate culture was resuspended in 1 ml of PBS to produce a dense suspension; this was heated at 100°C for 30 min. A standard dilution was made by adding the boiled suspension to 5 ml of PBS to an opacity equivalent to McFarland standard 4 (API, Vercieu, France). This suspension is stable at 4°C for at least 20 weeks. For serotyping, 25-μl aliquots of a 1:40 dilution of the reference antisera were dispensed into a U-bottom-well microtiter tray and 25 μl of test suspension was added. The tray was incubated at 50°C for 2.5 h in a moist atmosphere with gentle agitation on an orbital shaker. Agglutination was read at 1.5 h, and negative isolates were reincubated for a further 1 h. It was essential that agglutination be read within 30 min of removal of the tray from the incubator. Where agglutination was observed with more than one antiserum, the reacting antisera were titrated against the strain and titers of greater than 40 were regarded as positive.

## RESULTS

**Antiserum production and absorption.** Antisera were produced from all 66 Penner type strains (Table 1) with homologous titers, as measured by direct agglutination, varying from 80 to 2,560. Heterologous agglutination was seen with 47 of the 66 antisera (72.7%). The patterns of cross-reactions observed with the type strains did not necessarily match those observed with current clinical isolates, and an absorption strategy was therefore developed to eliminate the observed cross-reactions occurring with current clinical isolates.

Eighteen antisera gave monospecific reactions with both the type strains and the current clinical isolates. These antisera are used in the serotyping panel unabsorbed, as are a further nine antisera which gave cross-reactions with one or more type strains but not with current clinical isolates. Thirty antisera showed cross-reactions with both type strains and current clinical isolates, and these have been absorbed with one or more of the type strains listed in Table 1.

Three pairs and one trio of antisera gave the same results by the direct agglutination method. All current clinical isolates reacted with both antisera of the pairs or all three antisera of the trio, and absorption with any of the type strains in that group removed agglutination with all of the appropriate antisera. Only one of each of these four groups of antisera is therefore included in the panel of 61 antisera which constitute the routine serotyping panel, and the earlier of the type designations has been conserved (e.g., HS9 has been retained, and HS38 has been discarded).

**Typing of current clinical isolates.** The distribution of serotypes among 2,407 *C. jejuni* samples and 182 *C. coli* samples isolated in Wales between April 1996 and March 1997 is given in Table 2. Forty-seven serotypes were identified for *C. jejuni* (Table 2); for 20 of these, the numbers of samples were more than 1% of the total. For *C. coli* (Table 2), 15 serotypes were identified, six of which were represented by a single isolate. Four hundred and eighty-nine isolates were untypeable (18.9%), and the majority of these failed to agglutinate with any of the antisera used. Seventeen isolates reacted with two or more antisera and are reported as untypeable pending resolution of these cross-reactions. Ten of the 17 reacted with two or more of antiHS13, antiHS16, and antiHS29, i.e., the so-called "HS4 complex" (19).

The proportion of untypeable isolates will be reduced by raising antisera against untypeable isolates to define new types. For example, an antiserum raised against strain C001975 was tested against all other untypeable isolates in this study and against the type strains; five *C. jejuni* isolates agglutinated with anti-C001975. None of the type strains agglutinated with anti-C001975, and C001975 has therefore been adopted as the type strain for serotype HS67, which has been added to the scheme.

## DISCUSSION

Antisera were produced in rabbits against all 66 Penner serotyping type strains deposited in NCTC and used to develop a serotyping scheme for *C. jejuni* and *C. coli*, based on direct bacterial agglutination. Forty-seven antisera gave cross-reactions with the type strains and, where cross-reactions have also been observed among current clinical isolates, these have been removed by using agglutinin absorption against appropriate type strains (Table 1). The titers obtained from antisera raised against the freeze-dried type cultures were generally relatively low, whereas preparation of antisera against current clinical isolates of the same serotypes resulted in higher titers (unpublished data). Where more than one isolate of the same serotype was used to prepare antisera or when a number of rabbits were injected with the same vaccine, considerable variations in titer were observed (unpublished data).

The LEP serotyping scheme for *C. jejuni* and *C. coli* has addressed the two principal limitations of the Penner serotyping scheme for campylobacters. Reproducibility problems due to variations in erythrocytes (18) have been eliminated by adopting a direct bacterial agglutination method similar to that used for serogrouping schemes for other enteric pathogens. The nonspecific reactions observed in the original serotyping studies of *Vibrio fetus* (5) have been eliminated by incubating the reaction mixtures at 50°C with gentle shaking and reading the result immediately on removal from the shaker. The use of unabsorbed antisera in the Penner scheme resulted in a high proportion of isolates reacting with more than one antiserum (26), and these cross-reactions varied in expression (16). This problem has been addressed by using absorbed antisera to eliminate the cross-reactions observed among current clinical isolates. This has resulted in a scheme which defines 44 heat-stable serotypes for *C. jejuni* and 17 for *C. coli.*

The proportion of untypeable isolates is unsatisfactorily high at 19%. However, this level of typeability is comparable to that from a recent study of poultry campylobacter isolates in The Netherlands where 18% of isolates were found to be untypeable by using the Penner serotyping technique (11). The most recently published data from studies using the Penner scheme for isolates from humans have been those from studies carried out in China and Japan (17) and Ethiopia (4). The quoted untypeability rates are 58.8% for Chinese isolates, 13.0% for Japanese isolates, and 63.4% for Ethiopian isolates. These observations no doubt reflect wide geographical variations in the distribution of *C. jejuni* serotypes. The type strains are representative of campylobacter strains prevalent in the early 1980s, and the majority were isolated in Canada. Antisera raised against untypeable isolates from the present study will be used to extend the scheme and reduce the proportion of untypeable isolates in the United Kingdom by defining new types. One new type, HS67, has been added to date.

Comparability with the Penner scheme is not exact because of the methodological differences detailed above. Penner antisera were prepared with live vaccines, whereas the method described above uses heat-killed whole-cell vaccines. The original Penner scheme used only unabsorbed antisera; Jones et al. (12, 13) reported the use of absorbed antisera, but almost half of the isolates tested still showed some cross-reactions within serogroups 4, 13, 16, and 50. More recently, Jacobs-Reitma et al. (11) have developed a set of absorbed antisera and have used a formalin-fixed inoculum for rabbit immunization.

The principal difference between the Penner and LEP meth-

TABLE 1. Antisera raised against Penner type strains

| Vaccine strain used | | | Antiserum[a] | | | | |
|---|---|---|---|---|---|---|---|
| NCTC no. | Species | Penner serotype[b] | LEP designation | Homologous titer with[c]: I | Homologous titer with[c]: II | Penner type strain(s) (titer[s]) for which cross-reactions observed | Penner type strain(s) with which antiserum absorbed |
| 12500 | *C. jejuni* | 1 | antiHS1 | 160 | 160 | 5 (80) | 5 |
| 12501 | *C. jejuni* | 2 | antiHS2 | 640 | 320 | 7 (320), 8 (40), 10 (80), 13 (320), 22 (80), 44 (80) | 13, 44 |
| 12502 | *C. jejuni* | 3 | antiHS3 | 160 | | | |
| 12503 | *C. jejuni* | 4 | antiHS4 | 160 | | | |
| 12504 | *C. jejuni* | 5 | antiHS5 | 160 | 160 | 1 (20), 47 (80), 66 (160) | 1 |
| 12505 | *C. jejuni* | 6 | antiHS6 | 640 | 640 | 12 (80), 25 (320), 26 (80), 59 (80) | 25 |
| 12506 | *C. jejuni* | 7 | antiHS7 | 320 | | | |
| 12507 | *C. jejuni* | 8 | antiHS8 | 640 | 320 | 17 (160), 44 (40), 47 (80), 53 (80) | 17 |
| 12508 | *C. jejuni* | 9 | NI[d] | 160 | | 38 (80) | |
| 12509 | *C. jejuni* | 10 | antiHS10 | 160 | 160 | 50 (40), 65 (80) | 50 |
| 12510 | *C. jejuni* | 11 | NI | 160 | | | |
| 12511 | *C. jejuni* | 12 | antiHS12 | 1280 | 1280 | 6 (40), 25 (320), 26 (320), 37 (80), 59 (160) | 25 |
| 12512 | *C. jejuni* | 13 | antiHS13 | 1280 | 80 | 4 (640), 7 (160), 16 (640), 19 (640), 22 (320), 29 (320) | 29, 50 |
| 12526 | *C. coli* | 14 | antiHS14 | 320 | 160 | 46 (640), 47 (640), 49 (640), 52 (320) | 46, 49 |
| 12513 | *C. jejuni* | 15 | antiHS15 | 640 | 640 | 18 (80) | 18 |
| 12514 | *C. jejuni* | 16 | antiHS16 | 1280 | 1280 | 2 (160), 4 (160), 10 (80), 13 (320), 19 (320), 22 (160), 29 (80), 46 (320), 50 (80), 62 (80), 65 (40) | 13, 29 |
| 12515 | *C. jejuni* | 17 | antiHS17 | 320 | | | |
| 12516 | *C. jejuni* | 18 | antiHS18 | 320 | | | |
| 12517 | *C. jejuni* | 19 | antiHS19 | 80 | | | |
| 12527 | *C. coli* | 20 | antiHS20 | 320 | | | |
| 12518 | *C. jejuni* | 21 | antiHS21 | 640 | | | |
| 12519 | *C. jejuni* | 22 | antiHS22 | 320 | 160 | 23 (80), 35 (160) | 23 |
| 12520 | *C. jejuni* | 23 | antiHS23 | 320 | 160 | 22 (320) | 22 |
| 12528 | *C. coli* | 24 | antiHS24 | 160 | 160 | 25 (80), 26 (640) | 25 |
| 12529 | *C. coli* | 25 | antiHS25 | 1280 | 160 | 6 (640), 12 (640), 26 (640), 59 (160) | 6 |
| 12530 | *C. coli* | 26 | antiHS26 | 640 | 320 | 24 (80), 25 (160) | 24 |
| 12521 | *C. jejuni* | 27 | antiHS27 | 640 | | | |
| 12531 | *C. coli* | 28 | antiHS28 | 640 | | 30 (80), 34 (160) | ANR[e] |
| 12522 | *C. jejuni* | 29 | antiHS29 | 640 | 320 | 7 (40), 50 (320), 65 (320) | 50 |
| 12532 | *C. coli* | 30 | antiHS30 | 320 | 80 | 3 (160), 28 (640), 34 (320) | 3, 28 |
| 12523 | *C. jejuni* | 31 | antiHS31 | 320 | 160 | 11 (320), 17 (80), 64 (40) | 64 |
| 12524 | *C. jejuni* | 32 | antiHS32 | 160 | | 36 (160) | ANR |
| 12537 | *C. jejuni* | 33 | antiHS33 | 320 | | | |
| 12533 | *C. coli* | 34 | antiHS34 | 640 | 640 | 28 (20), 30 (160) | 28 |
| 12538 | *C. jejuni* | 35 | antiHS35 | 1280 | 160 | 22 (160), 29 (640) | 29 |
| 12547 | *C. jejuni* | 36 | antiHS36 | 160 | 80 | 32 (160) | 32 |
| 12539 | *C. jejuni* | 37 | antiHS37 | 640 | 160 | 12 (160), 25 (160) | 12 |
| 12540 | *C. jejuni* | 38 | antiHS9 | 320 | 160 | 63 (320) | 63 |
| 12534 | *C. coli* | 39 | antiHS39 | 320 | 80 | 51 (160), 61 (160), 66 (160) | 61 |
| 12541 | *C. jejuni* | 40 | antiHS40 | 640 | | 45 (80) | ANR |
| 12542 | *C. jejuni* | 41 | antiHS41 | 160 | | | |
| 12543 | *C. jejuni* | 42 | antiHS42 | 160 | | | |
| 12548 | *C. jejuni* | 43 | antiHS43 | 160 | 80 | 2 (80), 4 (80), 10 (80), 13 (80), 22 (160), 29 (640), 31 (80), 44 (80), 46 (80) | 44 |
| 12549 | *C. jejuni* | 44 | antiHS44 | 320 | 320 | 2 (80), 4 (320), 10 (160), 13 (320), 19 (80), 22 (80), 29 (80), 31 (80), 46 (320), 47 (160) | 43 |
| 12544 | *C. jejuni* | 45 | antiHS45 | 640 | | 40 (80) | |
| 12569 | *C. coli* | 46 | antiHS46 | 640 | 640 | 14 (80), 47 (640), 49 (80), 54 (160) | 14 |
| 12535 | *C. coli* | 47 | NI | 640 | | 14 (320), 46 (640), 47 (640), 49 (320), 54 (320) | |
| 12536 | *C. coli* | 48 | antiHS48 | 1280 | | 7 (320) | ANR |
| 12570 | *C. coli* | 49 | antiHS49 | 640 | | | |
| 12559 | *C. jejuni* | 50 | antiHS50 | 640 | 320 | 16 (80), 65 (640) | 16 |
| 12550 | *C. coli* | 51 | antiHS51 | 160 | | | |
| 12545 | *C. jejuni* | 52 | antiHS52 | 160 | | | |
| 12560 | *C. jejuni* | 53 | antiHS53 | 160 | | | |
| 12551 | *C. coli* | 54 | NI | 640 | | 14 (320), 26 (80), 46 (640), 47 (640), 49 (320) | |
| 12546 | *C. jejuni* | 55 | antiHS55 | 80 | | | |
| 12567 | *C. coli* | 56 | antiHS56 | 320 | | 37 (40) | ANR |
| 12552 | *C. jejuni* | 57 | antiHS57 | 160 | | 3 (160), 60 (80) | ANR |
| 12553 | *C. jejuni* | 58 | antiHS58 | 160 | 160 | 11 (640), 17 (160), 31 (320), 64 (160) | 64 |
| 12568 | *C. coli* | 59 | antiHS59 | 80 | 80 | 25 (320), 26 (320), 64 (80) | 25 |
| 12554 | *C. jejuni* | 60 | antiHS60 | 320 | | 7 (320) | ANR |
| 12570 | *C. coli* | 61 | antiHS61 | 160 | 160 | 39 (80), 51 (160), 66 (80) | 66 |
| 12555 | *C. jejuni* | 62 | antiHS62 | 80 | | | |
| 12556 | *C. jejuni* | 63 | antiHS63 | 640 | | 9 (40), 38 (640) | ANR |
| 12557 | *C. jejuni* | 64 | antiHS11 | 320 | | 11 (640), 17 (160), 31 (320) | ANR |
| 12558 | *C. jejuni* | 65 | NI | 640 | | 16 (40), 50 (320) | |
| 12525 | *C. coli* | 5 | antiHS66 | 320 | 160 | 5 (160), 39 (160), 51 (160), 61 (160) | 39 |

[a] Titers were measured by the direct agglutination technique.
[b] Penner serotype as given in the NCTC catalog. For Penner serotype 5, strain 12504 is hippurate positive and strain 12525 is hippurate negative.
[c] I, unabsorbed antiserum; II, absorbed antiserum if appropriate.
[d] NI, type not included in the LEP scheme because antisera were indistinguishable by direct agglutination: antiHS9 = antiHS38, antiHS11 = antiHS64, antiHS47 and antiHS54 = antiHS14, and antiHS65 = antiHS50.
[e] ANR, absorption not required for current clinical isolates; see text.

TABLE 2. Serotypes of *C. jejuni* and *C. coli* isolated in Wales between April 1996 and March 1997

| Serotype | No. of isolates | % of total |
|---|---|---|
| *C. jejuni* | | |
| HS50 | 245 | 10.2 |
| HS2 | 237 | 9.8 |
| HS44 | 183 | 7.6 |
| HS11 | 161 | 6.7 |
| HS16 | 98 | 4.1 |
| HS13 | 95 | 3.9 |
| HS6 | 82 | 3.4 |
| HS37 | 81 | 3.4 |
| HS21 | 71 | 2.9 |
| HS5 | 70 | 2.9 |
| HS1 | 61 | 2.5 |
| HS14 | 56 | 2.3 |
| HS8 | 46 | 1.9 |
| HS18 | 46 | 1.9 |
| HS12 | 41 | 1.7 |
| HS9 | 40 | 1.7 |
| HS35 | 37 | 1.5 |
| HS59 | 34 | 1.4 |
| HS15 | 33 | 1.4 |
| HS29 | 31 | 1.3 |
| HS3 | 21 | 0.9 |
| HS57 | 21 | 0.9 |
| HS60 | 18 | 0.7 |
| HS43 | 17 | 0.7 |
| HS10 | 13 | 0.5 |
| HS27 | 10 | 0.4 |
| HS42 | 7 | 0.3 |
| HS62 | 6 | 0.2 |
| HS7 | 4 | 0.2 |
| HS33 | 4 | 0.2 |
| HS52 | 4 | 0.2 |
| HS38 | 3 | 0.1 |
| HS45 | 3 | 0.1 |
| HS23 | 2 | 0.1 |
| HS41 | 2 | 0.1 |
| HS53 | 2 | 0.1 |
| HS55 | 2 | 0.1 |
| HS63 | 2 | 0.1 |
| HS30 | 2 | 0.1 |
| HS4 | 1 | 0.04 |
| HS22 | 1 | 0.04 |
| HS46 | 1 | 0.04 |
| HS48 | 1 | 0.04 |
| HS49 | 1 | 0.04 |
| HS56 | 1 | 0.04 |
| HS58 | 1 | 0.04 |
| HS61 | 1 | 0.04 |
| Untypeable | 467 | 19.4 |
| Rough | 41 | 1.7 |
| Total | 2407 | |
| *C. coli* | | |
| HS56 | 69 | 37.9 |
| HS66 | 33 | 18.7 |
| HS28 | 23 | 12.6 |
| HS14 | 6 | 3.3 |
| HS61 | 5 | 2.7 |
| HS48 | 4 | 2.2 |
| HS6 | 3 | 1.6 |
| HS49 | 2 | 1.0 |
| HS46 | 2 | 1.0 |
| HS25 | 1 | 0.5 |
| HS30 | 1 | 0.5 |
| HS31 | 1 | 0.5 |
| HS35 | 1 | 0.5 |
| HS39 | 1 | 0.5 |
| HS59 | 1 | 0.5 |
| Untypeable | 22 | 12.1 |
| Total | 182 | |

ods lies in the detection system. Whereas the LEP protocol uses direct bacterial agglutination, the Penner method uses PHA, that is, the supernatant from a boiled cell suspension is used to sensitize erythrocytes, which are in turn mixed with antisera in order to demonstrate agglutination. Although the PHA method will be most efficient for soluble antigens, it had been assumed that the *C. jejuni* heat-stable antigens were lipopolysaccharides (LPS) (24). While long-chain LPS have been detected in some serotypes of *C. jejuni* (25), only LPS core and short-chain polysaccharides have been detected in other studies (15), and it has been suggested that the antigen detected by PHA and direct agglutination is capsular (7).

Many workers have noted cross-reactions in isolates belonging to serotypes 4, 13, 16, 43, and 50 (11, 20, 26). These serotypes have sometimes been referred to collectively as the HS4 complex (19). By using the LEP protocol and unabsorbed antisera, cross-reactivity between serotypes 4, 13, 43, 50, and 65 was observed. However, serotypes HS4, HS13, HS16, HS43, and HS50 could be distinguished with absorbed antisera. Only one isolate in this study, other than the type strain, belonged to HS4. Serotypes HS50 and HS65 were indistinguishable by direct agglutination and reciprocal absorption, so HS65 has been dropped from the LEP scheme. A number of clinical isolates which react with both antiHS13 and antiHS16 but which are not resolved by absorption remain, and further studies are in progress to determine the antigenic structures of these isolates. These observations fit with a numerical analysis of pulsed-field patterns carried out to determine lineages within *C. jejuni* (9); this analysis identified three clonal lines within the HS4 complex, namely, HS4/HS13/HS16, HS43, and HS50/HS65.

The combination of absorbed antisera with a direct whole-cell agglutination technique has produced a method which gives a serotyping result in 2 h. The use of a microtiter agglutination technique maximizes the number of tests from each batch of antiserum and lends itself to automation. In order to investigate the epidemiology of an organism when there is a need to type large numbers of sporadic isolates, the chosen typing technique must lend itself to routine use on large numbers of isolates, produce easily interpreted data, and be capable of standardization across a number of users. Serotyping fulfills these requirements, and the LEP scheme for *C. jejuni* and *C. coli* has demonstrated its applicability in this pilot study in Wales. The scheme has now been adopted as the basis of reference typing in England and Wales.

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## REFERENCES

1. **Abbott, J. D., B. Dale, J. Eldridge, D. M. Jones, and E. M. Sutcliffe.** 1980. Serotyping of *Campylobacter jejuni/coli*. J. Clin. Pathol. **33:**762–766.
2. **Adak, G. K., J. M. Cowden, S. Nicholas, and H. S. Evans.** 1995. The Public Health Laboratory Service national case-control study of primary indigenous sporadic cases of campylobacter infection. Epidemiol. Infect. **115:**15–22.
3. **Advisory Committee on the Microbiological Safety of Foods.** 1993. Interim report on campylobacter. Her Majesty's Stationery Office, London, United Kingdom.
4. **Asrat, D. A., A. Hathaway, E. S. Sjögren, E. Ekwall, and B. Kaijser.** 1997. The serotype distribution of *Campylobacter jejuni* and *Campylobacter coli* isolated from patients with diarrhea and controls in Tikur Anbassa Hospital, Addis Ababa, Ethiopia. Epidemiol Infect. **118:**91–95.
5. **Bokkenheuser, V.** 1972. *Vibrio fetus* infection in man: a serological test. Infect. Immun. **5:**222–226.
6. **Bolton, F. J., D. R. A. Wareing, M. B. Skirrow, and D. N. Hutchinson.** 1992. Identification and biotyping of campylobacters, p. 151–161. *In* G. R. Board, D. Jones, and F. A. Skinner (ed.), Identification methods in applied and environmental microbiology. Blackwell Scientific Publications, Oxford, United Kingdom.

7. **Chart, H., J. A. Frost, A. N. Oza, R. T. Thwaites, S. A. Gillanders, and B. Rowe.** 1996. Heat-stable serotyping antigens expressed by strains of *Campylobacter jejuni* are probably capsular and not long-chain lipopolysaccharide. J. Appl. Bacteriol. **81:**635–640.
8. **Fricker, C. R., M. M. Alemohammed, and R. W. A. Park.** 1987. A study of factors affecting the sensitivity of the passive haemagglutination method for serotyping *Campylobacter jejuni* and *Campylobacter coli* and recommendations for a more rapid procedure. Can. J. Microbiol. **33:**33–39.
9. **Gibson, J., E. Lorenz, and R. J. Owen.** 1997. Lineages within *Campylobacter jejuni* defined by numerical analysis of pulsed-field gel electrophoretic DNA profiles. J. Med. Microbiol. **46:**157–163.
10. **Healing, T. D., M. H. Greenwood, and A. D. Pearson.** 1992. *Campylobacters* and enteritis. Rev. Med. Microbiol. **3:**159–167.
11. **Jacobs-Reitma, W. F., H. M. E. Maas, and W. H. Jansen.** 1995. Penner serotyping of Campylobacter isolates from poultry, with absorbed pooled antisera. J. Appl. Bacteriol. **79:**286–291.
12. **Jones, D. M., J. D. Abbott, M. J. Painter, and E. M. Sutcliffe.** 1984. A comparison of biotypes and serotypes of *Campylobacter* spp. isolated from patients with enteritis and from animal and environmental sources. J. Infect. **9:**51–58.
13. **Jones, D. M., E. M. Sutcliffe, and J. D. Abbott.** 1985. Serotyping of *Campylobacter* spp. by combined use of two methods. Eur. J. Clin. Microbiol. **4:**562–565.
14. **Lior, H., D. L. Woodward, J. A. Edgar, L. J. Laroche, and P. Gill.** 1982. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. J. Clin. Microbiol. **15:**761–768.
15. **Logan, S. M., and T. J. Trust.** 1984. Structural and antigenic heterogenicity of lipopolysaccharides of *Campylobacter jejuni* and *Campylobacter coli*. Infect. Immun. **45:**210–216.
16. **Mills, S. D., B. Kuznier, B. Shames, B. Kurjanczyk, and J. L. Penner.** 1992. Variation of the O antigen of *Campylobacter jejuni in vivo*. J. Med. Microbiol. **36:**215–219.
17. **Nishimura, M., M. Nukina, J. M. Yuan, B. Q. Shen, J. J. Ma, M. Ohta, T. Saida, and T. Uchiyama.** 1996. PCR-based restriction fragment length polymorphism (RFLP) analysis and serotyping of *Campylobacter jejuni* isolates from diarrheic patients in China and Japan. FEMS Microbiol. Lett. **142:**133–138.
18. **Owen, R. J., and J. R. Gibson.** 1995. Update on epidemiological typing of *Campylobacter*. PHLS Microbiol. Digest **12:**2–6.
19. **Owen, R. J., K. Sutherland, C. Fitzgerald, J. Gibson, P. Borman, and J. Stanley.** 1995. Molecular subtyping scheme for serotypes HS1 and HS4 of *Campylobacter jejuni*. J. Clin. Microbiol. **33:**872–877.
20. **Patton, C. M., T. J. Barrett, and G. K. Morris.** 1985. Comparison of the Penner and Lior methods for serotyping *Campylobacter* spp. J. Clin. Microbiol. **22:**558–565.
21. **Patton, C. M., and I. K. Wachsmuth.** 1992. Typing schemes—are current methods useful? p. 110–128. *In* I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni*: current status and future trends. American Society for Microbiology, Washington, D.C.
22. **Patton, C. M., I. K. Wachsmuth, G. M. Evins, J. A. Keihlbauch, B. D. Plikaytis, N. Troup, L. Tompkins, and H. Lior.** 1991. Evaluation of 10 methods to distinguish epidemic-associated *Campylobacter* strains. J. Clin. Microbiol. **29:**680–688.
23. **Penner, J. L.** 1988. The genus *Campylobacter*: a decade of progress. Clin. Microbiol. Rev. **1:**157–172.
24. **Penner, J. L., and J. N. Hennessy.** 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. J. Clin. Microbiol. **12:**732–737.
25. **Preston, M. A., and J. L. Penner.** 1987. Structural and antigenic properties of lipopolysaccharides from serotype reference strains of *Campylobacter jejuni*. Infect. Immun. **55:**1806–1812.
26. **Preston, M. A., and J. L. Penner.** 1989. Characterization of cross-reacting serotypes of *Campylobacter jejuni*. Can. J. Microbiol. **35:**265–273.
27. **Thomson-Carter, F.** 1996. Campylobacter typing. SCIEH Weekly Rep. **30**(96/17)**:**95.