Lectin typing of Campylobacter isolates

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Abstract

Isolates of *Campylobacter jejuni, C coli, C fetus* and *C laridis* were tested for agglutination reactions with a panel of five lectins: *Arachis hypogaea, Bauhinia purpurea, Solanum tuberosum, Triticum vulgaris* and *Wisteria floribunda*. Twenty three patterns of agglutination (lectin types) were recorded among 376 isolates. Patterns were consistent and reproducible. Only 4·5% of isolates were untypable because of autoagglutination. Some lectin types were found exclusively or predominantly in a species, but others were shared between species. Forty two per cent of *C jejuni* and 35% of *C coli* isolates belonged to lectin type 4. There was no apparent correlation between lectin type and serotype; different lectin types were found among strains of single Penner and Lior serotypes. Lectin typing is a simple and economical procedure suitable for use in non-specialist laboratories, either as an adjunct to serogrouping or, after further development, as a sole typing scheme.

Campylobacters are microaerophilic Gram negative rods now recognised as the commonest cause of acute enteritis in the developed world. Less commonly, *Campylobacter* species may cause septicaemia, meningitis, and endocarditis. Any campylobacter infection may be followed by a reactive arthritis.

Many of the biological properties of bacteria are determined by their outer membrane lipopolysaccharide structure. Serological specificity is conferred in part by the variable polysaccharide portion of the lipopolysaccharide or somatic O antigen. The lipopolysaccharide portion of *Campylobacter* species includes the following carbohydrate moieties: glucose (Glc), N-acetyl-glucosamine (GlcNAc), galactose (Gal), N-acetyl-galactosamine (GalNAc), mannose (Man), L-glycero-D-mannoheptose, 3-amino-3,6-dideoxy-D-glucose and 2-keto-3-deoxy-octulosonic acid.[1 2] Lectins chosen for the study of campylobacters must express a specificity for at least one of these carbohydrate moieties.

A simple method for typing Campylobacter isolates that does not depend on raising antisera in animals would be invaluable. Lectins (from the latin *legere*, to pick out or choose) are possible candidates. They are sugar binding proteins or glycoproteins of non-immune origin which agglutinate or precipitate glycoconjugates.[3] The specificity of lectin binding to bacterial surface carbohydrates has been described[4] and may be exploited as a tool for typing micro-organisms, such as *Neisseria*,[5] *Legionella*,[6] *Bacillus*[7] and *Staphylococcus* species.[8]

Preliminary results of lectin typing of *C jejuni* and *C coli* have been described by Wong *et al*.[9 10]

The aims of our study were to evaluate the feasibility and reproducibility of lectin typing of Campylobacter isolates in a routine medical microbiology laboratory and to compare the results of lectin and serological typing methods.

Methods

Campylobacter isolates were either fresh cultures (subcultured not more than three times and checked for purity), or were obtained from stocks which had been stored in liquid nitrogen immediately after primary isolation.

The organisms were grown on horse blood agar in a microaerophilic atmosphere, produced by evacuating a jar to a negative pressure of 500 mm Hg and refilling with a gas mixture of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide, at either 37°C or 42°C for 48 hours. Bacteria were suspended in 3 ml amounts of pH 7·2 phosphate buffered saline (PBS) in 5 ml bijou bottles.

Suspensions were placed in a boiling water bath for one hour and then centrifuged for 15 minutes at 3000 rpm. The supernatant was discarded and fresh PBS was added to resuspend the deposit to a turbidity giving 50% transmission at 560 nm in a spectrophotometer.

The lectins were obtained from EY Laboratories (San Mateo, California, USA) and Sigma (Sigma Chemical Co, St Louis, Missouri). The major sugar specificities of each lectin are shown in table 1. Lectins were dissolved in either PBS (pH 6·8 or 7·2), or in

Table 1 Major sugar specificities of lectins used in this study

Lectin from	*Abbreviation*	*Major specificity*
Arachis hypogaea	PNA	β-D-gal-(1,3)-D-GalNAc
Bauhinia purpurea	BPA	D-GalNAc
Solanum tuberosum	STA	$(D\text{-}GlcNAc)_2$
Triticum vulgaris	WGA	$(\beta\text{-}(1\text{-}4)\text{-}D\text{-}GlcNAc)_2$
Wisteria floribunda	WFA	D-GalNAc

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Accepted for publication
6 July 1990

TRIS hydrochloride buffer (0·01M, pH 7·2) according to the manufacturer's instructions. Both buffers contained calcium chloride and magnesium chloride (0·02% each). Stock solutions containing 1 mg lectin/ml were stored at −20°C. Working solutions were prepared by diluting stock solutions in the appropriate buffer to a concentration of 200 μg/ml.

Fifty microlitres of each bacterial suspension and 50 μl of each lectin solution were added to U-bottomed wells in a microtitre tray. A negative control was included for each organism by adding 50 μl of PBS in place of lectin solution. Known positive control organisms were included in each test batch. The trays were agitated on a mechanical shaker for 15 seconds, left undisturbed at room temperature for one hour, and then incubated at 4°C for 18 hours.

Plates were read with the aid of a × 4 hand lens against a dark background. The presence or absence of agglutination was assessed with reference to the negative controls. Strains that gave an agglutination reaction in the negative control well were recorded as showing autoagglutination and considered non-typable. Thirty three isolates had been previously serotyped according to the Penner and Lior systems.

Results

The Campylobacter isolates tested are listed in table 2.

A panel of five lectins permits 32 possible agglutination patterns. Twenty three patterns were obtained among the 376 isolates. The patterns were arbitrarily numberd 1 to 23 (table 3). Comparison of results using lectins from Sigma and EY laboratories yielded identical results.

Figures 1–5 show the distribution of the 23 patterns among each of the *Campylobacter* species. Autoagglutination reactions occurred in 4·5% of tests.

There was no apparent correlation between lectin and serological types in the 33 isolates which were assessed (table 4). Lectin typing, however, distinguished between two strains of identical Penner and Lior types.

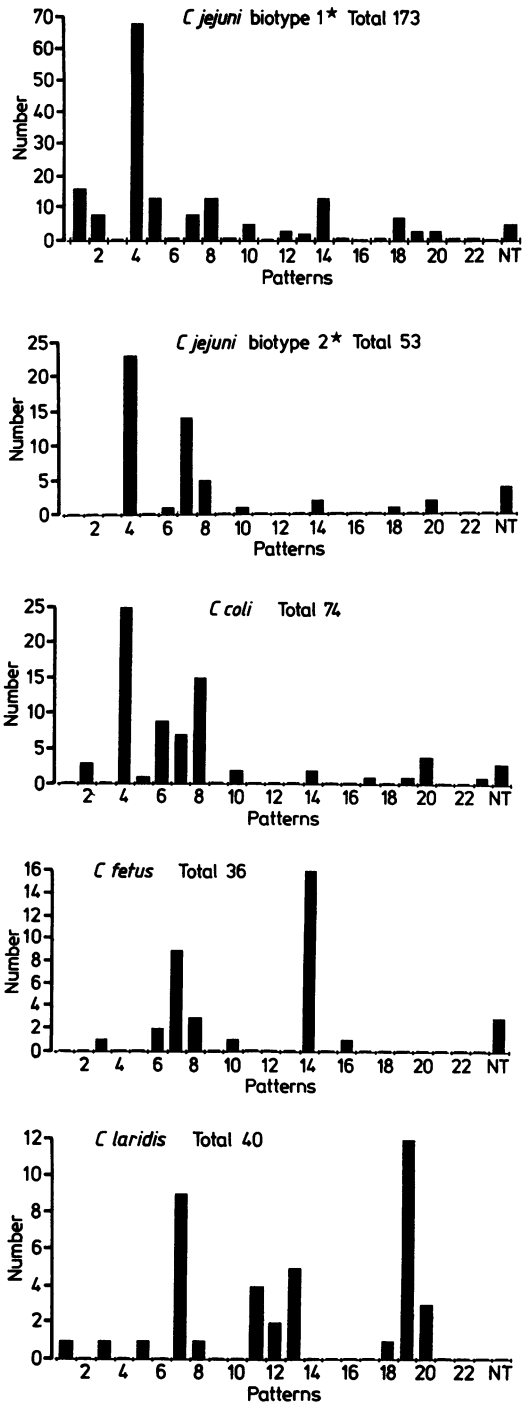
Table 2 Source of Campylobacter isolates

Organism	*Number of isolates*	*Source*
C jejuni biotype 1	173	Human
C jejuni biotype 2	53	Human
C coli	74	Human
C laridis	40	Animal
C fetus	36	Human and animal
Total	376	

Table 3 Patterns of lectin agglutination

Lectin type	*WFA*	*WGA*	*PNA*	*BPA*	*STA*
1	+	+	+	+	+
2	+	+	−	+	+
3	+	+	−	+	−
4	+	+	−	−	+
5	+	+	−	−	−
6	−	−	−	−	+
7	−	−	−	−	−
8	−	+	−	−	+
9	+	−	−	+	+
10	−	+	−	+	+
11	+	−	−	−	−
12	−	−	−	+	−
13	+	+	+	+	−
14	+	+	+	−	+
15	−	+	−	−	−
16	−	+	+	−	−
17	−	+	+	−	+
18	+	−	−	+	−
19	+	−	+	+	−
20	+	−	+	+	+
21	+	−	−	−	+
22	+	−	+	−	−
23	−	−	+	+	+

+ = Agglutination.
− = Absence of agglutination.



*Figures 1–5 Distribution of 23 lectin binding patterns. NT = non-typable. *Skirrow and Benjamin Scheme, 1980.*

Table 4 Comparison of lectin and serological typing for 33 Campylobacter isolates

Strain	Lectin type	Penner type	Lior type
C jejuni biotype 1 (20 isolates)	2	31	32
	2	35	26
	4	1	2
	4	1,44	1
	4	1	4
	4	—	4
	4	2	4
	4	4,13,16,50	1
	4	4,13,16,50	1
	4*	4,16,50	1
	4	16,50	1
	4	16,50	1
	4	5,50	7
	5*	4,16,50	1
	7	15	13
	7	—	36
	11	NT	—
	14	40	22
	14	41	27
	19	2	—
C jejuni biotype 2 (4 isolates)	4	52	30
	7	6	—
	7	19	17
	8	21	40
C coli (9 isolates)	4	—	46
	6	20	14
	6	28	—
	6	NT	8
	7	9,37	NT
	8	9,37	1
	8	51	45
	20	—	9
	23	47	47

*Example of isolates with identical Penner and Lior types but differing lectin types.
NT Not typable.

Once the organisms were harvested from agar plates and diluted in PBS, it was possible to delay until convenient any subsequent stage of preparation up to the addition of lectins. Parallel experiments using freshly prepared campylobacter solutions and solutions stored at 4°C for up to seven days gave identical results.

Discussion

The lectins evaluated in this study were selected because their major specificities were for common saccharides present at the bacterial cell surface. The results of this study indicate that the campylobacter cell surface contains terminal α- and β-GalNAc residues (BPA and WFA binding), β-GlcNAc residues (WGA and STA binding), β-Gal residues (PNA binding).

The lack of correlation between lectin type and traditional serogrouping is not surprising. The Penner scheme is known to depend on extracted thermostable antigens which have been identified as somatic lipopolysaccharide (LPS) somatic O antigen.[11] The Lior scheme is based on thermolabile antigens believed to be proteins.[12] Lectins bind exclusively to carbohydrates, hence the lack of correlation with Penner and Lior schemes.

The exact sequence of carbohydrates to which a particular lectin binds is unknown, although the major specificity of each lectin may be determined using "blocking" sugars. It is interesting to note that lectins inhibited by the same monosaccharides, such as BPA and WFA, show different patterns of binding to *Campylobacter* species. This observation confirms that BPA and WFA, although inhibited by the same monosaccharide, do not bind to identical GalNAc- containing carbohydrate sequences. This also illustrates that the carbohydrate sequences to which different lectins bind are more complex than the simple monosaccharides which are commonly used to describe lectin specificity. When lectins bind to carbohydrates in glycoproteins and glycolipids they interact with specific domains of relatively complex oligosaccharides. This is so even when lectins are inhibited by dilute solutions of free sugars. Based on their sensitivity to different carbohydrate inhibitors, two modes of oligosaccharide recognition may be identified.[13] In the simpler case lectin reactivity is directed principally towards a particular monosaccharide constituent in the ligand. This is so with lectins which are strongly inhibited by low concentrations of the appropriate free sugars. In the more complex recognition mode, lectins react only with specific carbohydrate sequences, and none of the individual sugars in the sequence has a predominant role in the binding process.

In this study 95·5% of strains were typable. This compares favourably with Penner and Lior typing. Lectin typing is not suitable for species differentiation, although certain patterns were seen only with certain species.

The discriminating power of lectin typing with this set of five lectins was not ideal; 42% of *C jejuni*, and 35% of *C coli* isolates belonged to lectin type 4. Type 4, however, was not found in any of *C fetus* or *C laridis* isolates tested. Even the Penner and Lior serotyping schemes are not free from this problem when used individually; some 20% of sporadic isolates commonly fall into one subgroup. As lectin typing was able to recognise strains with a single Penner and Lior serotype it could be a useful adjunct to serotyping.

In essence, each lectin will recognise only a small proportion of the carbohydrate on the cell surface. Hence it is possible to improve discrimination of lectin typing by including other appropriate lectins in the panel. For example, we have succeeded in dividing *C jejuni* of lectin type 4 into five subtypes using an additional three lectins: *Bandeiraea simplicifolia* l, *Glycine max* (soyabean), and *Ricinus communis* (unpublished results). Further work is progressing in our laboratory to refine this technique.

Routine serotyping of *Campylobacter* species in busy clinical laboratories is not practical.[14] Lectin typing is simple to perform and does not require specialised equipment. Lectins are inexpensive (£0·50p per isolate with a panel of five lectins), easy to obtain, and stable. Identical results were observed with all batches from two manufacturers. This contrasts with the expense and rigorous quality control necessary when using antisera from mammals. Lectins may be stored at −20°C for more than a year with no apparent loss of reactivity. The method described may be interrupted at any stage and resumed when convenient without affecting results.

We thank Dr K H Wong for suplying the positive control organisms.

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