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The cellular fatty acid composition of *Campylobacter* species isolated from cases of enteritis in man and animals

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SUMMARY

The cellular fatty acid composition of 41 strains of suspected *Campylobacter jejuni*, 23 from human cases of gastroenteritis and 18 from animals, was examined by gas–liquid chromatography. Three of the 23 human isolates and 2 of 18 animal isolates did not contain 19:0 cyclopropane fatty acid and were identified as *C. laridis*. The remaining 36 strains had cellular fatty acid profiles consistent with *C. jejuni* but could be divided into three groups on the ratio of the concentration of 18:1 and 19:0 cycloproprane. Most human isolates (85%) were in groups II or III whereas most animal isolates (56%) were in group I. It is proposed that gas–liquid chromatographic analysis of cellular fatty acids is a relatively easy method for epidemiological typing of *C. jejuni* isolates.

INTRODUCTION

Campylobacter spp. are now recognized as major enteric pathogens in animals and man, causing diarrhoea, enteritis and occasionally septicaemia, and equalling or exceeding *Salmonella* and *Shigella* spp. in prevalence (Munroe, Prescott & Penner, 1983). In most instances of campylobacter diarrhoea in man, *C. jejuni* has been identified as the cause, but recent publications on the occurrence of *C. coli* (Blaser & Reller, 1981) and *C. fetus* subsp. *fetus* as a cause of gastroenteritis (Harvey & Greenwood, 1983), and the isolation of *C. laridis* (Benjamin *et al.* 1983) from the faeces of patients with diarrhoea indicates that several *Campylobacter* spp. may cause gastroenteritis in man. With this increased awareness of *Campylobacter* spp. as human pathogens the speciation and typing of campylobacter isolates has become more important from both a diagnostic and epidemiological view point.

Recently there have been attempts to develop chemical, serological and fluorescent antibody techniques for identification of campylobacter isolates, so

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that the source of infection can be determined. Blaser, Moss & Weaver (1980) reported on the cellular fatty acid composition of 13 strains of *C. jejuni*, 9 from man and 4 from fowls. They found that all strains had a characteristic fatty acid composition that enabled them to be differentiated from '*C. fetus* ss *intestinalis*' and '*C. fetus* ss *fetus*'. However, these authors did not analyse any *C. laridis* strains in their study, nor did they attempt to group isolates on the basis of their cellular fatty acid composition and so the usefulness of the technique for epidemiological investigations was limited.

The several serotyping methods that have been described are either time consuming to perform or they require the production of absorbed antisera; consequently facilities are limited. There is a need for a more simple typing system.

In this paper we describe the use of gas–liquid chromatographic analysis of cellular fatty acids in order to group strains of *C. jejuni* and *C. laridis* isolated from cases of gastroenteritis in man and to compare those strains with isolates obtained from the intestinal tract of animals.

MATERIALS AND METHODS

Forty-one strains of suspected *C. jejuni* were investigated. The strains consisted of 23 isolated from stools of patients with gastroenteritis and 18 isolated from intestinal tracts of food animals. All strains were identified tentatively as *C. jejuni* at primary isolation on morphology and staining characteristics, but were identified further using selected biochemical tests, which included growth at 25 and 42 °C, growth in the presence of 1 % glycine, production of H_2S, hippurate hydrolysis and nalidixic acid sensitivity. *C. laridis* isolates were differentiated from *C. jejuni* on the basis of resistance to nalidixic acid (30 μg) and by the inability to hydrolyse hippurate.

Bacterial cells intended for fatty acid analysis were grown on duplicate plates of Columbia agar (Oxoid) containing 5 % defibrinated horse blood. The plates were incubated for 48 h at 37 °C in a sealed metal tin in an atmosphere of 5 % O_2, 10 % CO_2 and 85 % N_2. The cells were removed from the plates and processed to prepare fatty acid methyl esters according to the method of Moss (1979). Fatty acids were separated and measured by a gas–liquid chromatograph equipped with a 6 ft × $\frac{1}{4}$ in glass column packed with 3 % SP 2100 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, Pa.). Fatty acids were identified by comparison of g.l.c. retention times with those of purified fatty acid methyl ester standards (Supelco, Inc., Bellefonte, Pa.).

RESULTS

The cellular fatty acid profiles of representative strains of *C. jejuni* and *C. laridis* are shown in Fig. 1 and the relative fatty acid composition of human and animal isolates are shown in Tables 1 and 2 respectively. All strains of *C. jejuni* isolated from man or animals contained the cellular fatty acid 14:0, 3-OH 14:0, 16:0, 18:1 and 19:0 cyclopropane (cyc). In addition some strains contained varying amounts of 16:1 and 18:0. All the *C. laridis* isolates contained 14:0, 3-OH 14:0, 16:1, 16:0 and 18:1, but 19:0 cyc was absent.

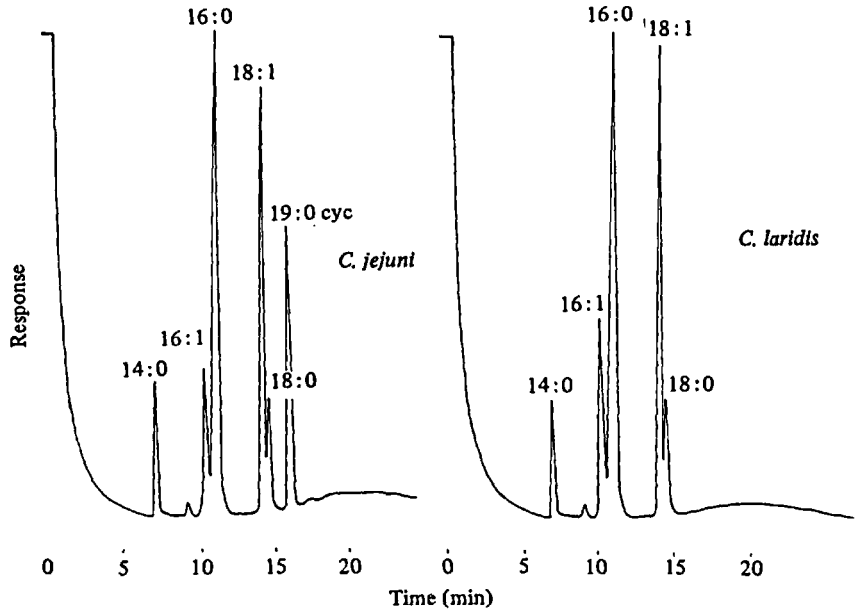

Fig. 1. Cellular fatty-acid profiles of *C. jejuni* and *C. laridis*.

Table 1. *Cellular fatty acid composition of strains of* Campylobacter jejuni *and* C. laridis *isolated from cases of human gastroenteritis*

G.l.c. group	Organism	Fatty acids (% of total) 14:0	3-OH 14:0	16:1	16:0	18:1	18:0	19:0 cyc	Ratio 18:1/ 19:0 cyc
I	*C. jejuni* ($n = 3$)	7·3	1·1	4·3	38·7	36·7	0	11·9	> 2
II	*C. jejuni* ($n = 9$)	10·6	3·4	6·3	38·7	20·9	0	20·1	0·5–2·0
II	*C. jejuni* ($n = 8$)	8·6	2·1	5·1	41·1	13·7	0	29·4	< 0·5
V	*C. laridis* ($n = 3$)	7·1	4·4	4·4	43·1	39·6	7·4	0	0

Table 2. *Cellular fatty acid composition of* Campylobacter jejuni *and* C. laridis *strains isolated from animal sources*

G.l.c. group	Organism	Fatty acids (% of total) 14:0	3-OH 14:0	16:1	16:0	18:1	18:0	19:0 cyc	Ratio 18:1/ 19:0 cyc
I	*C. jejuni* ($n = 9$)	7·3	4·8	5·8	41·6	28·0	0	12·5	> 2
II	*C. jejuni* ($n = 5$)	13·7	2·6	7·1	37·1	18·9	0	30·6	0·5–2
III	*C. jejuni* ($n = 2$)	4·9	4·6	0	42·6	12·2	3·1	32·6	< 0·5
IV	*C. laridis* ($n = 2$)	6·7	2·4	12·2	39·2	39·5	0	0	0

It was possible to divide the 23 human campylobacter isolates into four groups based on the ratio of the relative proportions of 18:1 to 19:0 cyc. Groups I, II, and III, which included all the *C. jejuni* isolates, had 18:1/19:0 cyc ratios of greater than 2:1, about 1:1 and less than 1:2 respectively, whereas group IV isolates (*C. laridis*) were clearly differentiated because of the absence of 19:0 cyc. To confirm the reproducibility of these findings one strain of each group of human

and animal isolates was analysed on five separate occasions. The relative fatty acid concentrations of the strains on each occasion was within 2%.

Of the 18 animal isolates of suspect *C. jejuni*, 16 were confirmed as *C. jejuni* and 2 as *C. laridis*. The fatty acid profiles of the isolates were identical to those obtained from the human isolates and they could be placed into the same groups on the basis of their 18:1 to 19:0 cyc ratios. However, 85% of the *C. jejuni* isolates were of groups II and III, whereas most (56%) of the animal isolates were of group I.

DISCUSSION

All of the human isolates studied in this paper were obtained from patients with acute gastroenteritis in whom other potential causes of the gastroenteritis had been eliminated. The isolates were tentatively identified as *C. jejuni* on the basis of morphology and limited biochemical tests of the type that would normally be used in a hospital diagnostic laboratory, yet g.l.c. analysis of the strains showed that 13% of the human isolates did not produce 19:0 cyc and that these isolates should be classified as *C. laridis* not *C. jejuni*.

Four fatty acids (14:0, 3-OH 14:0, 16:1, 16:0 and 18:1) were common to both *Campylobacter* spp., but 19:0 cyc was always found in *C. jejuni* and was always absent in *C. laridis*. These results are in general agreement with those of Blaser, Moss & Weaver (1980) and Moss, Lambert & Patton (1984). We are also in agreement with Blaser, Moss & Weaver (1980) and Curtis (1982) that both *C. jejuni* and *C. laridis* contain 3-OH 14:0, but not with Smibert (1978) and Tornabene & Ogg (1971) who did not detect any 3-OH 14:0. However, the particular feature of interest in our results is the finding of significant variation in the proportions of the component fatty acids which enabled us to divide the *C. jejuni* isolates into three groups. These groups could be used for epidemiological tracing of the source of campylobacter gastroenteritis outbreaks.

Further analysis of the results of Curtis (1982) by our criteria for classification, namely the ratio of 18:1/19:0 cyc, shows that his isolates fall into group I. Similarly, the isolates of Blaser, Moss & Weaver, (1980) fall into group II, perhaps reflecting the variation in *C. jejuni* strains in different parts of the world. However, further studies would be necessary to verify this hypothesis.

With *C. laridis* strains isolated from up to 13% of cases of human gastroenteritis, and the reports of *C. fetus* ss *fetus* and *C. coli* as causes of gastroenteritis in man (Harvey & Greenwood, 1983; Blaser & Reller, 1981), there is a need for more deliberate speciation and typing of campylobacter isolates. G.l.c. analysis of cellular fatty acids, when used with accepted biochemical tests and seriological typing, can provide improved, rapid identification of isolates and hasten epidemiological tracing of the sources of campylobacter infection. Similar techniques have been used successfully for the identification of *Legionella* spp. (Moss, 1979).

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