Distribution of sero-biotypes of *Campylobacter jejuni* and *C. coli* isolated from paediatric patients

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Summary. During a one-year period, 258 isolates of *Campylobacter jejuni* and *C. coli* were obtained from children with gastroenteritis or bacteraemia at the Red Cross Children's Hospital, Cape Town, South Africa. These isolates were biotyped by hippurate hydrolysis, H₂S production and tolerance to 2,3,5-triphenyltetrazolium chloride (TTC). Our study indicated that 95.4% of the isolates were *C. jejuni* biotype 1, 1.5% were *C. jejuni* biotype 2 and 3.1% were *C. coli*; 70% of the isolates were resistant to TTC. Serotyping on the basis of soluble, thermostable antigens detected by a passive-haemagglutination technique revealed that 79% of the Cape Town isolates were typable and that the most common serotypes, in order, were: 4, 2, 12, 23/36 and 19, together comprising 25% of the isolates. About 37% of the typable isolates belonged to nine serotypes. The finding that 21% of the isolates were non-typable suggests the existence of antigenic specificities different from those defined by the 60 antisera in current use.

Introduction

Campylobacter spp. are firmly established as common causes of gastroenteritis in man, particularly in children under the age of two years (Butzler and Skirrow, 1979; Rettig, 1979; Anders *et al.*, 1981). *Campylobacter* spp. have also been implicated in bacteraemia in young children (Lastovica and Penner, 1983). These organisms have been found in a wide variety of animals, including cattle, sheep, gulls, monkeys, pigs and domestic pets such as cats and dogs (Fernie and Park, 1977; Butzler and Skirrow, 1979; Skirrow and Benjamin, 1980*b*). Epidemiological studies have shown that campylobacteriosis may be acquired by contact with animals or by the ingestion of contaminated water or foods, in particular poultry, unpasteurised milk and red meat (Butzler and Skirrow, 1979; Skirrow and Benjamin, 1980*b*). Furthermore, it is clear that *Campylobacter*-associated gastroenteritis is an important medical problem in many countries (Schewitz and Roux, 1978; Bokkenheuser *et al.*, 1979; Karmali and Fleming, 1979; Anders *et al.*, 1981).

Further discrimination of clinical isolates of *Campylobacter* by biotyping and serotyping should greatly assist our understanding of their epidemiology. Harvey (1980) has demonstrated that *C.*

jejuni and *C. coli* can be discriminated by a test for hippurate hydrolysis; isolates of *C. jejuni* are hippurate positive and those of *C. coli* are hippurate negative. Skirrow and Benjamin (1980 *a* and *b*) extended this biochemical profile by adding a test for H₂S production that enabled isolates to be designated as *C. jejuni* biotype 1 or 2 or as *C. coli*. They also found that tolerance of 2, 3, 5-triphenyltetrazolium chloride was useful in identifying isolates of *Campylobacter*. The usefulness of serotyping *Campylobacter* spp. by determination of heat-stable antigens has been reported (Penner and Hennessy, 1980; McMyne *et al.*, 1982). In this report we present the sero-biotype distribution of strains of *C. jejuni* and *C. coli* isolated from patients with gastroenteritis or bacteraemia, who were seen at a Children's Hospital in Cape Town during a one-year period.

Materials and methods

Isolation and growth of campylobacters

Diarrhoeal stools were submitted to the routine-microbiology laboratory of the Red Cross War Memorial Children's Hospital, Cape Town, and were examined for species of *Campylobacter*, *Salmonella* and *Shigella* and for *Yersinia enterocolitica*. For the isolation of *Campylobacter* spp., stools were cultured on plates of Tryptose Blood Agar Base (CM 233, Oxoid) containing lysed horse

blood 5% v/v, Skirrow's Selective supplement (SR 69, Oxoid) and fungizone 1 µg/ml. Between September 1982 and February 1983, blood was cultured in a biphasic medium consisting of Mueller-Hinton Agar and Tryptic Soy Broth with Thioglycollate (all from Difco) and supplemented with polyanethol sulphate. Between March and August 1983, blood was cultured in Bactec 16B and 7C bottles (Johnston Laboratories, Cockeysville, MD, USA). Single colonies were picked from the selective medium for subculture on to plates of tryptose blood agar without antibiotics. Cultures obtained after growth at 37°C and 42°C in micro-aerophilic conditions for 48–72 h were identified as *C. jejuni* or *C. coli* on the basis of published criteria (Butzler and Skirrow, 1979; Skirrow and Benjamin, 1980b).

Biotyping

All isolates were biotyped in the Cape Town laboratory. Before serotyping, isolates were retested for hippurate hydrolysis in the Toronto laboratory. Biotyping was performed by the method of Skirrow and Benjamin (1980a). On the basis of hippurate hydrolysis and H₂S production in an iron-containing medium, each isolate was designated as *C. jejuni* biotype 1 or 2, or as *C. coli*. Each isolate was also tested for tolerance to TTC (2,3,5-triphenyltetrazolium chloride; Sigma) 400 µg/ml incorporated in plates of tryptose blood agar. On this medium, sensitive strains did not grow, or produced only a few colonies after prolonged cultivation, whereas resistant strains grew and formed the red pigment formazan.

Serotyping

All isolates were serotyped in the Toronto laboratory by the scheme of Penner and Hennessy (1980) now extended to include 60 typing antisera (Penner *et al.*, 1983). All isolates were tested with these 60 antisera and those found to be non-typable were tested with 16 other provisional antisera recently prepared against infrequently occurring serotypes and not yet included in the serotyping scheme. Antigens were extracted from bacterial-cell suspensions heated at 100°C for 1 h. Type-specific antisera were titrated by a passive-haemagglutination test against antigen-sensitised sheep erythrocytes. The serotype of the isolate was designated according to the antisera in which agglutination was observed. Only one isolate from each patient was included in our study.

Results

Of 300 clinical strains (294 from diarrhoeal stools and 6 from blood cultures) of *C. jejuni* and *C. coli* isolated between 1 Sep. 1982 and 31 Aug. 1983, 86% (258) were available for biotyping and serotyping. *Campylobacter* spp. were the bacterial pathogens most frequently isolated from the stools of children with diarrhoea (table I). Of these patients, 52% were

Table I. Relative frequency of isolation of enteric bacterial pathogens from 2951 stools* of patients with gastroenteritis

Species	Number (%) of isolates
<i>C. jejuni</i> and <i>C. coli</i>	294 (10.0)
<i>Salmonella</i> spp.	239 (8.1)
<i>Shigella</i> spp.	53 (1.8)
<i>Y. enterocolitica</i>	10 (0.3)

* Received at the Red Cross War Memorial Children's Hospital, Cape Town, South Africa during 1982–1983.

male and about one-third were out-patients. Cases of *Campylobacter*-associated gastroenteritis and bacteraemia were diagnosed throughout the year; the most occurred in February, the hottest month of the South African summer, and the least in July, the coldest and wettest month of the winter. The ages of the patients ranged from 1 week to 10 years (mean 12.7 months). Thus, 68% of the children were less than 1 year old, 25% of them 1–2 years old, 4% were 2–3 years old and only c. 3% were 4–10 years old (fig. 1).

Biotyping

Isolates of *C. jejuni* of biotypes 1 and 2 constituted, respectively, 95.4% and 1.5% of the series. Only eight isolates (3.1%) were hippurate negative; these were designated *C. coli*. All four isolates of *C. jejuni* biotype 2 were resistant to TTC whereas *c.*

Fig. 1. Distribution of isolates of *C. jejuni* and *C. coli* from children according to age.

70% of the isolates of *C. jejuni* biotype 1 and *C. coli* were resistant to TTC.

Serotyping

Of the isolates that were serotypable, 46% reacted with one or more of 38 of the 60 antisera in the established typing scheme, or with one or more of 6 of 16 antisera in the provisional scheme; *c.* 21% of the isolates reacted with two antisera and 12% reacted with three or more antisera. The frequency distribution of the most commonly occurring serotypes in our study is shown in table II. Together, non-typable isolates constituted the largest single group (21.3%) in this series. The nine most common serotypes amongst the typable isolates comprised *c.* 37% of all isolates. A further 61 serotypes (only six of which are listed, table II), each containing one to five representatives (<2%), accounted for the remainder of the typable isolates. Some of the latter typable strains reacted with only one antiserum, others with two or more antisera. Of the typable isolates from four blood cultures, one isolate each was of serotypes 1/33/6/7 and 2, and two were non-typable. Whereas six of the eight hippurate-negative isolates provisionally classified as *C. coli* were agglutinated by antisera against *C. coli* serotype-reference strains 20, 24, 34 and 49 (one strain each)

and 46 (two strains), another two isolates reacted only with *C. jejuni* antisera and were of serotypes 23/36 and 1/4/21/42. Conversely, five hippurate-positive strains, provisionally classified as *C. jejuni*, reacted with *C. coli* antisera and were of serotypes 34 and 46 (one strain each) and 49 (three strains). These observations were consistent with a previously reported finding that isolates generally react only with antisera prepared against strains of the same species (Penner *et al.*, 1983). Only four isolates were H₂S-positive and provisionally designated as *C. jejuni* biotype 2; they belonged to serotypes 33 and 1/33/6/7 (one strain each) and 42 (two strains). Of eight H₂S-negative isolates of *C. jejuni* biotype 1 and *C. coli*, four strains each belonged to serotypes 33 and 41. The most common serotypes of isolates found in stools with frank blood or mucus were, in order, 4, 2, 19, 12 and 33.

The serotype distribution of the TTC-sensitive and TTC-resistant isolates generally followed the serotype distribution shown in table II. Table III indicates the serotype distribution according to the age group of the patients, non-typable isolates predominating in each age group. Serotypes 1, 2 and 4 were most common in older children accounting for only 11.8% of the isolates in children <6 months old but for >19% in those >2 years old. Isolates reacting with two or more antisera accounted for 10.8% in the 7–12 month age-group and 38% in children >2 years old. Isolates of serotype 33 were not found in children >6 months old.

Seasonal variation of the different serotypes is depicted in fig. 2. Non-typable isolates were more common (24–30%) in summer and autumn and less common (4%) in the spring. Isolates of serotypes 1, 2, 3, 4 and 5 were most common (24%) in the winter and least common (13%) in the autumn. Serotypes that reacted with two or more antisera were common in spring (45%) but not in winter (5%).

Discussion

Karmali *et al.* (1983), in a 3-year study of 289 campylobacters isolated from paediatric patients, reported that 60% of the patients were <5 years old, 20% were 5–10 years old and the remainder were ≥10 years old. Our findings indicated that *Campylobacter*-associated gastroenteritis was much more prevalent in very young children in Cape Town; thus, 97% of our patients were <3 years old and 40% were <6 months old. The Cape Town isolates may have been more virulent for younger children because of plasmid-mediated factors; in a separate study, Ambrosio and Lastovica (1984) reported

Table II. Distribution of the most prevalent serotypes of *C. jejuni* and *C. coli* isolated from patients with gastroenteritis

Percentage of isolates of <i>Campylobacter</i> spp. belonging to the given serotype in three studies					
1* (n = 258)		2† (n = 289)		3‡ (n = 1586)	
Sero-type	Percent-age	Sero-type	Percent-age	Sero-type	Percent-age
NT	21.3	2	18.6	2	14.4
4	6.6	4	11.2	4	14.3
2	4.7	3	9.8	1	10.3
12	4.7	1	8.8	3	7.0
23/36	4.7	8	6.6	5	4.0
19	3.9	13/16	5.6	13/16	3.7
5	3.5	NT	3.0	NT	3.5
5/31	3.5	5	2.8	8	2.9
1	3.1	18	2.8	11	2.4
13/16/50	2.3	21	2.8	18	2.1
41	1.9	23	2.8	21	1.9
8/17	1.9	36	2.8	23	1.7
23/53	1.9			31	1.4
8	1.6			33	0.6
18	1.6			12	0.4
4/13/16	1.6			36	0.3

* 1 = This study, see footnote table I.

† 2 = Karmali *et al.* (1983).

‡ 3 = Penner *et al.* (1983).

NT = non-typable.

Table III. Distribution of serotypes of *C. jejuni* and *C. coli* isolated from patients* grouped by age

Percentage of isolates of <i>Campylobacter</i> spp. in the given serotypes from patients in age groups									
0-6 months (n = 102)		7-12 months (n = 74)		13-18 months (n = 36)		19-24 months (n = 25)		25-118 months (n = 21)	
Sero- type	Percent- age	Sero- type	Percent- age	Sero- type	Percent- age	Sero- type	Percent- age	Sero- type	Percent- age
NT	19.6	NT	17.6	NT	27.7	NT	20.0	NT	23.8
2	6.9	4	9.5	2	8.3	23/36	16.0	4	9.5
4	4.9	12	5.4	19	5.6	1	8.0	41	9.5
12	3.9	19	5.4	37	5.6	4	8.0	8/17	9.5
19	2.9	5/31	5.4	4	2.8	5	8.0	13/16/50	9.5
41	2.9	1	4.0	5	2.8	4/13/16/43	4.0	23/36	9.5
5	2.9	2	4.0	10	2.8	8	4.0	1	4.8
33	2.9	16	4.0	12	2.8	12	4.0	2	4.8
23/36	2.9	4/13/16/50	2.7	58	2.8	18	4.0	5	4.8
13/16/50	2.0	5	2.7	2/4	2.8	13/16/22	4.0	12	4.8
1/44	2.0	18	2.7	2/13/43	2.8	13/16/50	4.0	4/16/43	4.8
5/31	2.0	23	2.7	5/31	2.8	13/16/43/50	4.0	13/16/43	4.8
49	2.0	23/53	2.7	13/16/22	2.8	16/22/50	4.0		
8/17	2.0	10	1.4	13/16/50	2.8	23	4.0		
10	1.0	34	1.4	23/36	2.8	31	4.0		

* See table I.

NT = non-typable.

Fig. 2. Seasonal variation of *C. jejuni* and *C. coli* isolates that were non-typable ■, of serotypes 1-5 □, ■ agglutinated by multiple antisera.

that 48% of these isolates carried at least one plasmid.

Generally, our results indicated that the distribution of strains of *C. jejuni* of biotypes 1 and 2 in Cape Town was similar to that in Europe and North

America (Skirrow and Benjamin, 1982; Karmali *et al.*, 1983; Jones *et al.*, 1984a). From this and other studies, which indicated that the frequency of *C. coli* was 2-5% of all *Campylobacter* isolates, it is apparent that *C. coli* is a much less important cause of human gastroenteritis than *C. jejuni*. Whereas Skirrow and Benjamin (1980b) reported that one-third of 1120 isolates of *C. jejuni* and *C. coli* were resistant to TTC, we found that c. 70% of the Cape Town isolates were resistant to TTC. Although useful, the array of biotyping tests available at present does not provide sufficiently fine differentiation for precise epidemiological studies, especially among isolates of the same serotype.

Many of the serotypes present in North America and elsewhere were also found in our study. Of the Cape Town isolates, 79% were typable by a passive-haemagglutination method, indicating the usefulness of the serotyping scheme of Penner and Hennessy (1980) and the universality of the relevant typable antigens. Jones *et al.* (1984b) have demonstrated that the antigens involved in serotyping *Campylobacter* isolates by passive haemagglutination are lipopolysaccharides and that other cell-surface components are inactive. In some respects our serotyping results are very different from those published for isolates from Canada and elsewhere (Karmali *et al.*, 1983; Penner *et al.*, 1983), the most striking difference being the many non-typable isolates (21.3%) in our study as compared with only 3% in the other two reports. In other investigations

it was reported that about one-half of the isolates were of serotypes, 1, 2, 3 or 4, and Jones *et al.* (1984b) reported that just over half of their isolates were of serotypes 1/44, 2 and 4. In the present investigation, serotypes 1, 2, 3 or 4 comprised only c. 15% of the Cape Town isolates. Whereas serotype 3 was found only twice among our 258 patients (0.8%), Karmali *et al.* (1983) and Penner *et al.* (1983) reported frequencies of 9.7% and 7% respectively. Another pronounced difference in the serotype distribution of our isolates is the large number that were agglutinated by more than one antiserum. Our study has indicated that 33% of the Cape Town isolates reacted with two or more antisera whereas comparable studies of Karmali *et al.* (1983) and Penner *et al.* (1983) have reported, respectively,

frequencies of 12% and 18.6%. These observed differences probably indicate the geographical variability of *Campylobacter* spp.

Our finding that 21.3% of the Cape Town isolates were non-typable in tests with the 60 antisera currently used suggests the existence of, as yet, undefined antigenic specificities. Work is in progress to define serologically these non-typable isolates, to extend the usefulness of the serotyping scheme and to make it universally applicable.

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REFERENCES

- Ambrosio R E, Lastovica A J 1984 Improved plasmid yield from clinical isolates of *Campylobacter*. *South African Medical Journal* **65**:500-501.
- Anders B J, Lauer B A, Paisley J W 1981 *Campylobacter* gastroenteritis in neonates. *American Journal of Diseases of Childhood* **135**:900-902.
- Bokkenheuser V D, Richardson N J, Bryner J H, Roux D J, Schutte A B, Koornhof H J, Freiman I, Hartman E 1979 Detection of enteric *Campylobacteriosis* in children. *Journal of Clinical Microbiology* **9**:227-232.
- Butzler J P, Skirrow M B 1979 *Campylobacter* enteritis. *Clinics in Gastroenterology* **8**:737-765.
- Fernie D S, Park R W A 1977 The isolation and nature of campylobacters (microaerophilic vibrios) from laboratory and wild rodents. *Journal of Medical Microbiology* **10**:325-329.
- Harvey S M 1980 Hippurate hydrolysis by *Campylobacter fetus*. *Journal of Clinical Microbiology* **11**:435-437.
- Jones D M, Abbott J D, Painter M J, Sutcliffe E M 1984a A comparison of biotypes and serotypes of *Campylobacter* sp. isolated from patients with enteritis and from animal and environmental sources. *Journal of Infection* **9**:51-58.
- Jones D M, Fox A J, Eldridge J 1984b Characterization of the antigens involved in serotyping strains of *Campylobacter jejuni* by passive hemagglutination. *Current Microbiology* **10**:105-109.
- Karmali M A, Fleming P C 1979 *Campylobacter* enteritis in children. *Journal of Pediatrics* **94**:527-533.
- Karmali M A, Penner J L, Fleming P C, Williams A, Hennessy J N 1983 The serotype and biotype distribution of clinical isolates of *Campylobacter jejuni* and *Campylobacter coli* over a three-year period. *Journal of Infectious Diseases* **147**:243-246.
- Lastovica A J, Penner J L 1983 Serotypes of *Campylobacter jejuni* and *Campylobacter coli* in bacteremic, hospitalized children. *Journal of Infectious Diseases* **147**:592.
- McMyne P M S, Penner J L, Mathias R G, Black W A, Hennessy J N 1982 Serotyping of *Campylobacter jejuni* isolated from sporadic cases and outbreaks in British Columbia. *Journal of Clinical Microbiology* **16**:281-285.
- Penner J L, Hennessy J N 1980 Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. *Journal of Clinical Microbiology* **12**:732-737.
- Penner J L, Hennessy J N, Congi R V 1983 Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. *European Journal of Clinical Microbiology* **2**:378-383.
- Rettig P J 1979 *Campylobacter* infections in human beings. *Journal of Pediatrics* **94**:855-864.
- Schewitz I A, Roux E 1978 *Campylobacter* infections. First reports from Red Cross War Memorial Children's Hospital, Cape Town. *South African Medical Journal* **54**:385-388.
- Skirrow M B, Benjamin J 1980a Differentiation of enteropathogenic *Campylobacter*. *Journal of Clinical Pathology* **33**:1122.
- Skirrow M B, Benjamin J 1980b '1001' *Campylobacter*s: cultural characteristics of intestinal campylobacters from man and animals. *Journal of Hygiene* **85**:427-442.
- Skirrow M B, Benjamin J 1982 The classification of "thermophilic" campylobacters and their distribution in man and domestic animals. In: Newell D G (ed) *Campylobacter: epidemiology, pathogenesis and biochemistry*, MTP Press, Lancaster, pp 40-44.