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

A. J. LASTOVICA, ELZA LE ROUX, ROSA V. CONGI* and J. L. PENNER*

Department of Microbiology, Red Cross War Memorial Children's Hospital, Rondebosch 7700, Cape Town, South Africa, and *Department of Medical Microbiology, University of Toronto, Toronto, Ontario, M5G 1L5, Canada

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, 1980b). 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, 1980b). 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 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. coli.
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 sero-
types 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 remain-
er 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-
typable. Whereas six of the eight hippurate-negative
isolates provisionally classified as C. coli were
agglutinated by antisera against C. coli serotype-
typable. Whereas six of the eight hippurate-negative
isolates provisionally classified as C. coli were
agglutinated by antisera against C. coli serotype-
typable. Whereas six of the eight hippurate-negative
isolates provisionally classified as C. coli were
agglutinated by antisera against C. coli serotype-
typable. Whereas six of the eight hippurate-negative
isolates provisionally classified as C. coli were
agglutinated by antisera against C. coli serotype-
typable. Whereas six of the eight hippurate-negative
isolates provisionally classified as C. coli were
agglutinated by antisera against C. coli serotype-

Table II. Distribution of the most prevalent sero-
types of C. jejuni and C. coli isolated from patients
with gastroenteritis

<table>
<thead>
<tr>
<th>Sero-</th>
<th>Percentage of isolates of Campylobacter spp. belonging to the given serotype in three studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>* (* n = 258)</td>
<td>** (n = 289)</td>
</tr>
<tr>
<td>Sero-</td>
<td>Percentage</td>
</tr>
<tr>
<td>type</td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>23/36</td>
<td>4</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>5/31</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>13/16/50</td>
<td>2</td>
</tr>
<tr>
<td>41</td>
<td>1</td>
</tr>
<tr>
<td>8/17</td>
<td>1</td>
</tr>
<tr>
<td>23/53</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>4/13/16</td>
<td>1</td>
</tr>
</tbody>
</table>

1 = This study, see footnote table I.
2 = Karmali et al. (1983).
3 = Penner et al. (1983).
NT = non-typable.

Discussion

Karmali et al. (1983), in a 3-year study of 289
Campylobacter 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 Campy-
lobacter-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 III. Distribution of serotypes of *C. jejuni* and *C. coli* isolated from patients* grouped by age

| Percentage of isolates of *Campylobacter* 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) |
| Serotype | Percentage | Serotype | Percentage | Serotype | Percentage | Serotype | Percentage | Serotype | Percentage |
| 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 | 2/31 | 5.4 | 4 | 2.8 | 5 | 8.0 | 13/16/50 | 9.5 |
| 41 | 2.9 | 1/40 | 5.4 | 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 | 4 | 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 | 14 | 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 | 4/16/43 | 4.8 |
| 8/17 | 2.0 | 10 | 1.4 | 13/16/50 | 2.8 | 23 | 4.0 | 13/16/43 | 4.8 |
| 10 | 1.0 | 34 | 1.4 | 23/36 | 2.8 | 31 | 4.0 | 13/16/43 | 4.8 |

* See table I.

NT = non-typable.

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.

This study was supported by research grants from the South African Medical Research Council to AJL, and from Health and Welfare, Canada, to JLP. We thank Dr D. Hanslo, Microbiology Dept, Red Cross Hospital, Cape Town, for constructive criticism of the manuscript.

REFERENCES


