

Original Article

Clinical Characteristics and Serotype Distribution of *Campylobacter jejuni* and *Campylobacter coli* Isolated from Diarrhoeic Patients in Dhaka, Bangladesh, and Cape Town, South Africa

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Clinical characteristics and serotype distribution of *Campylobacter jejuni* and *Campylobacter coli* isolated from paediatric diarrhoeic patients over a three-year period in two geographically diverse areas, Dhaka, Bangladesh, and Cape Town, South Africa, were compared. Both Dhaka and Cape Town patients had similar rates of diarrhoea, vomiting and fever. However, the Dhaka patients were younger (11.8 vs. 13.0 months), had more boys than girls infected (male/female ratio 1.78:1 vs. 1.25:1), had more predisposing conditions (26% vs. 15%) and had more additional stool pathogens co-isolated with *C. jejuni/coli* than the Cape Town patients. While some *C. jejuni* serotypes were common to both areas, i.e., O:4, other serotypes were present in one location, but not the other. Differences in clinical presentations and serotype distribution in Dhaka and Cape Town are suggestive of different reservoirs for *Campylobacter*, and different patterns of infection.

Keywords: *Campylobacter jejuni*, *Campylobacter coli*, Serotyping, Diarrhoea

Introduction

Campylobacter species are universally acknowledged as the most frequently isolated bacterial pathogens associated with human gastroenteritis, particularly in young children¹. *Campylobacter* has also been associated with other clinical conditions such as bacteraemia²⁻³, the Guillain-Barré syndrome⁴, reactive arthritis and pancreatitis¹.

Most epidemiological investigations of *Campylobacter* infection have been done in developed countries, and the distribution, epidemiology and clinical relevance of *Campylobacter* species are not fully understood in developing countries. The aim of this study was to compare clinical details and the serotype distribution of *C. jejuni* and *C. coli* isolates recovered from diarrhoea patients in two geographically diverse areas with a high incidence of paediatric diarrhoea, Dhaka, Bangladesh, and Cape Town, South Africa.

Materials and Methods

This study, conducted in 2000-2002, included 7,128 diarrhoea patients of all age groups admitted to the Dhaka Hospital of the ICDDR,B: Centre for Health and Population Research. During

this same time period, 5,635 diarrhoea patients were admitted to the Red Cross Children's Hospital in Cape Town, South Africa.

In the Dhaka laboratory, *Campylobacter* organisms were isolated from stools by inoculation onto culture plates containing *Brucella* selective agar (SR83, Oxoid, Basingstoke, UK), containing 5% lysed sheep blood and antimicrobial agents including vancomycin (10 mg/l), trimethoprim (5 mg/l), polymyxin B (2,500 U/l), amphotericin B (2 mg/l) and cephalothin (15 mg/l). Culture plates were incubated under microaerobic conditions at 42°C in a candle jar and examined 48 h later. Culture plates considered negative were re-incubated for a further 24 h. *Campylobacter* isolates were identified by following standard methods⁵. Isolates were preserved in tryptic soy broth (BBL, New Delhi) plus 25% glycerol and stored at -80°C, and subsequently serotyped by the soluble heat-stable scheme of Penner⁶. The Cape Town laboratory supplied the 60 different reference antisera used for serotyping.

In the Cape Town laboratory, *Campylobacter*, *Arcobacter* or *Helicobacter* isolates were recovered from diarrhoeic stools by the "Cape Town" protocol⁷, which employs membrane filtration onto antibiotic-free tryptose blood agar (CM 233, Oxoid, Basingstoke, UK) containing 10% un-lysed horse blood, and incubation at 37°C

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in an H₂-enriched microaerobic atmosphere (BR38, Oxoid) for up to 6 days. Isolates were characterized as *C. jejuni* or *C. coli* on the basis of established tests^{1,5}. Serotyping was done with antisera raised against 60 reference bacteria of the Penner scheme⁶.

Results and Discussion

Of the 7,128 diarrhoeic stool specimens examined in the Dhaka laboratory, 604 (8.5%) were positive for *Campylobacter jejuni* and *Campylobacter coli*. One hundred and eighty one (30%) of the *Campylobacter* isolates were randomly selected for clinical assessment and serotyping. Ages of the patients ranged from 1 month to 60 years, and 115 (64%) of the 181 patients were less than 6 years old (Table 1). The male/female ratio was 1.78 : 1.00. Almost all (96%) of the Dhaka patients had diarrhoea, 10% had vomiting and 7% had fever. For 50% of the stools from patients in Dhaka, *Campylobacter* was the sole pathogen isolated. In the remaining stools, *Campylobacter* was co-isolated with one or more of isolates of *Vibrio*, *Shigella*, *Salmonella*, *Aeromonas*, *Plesiomonas*, or *Hafnia*. Most *Campylobacter* isolations occurred in March to May, the hottest season of the year, and preceding the annual monsoon season.

Table 1. Clinical characteristics of patients with *Campylobacter jejuni* or *Campylobacter coli* infection in Dhaka, Bangladesh and Cape Town, South Africa

Feature	Dhaka, Bangladesh	Cape Town, South Africa
No. of patients	181	348
Age range	1 month - 60 years	1 month - 12 years
Male/female ratio	1.78 / 1.00	1.25 / 1.00
<2-year-old % (mean age)	50.3 (11.8 months)	65.1 (13.0 months)
<i>Campylobacter</i> as sole pathogen (%)	50.0	89.0
Stool characteristics (%) (watery, loose, dysenteric)*	95.5, 0.0, 4.5	10.6, 78.2, 11.2
Diarrhoea, Vomiting, Fever >38°C (%)	96.0, 10.0, 7.0	93.0, 8.0, 5.0
Pre-existing clinical conditions (%)**	26.0	15.0

* = Dhaka patients, loose and watery stools were grouped together;

** = Anaemia, respiratory distress, HIV positive, malnourished

Of the 5,635 diarrhoea stool specimens examined in the Cape Town laboratory, 999 (17.8%) were positive for 10 species of *Campylobacter*, 2 species of *Helicobacter* and *Arcobacter butzleri*. Of these 999 isolates, 316 were identified as *C. jejuni* and 32 as *C. coli* that formed 34.8% (348/999) of the total campylobacteraceae or helicobacteraceae isolated (Table 2). These 348 *C. jejuni* or *C. coli* isolates were selected for clinical assessment and serotyping⁶, for a direct comparison with the

C. jejuni and *C. coli* isolates recovered in Dhaka. The Penner serotyping system is only effective in typing *C. jejuni*, *C. coli* or *C. lari*, and not other species of *Campylobacter*, *Helicobacter*, or *Arcobacter*. Patient ages ranged from 1 month to 12 years of age. Sixty-five percent of the Cape Town patients were 2 years old or less, and the male/female ratio was 1.25:1.00.

Table 2. Distribution of *Campylobacter* species and related microorganisms isolated from 5,635 diarrhoeic stools at the Red Cross Children's Hospital, Cape Town, South Africa, 2000-2002

Bacterium	Isolation rate, No. (%)
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i> biotypes 1 & 2*	316 (31.6)
<i>Campylobacter concisus</i>	234 (23.4)
<i>Campylobacter upsaliensis</i>	226 (22.6)
<i>Campylobacter jejuni</i> subsp. <i>doylei</i>	91 (9.1)
<i>Helicobacter fennelliae</i>	64 (6.4)
<i>Campylobacter coli</i>	32 (3.2)
<i>Campylobacter hyointestinalis</i>	13 (1.3)
<i>Helicobacter cinaedi</i>	10 (1.0)
CLO/HLO†	5 (0.5)
<i>Arcobacter butzleri</i>	4 (0.4)
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	1 (0.1)
<i>Campylobacter lari</i>	1 (0.1)
<i>Campylobacter curvus</i>	1 (0.1)
<i>Campylobacter sputorum</i> biovar. <i>sputorum</i>	1 (0.1)
Total	999 (100.0)

* = Biotyping scheme of Skirrow and Benjamin⁵; † = CLO/HLO: *Campylobacter* or *Helicobacter* organisms that could not be fully characterized.

In 89% of the Cape Town patients with campylobacteriosis, *Campylobacter* was the sole pathogen isolated, while in the remaining stool samples, *Campylobacter* were co-isolated with *Salmonella*, or *Shigella*. Most Cape Town patients with *C. jejuni* or *C. coli* infection had diarrhoea (93%), 8% were vomiting and (5%) had fever >38°C. More than 11% of the patients had dysentery.

Fifteen percent of the South African patients had pre-existing clinical conditions such as, anaemia, respiratory diseases, HIV+ status and malnutrition (Table 1). The majority of *Campylobacter* isolations occurred during December to March, which is the late summer/early autumn in South Africa, and a pattern that is reflective of developed countries⁸.

Of the 181 isolates serotyped in Dhaka, 112 isolates reacted with the reference antisera. Eighty-two (45.3%) isoaltes reacted with a single serotype, 30 (16.6%) reacted with multiple (2 or more), serotypes, and 69 (38.1%) were non-typable. Among the 82 single serotype isolates, 26 different serotypes were identified; serotype O:33 was the most frequent, followed by serotypes O:22, O:3,

O:55, O:26 and O:1. Among the isolates with multi-antigenic expression, 36 different serotypes were noted; serotype O:23/O:36 was the most common, followed by serotype O:1/O: 8 (Table 3). One isolate typed as serotype O:41, which has been recognized to be associated with the Guillain-Barré Syndrome in South Africa and elsewhere⁴.

Table 3. Serotype distribution of *Campylobacter jejuni* isolated from diarrhoeic patients in Dhaka, Bangladesh and Cape Town, South Africa

Serotype	Isolate No.	Frequency (%)
Dhaka, Bangladesh (n = 181)		
O:33	12	6.6
O:22	8	4.4
O:55	7	3.8
O:1	6	3.3
O:3	6	3.3
O:26	6	3.3
O:42	5	2.8
O:53	4	2.2
O:40	4	2.2
O:4	1	0.5
O:41	1	0.5
NT	69	38.1
Multi-reactive*	30	16.6
Other**	23	12.9
Cape Town, South Africa (n = 316)		
O:2	18	5.8
O:1	13	4.2
O:4	10	3.5
O:37	9	2.9
O:5	8	2.6
O:12	8	2.6
O:22	7	2.2
O:33	1	0.3
O:3	1	0.3
NT	56	17.9
Multi-reactive*	70	22.4
Other***	112	35.6

NT = Non-typable; * = Reacts in two or more antisera; Other** = Reacts in 15 additional antisera; Other*** = Reacts in 21 additional antisera

These results contrast with the serotyped Cape Town *C. jejuni* isolates, among which 18% were non-typable and 22% reacted with two or more antisera. Antigenic extracts of 60% of the Cape Town *C. jejuni* isolates reacted with only one test antisera, and the most frequent serotype detected was O:2, followed by O:1, O:4 and O:37 (Table 3). Non-typable isolates predominated for *C. coli* isolates from both Cape Town and Dhaka, and the remaining *C. coli* isolates represented a variety different serotypes (Table 4).

Table 4. Serotype distribution of *Campylobacter coli* isolates recovered from diarrhoeic patients in Dhaka, Bangladesh and Cape Town, South Africa

Serotype	Isolate No.	Frequency (%)
Dhaka, Bangladesh (n = 15)		
NT	12	80.0
O:20/O:26	1	6.6
O:28/O:55	1	6.6
O:64	1	6.6
Cape Town, South Africa (n = 32)		
NT	6	18.7
O:30	4	12.5
O:46	4	12.5
O:49	3	9.4
O:5	3	9.4
O:5/O:30	2	6.3
O:20	2	6.3
O:24	2	6.3
O:26/O:34	1	3.1
O:34	1	3.1
O:24/O:30	1	3.1
O:26	1	3.1
O:66	1	3.1
O:54	1	3.1

NT = Non-typable

Our data indicates a high level of diversity among serotyped isolates of *C. jejuni* and *C. coli* isolated from diarrhoeic patients in both Dhaka and Cape Town. The high incidence of non-typable isolates in Dhaka implies that these isolates have antigenic specificities, which are undetectable by the current serotyping system. Some serotypes were found in one study area but not in the other (serotype O:55 in Dhaka, serotype O:2 in Cape Town). This could be due to different animal reservoirs for *Campylobacter* in these areas, e.g., pigs are much more commonly consumed in Cape Town than Bangladesh. Serotype O:2 was not detected in Dhaka in this study as well as not in a previous one⁹, but it was the most commonly detected serotype (5.8%) in the Cape Town. Serotype O:2 is one of the most commonly and consistently detected serotypes world-wide¹⁰⁻¹¹. Antigenic variability (i.e., serotypes O:55 in Dhaka, and O:2 in Cape Town) indicates persistence over time of virulent *C. jejuni* clones in a confined geographical area.

The clinical and serological observations of patients in Dhaka and Cape Town indicated both similarities and differences. Our observations suggest dissimilarity in patterns of infection, and the prevalence and persistence in environmental and animal reservoirs of *Campylobacter* between these two population groups. One of the Bangladeshi *C. jejuni* isolates was serotyped as O:41. This serotype is known to be associated with the Guillain-Barré syndrome in patients in Cape Town and elsewhere⁴, but the true incidence and causative agents of the Guillain-Barré syndrome in Bangladesh remains unknown at present. Additional

epidemiological, serological and molecular studies are essential for a more complete understanding of the disease potential and other aspects of *Campylobacter* infection in our patients.

Different isolation protocols in the two laboratories yielded different *Campylobacter* prevalence rates. With the candle jar method used in Dhaka, only *C. jejuni* and *C. coli* were isolated, while in Cape Town, the “Cape Town” protocol⁷ yielded isolates of 10 *Campylobacter* species, 2 *Helicobacter* species and *Arcobacter butzleri*. Many of these species are seldom isolated elsewhere and their true disease is largely unknown. Possibly altering the isolation protocol in Dhaka could be more efficient for the isolation of all *Campylobacter* species and provide a better appreciation of the disease potential of all *Campylobacter* species.

In both Cape Town and Dhaka the high diversity of serotypes recognized is suggestive of multiple source of infections. This high rate of infection with multiple pathogens could indicate *Campylobacter* transmission from reservoirs with little seasonal variation, such as infected chicken, rather than contamination of previously uninfected food sources which may be enhanced at higher temperatures⁸.

In 1987, Neogi and Shahid⁹ documented the serotype distribution of 102 *C. jejuni* isolates isolated from diarrhoeic patients attending the ICDDR,B in Bangladesh. *C. jejuni* was recovered from 7% of the patients tested. Of the *Campylobacter* isolates examined, 74% were typable, and serotypes O:53, O:15 and O:22 predominated⁹. Our current results indicated that 62% of the Bangladeshi *C. jejuni* isolates tested was typable. This high incidence of non-typable isolates (38%) in Dhaka implies these isolates have antigenic specificities unable to be detected by the current serotyping system. While serotypes O:53 and O:22 predominated in our study, serotype O:15 was not detected (Table 3). The persistence of serotypes O:22 and O:53 in Dhaka over a 17-year period may indicate a stable reservoir in Dhaka for *Campylobacter* isolates of these antigenic specificities.

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