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Campylobacter jejuni Bacteraemia in Children with Diarrhoea in Bangladesh: Report of Six Cases

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ABSTRACT

Campylobacter jejuni **was isolated from blood cultures from 6 of 6,275 diarrhoeal children seeking treatment at the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) between April 1989 and December 1990. The clinical records of these 6 children were reviewed. All children were male; 5 were less than 1 – year old and were severely malnourished. Five patients presented with watery diarrhoea and one with bloody diarrhoea. Two children died in the hospital. All strains of *Campylobacter* isolated from the 6 children were negative for cell invasive properties as tested by the Sereny test. Of the two strains tested for serum bactericidal activity, one strain was serum sensitive (growth inhibition), and the other resistant. The ability of *C. jejuni* to cause bacteraemia suggests that the organisms may be responsible for diarrhoeal diseases especially in young and malnourished children. An early attempt to detect *Campylobacter* and start effective antimicrobial therapy is indicated.**

Key words: Diarrhoea; *Campylobacter jejuni*; Infantile diarrhoea; Actiology; Bacteraemia.

INTRODUCTION

Infections by *Campylobacter* species may cause a wide variety of clinical syndromes in man (1 – 5). *C. jejuni* and *C. coli* are well recognised causes of diarrhoea in children and adults (6 – 11). Although there are several reports of bloodstream infections due to *C. fetus* subspecies *fetus*, *C. coli*, and non – typable *Campylobacter* (6,10,12 – 15), bacteraemia due to *C. jejuni* is relatively uncommon (11,16). We report here, for the first time, six children who had *C. jejuni* bacteraemia in Bangladesh, where *Campylo – bacter* infection is common but the enteropathogenicity of the organism was unclear (17 – 19).

MATERIALS AND METHODS

From 6,275 children with diarrhoea seeking treatment at the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), between April 1989 and December 1990, 6,303 blood samples were obtained. Blood was withdrawn by puncture of the femoral vein with a sterile needle fitted to a plastic syringe after cleaning the area with iodine and 70% ethanol. Before the blood was injected into the culture bottle the needle was discarded and a fresh one fitted.

C. jejuni were grown from the blood samples of 6 children. The case records of these 6 children were reviewed. On admission, a stool specimen from each patient was examined microscopically and cultured for *Salmonella*, *Shigella*, and/or Vibrio. A complete blood cell count (CBC) was done and the serum electrolytes were determined.

Of the 6 blood culture isolates of *C. jejuni*, five were obtained using trypticase soy broth (DIFCO, Detroit, MI) and one using a lysis – centrifugation system DuPont isolator 1.5 (Diagnostica, MERCK, Darmstadt, Germany). The trypticase soy broth cultures were incubated aerobically at 37 °C and examined for turbidity daily for 7 – 8 days. They were then subcultured onto blood agar and chocolate agar plates, which were incubated at 37 °C aerobically and in CO_2 atmosphere, respectively, for 24 – 48 hr. Colonies were Gram – stained, and oxidase and catalase tests were performed. Organisms

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resembling *Campylobacter* were subcultured onto *Brucella* agar and incubated at 42°C in an increased CO_2 atmosphere for further characterisation as described elsewhere (5). Gram-staining, and catalase and oxidase tests were repeated. Hippurate-hydrolysis test and susceptibility test to nalidixic acid and cephalothin were performed (5).

Susceptibility of organisms to antibiotics was tested by the standard disk-diffusion method against ampicillin, chloramphenicol, erythromycin, furazolidone, gentamicin, nalidixic acid, and tetracycline.

Sereny test was performed to test the ability of the organisms to invade the epithelial cells of rabbit conjunctiva and cornea (20). During storage four strains were lost. Serum bactericidal assay with the two remaining isolates was carried out using pooled unheated normal adult human sera by the method of Blaser and colleagues (16).

RESULTS

There was bacterial growth on chocolate agar plates of all subcultures from blood culture bottles of all six patients. On subculture, the bacteria also grew on *Brucella* agar. The organisms were Gram-negative, spiral, S-shaped, and/or seagull-wing-like bacilli, oxidase and catalase positive, susceptible to nalidixic acid but resistant to cephalothin, and were positive for hippurate-hydrolysis test. Based on these characteristics, the organisms were identified as *C. jejuni*.

The major clinical characteristics and the results of antibiotic therapy of these 6 children are shown in Table I. All children were male, five were less than one year old (2-7 months), and one aged four years. Five children presented with watery diarrhoea, and one with bloody diarrhoea. All the patients had histories of repeated attacks of diarrhoea before coming to the hospital. History of vomiting and fever was present in all cases. Three children had respiratory symptoms including fever, cough, and chest indrawing during breathing. Five patients were severely malnourished (weight-for-age: 39%-56% of NCHS median) and the remaining one was normal (weight-for-age: 69% of NCHS median). In four patients, the liver was palpable, and so was the spleen in two cases. All patients received parenteral ampicillin (200 mg/kg body weight/day in 4 doses) and gentamicin (6 mg/kg body weight/day in 3 doses) for 4-10 days after hospital admission. In addition, case no. 2 received nalidixic acid (3 oral doses, 100 mg per dose every 6 hrs), followed by pivmecillinam (5 oral doses, 125 mg per dose every 6 hrs); case no. 3 received tetracycline (40 mg orally every 6 hrs for 3 days); and case no. 6 received cloxacillin (250 mg parenterally every 6 hrs for 10 days) and erythromycin (70 mg orally every 6 hrs for 7 days) (Table I). Two patients died in the hospital due to septicaemia.

Table I. Clinical characteristics on admission, therapy, and results of treatment of six children with *C. jejuni* bacteraemia in Bangladesh

Parameters	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Types of diarrhoea	Watery	Bloody mucoid	Watery	Watery	Watery	Watery
Duration of diarrhoea before admission	3 days	7 days	7 days	3 days	3 days	7 days
Dehydration	Mild	Mild	Severe	Mild	Mild	Moderate
Duration of vomiting	2 days	1 day	1 day	2 days	2 days	3 days
History of fever with duration	3 days	2 days	2 days	5 days	2 days	Present*
Body temperature on admission (°c)	37.4	37.0	38.0	39.0	38.0	37.0
Lung X-ray	Infiltration	Not done	Infiltration	Clear	Clear**	Infiltration
Antibiotic therapy	Amp,Gen	NA,Mec, Amp,Gen	Tet,Amp, Gen	Amp,Gen	Amp,Gen	Clx,Gen, Ery
Outcome	Died	Improved	Improved	Improved	Died	Improved

Amp = Ampicillin; Clx = Cloxacillin; Ery = Erythromycin; Gen = Gentamicin; Mec = Pivmecillinam; NA = Nalidixic acid; Tet = Tetracycline; *Duration not available; **This patient developed pneumonia on 5th day of hospitalisation

Laboratory findings on admission are shown in Table II. The peripheral blood cell count showed leukocytosis with a shift to the left in three patients. Mild toxic granules were present in blood in four cases. Two patients had hyponatraemia, and three

patients had evidence of metabolic acidosis. Stool microscopic examination showed dysentery-like characteristics in all cases. Stool cultures yielded non-01 *Vibrio cholerae* in one child. However, stool cultures for *Campylobacter* were not done.

no bacteraemic case has been detected thereafter in spite of regular blood cultures of suspected cases. The reasons for the clustering of cases in this period are not known. The mechanism(s) of bloodstream infection due to *C. jejuni* is not clearly understood.

Table II. Laboratory features of 6 children with *C. jejuni* bacteraemia

Parameters	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
BLOOD						
Haematocrit (%)	28.0	11.5	36.0	26.0	30.0	34.0
Total white blood cell count/cmm	21,300	14,200	15,900	15,800	20,000	5,200
Polymorhps (%)	21	69	40	50	43	59
Band cell (%)	9	0	2	0	7	1
Lymphocytes (%)	74	26	56	35	55	41
Metamyelocytes (%)	2	2	1	0	2	0
SERUM ELECTROLYTES (mmol/l)						
Sodium	146	132	135	124	126	136
Potassium	4.43	4.89	6.53	5.11	4.90	2.57
Chloride	130	100	110	87	104	114
Bicarbonate	13.2	18.8	9.6	22.8	16.0	8.2
STOOL						
Presence of mucus and blood	Mucus	Blood and mucus	Mucus	Absent	Mucus	Mucus
Pus cell/HPF	11-20	11-20	11-20	11-20	11-20	11-20
Red blood cell/HPF	11-20	0	1-10	0	1-10	0

HPF = High power field of microscope

In vitro drug-susceptibility tests showed that all 6 strains of *C. jejuni* were susceptible to chloramphenicol, erythromycin, furazolidone, gentamicin, nalidixic acid, and tetracycline. However, isolates from three cases (nos. 2, 4, and 6) were susceptible to ampicillin, whereas the remaining 3 isolates were not.

Sereny test was negative for all the strains. The serum bactericidal activity on the two strains tested (cases 3 and 4) showed that the strain from case 3 was serum-sensitive (2.7×10^6 cfu/ml without serum vs. no growth after incubation with serum), and the strain from case 4 was serum-resistant (1.2×10^7 cfu/ml without serum vs. 2×10^5 cfu/ml with serum).

DISCUSSION

Our results indicate that 6 bacteraemic strains of *C. jejuni* were isolated from diarrhoeal children within a short period in the early part of 1989 and

Blaser and colleagues suggested that extraintestinal or bloodstream infections are due to compromised defence mechanisms of the host which enable *C. jejuni* and *C. coli* to invade the bloodstream (16). Host defence mechanisms, including age, nutritional status, immune status, and underlying diseases are also important factors. In our study, all patients, except one, were infants aged 2-7 months and were severely malnourished and possibly immunocompromised. Bacteraemia in these patients might have been secondary to intestinal infection. However, stool cultures for *Campylobacter* were not performed and it is difficult to rule out the intestine as the possible source of bacteraemia. Previous studies in the Centre have shown similar frequencies of isolation of *Campylobacter* spp. from both diarrhoeal and healthy subjects, thus questioning the significance of the isolates as a cause of diarrhoeal diseases in this population (17,18).

All 6 strains of *C. jejuni* were negative for Sereny

test. This finding is consistent with the previous observations that *C. jejuni* isolated from blood and stool did not produce keratoconjunctivitis in guinea pig eyes as detected by Sereny test (12,21). Of the two strains tested, one was susceptible (inhibition of growth) to normal human sera and the other was not. However, since the majority of children were malnourished and had repeated enteric infections with possible immunosuppression, it was likely that the strains were resistant to the activity of the patients' own sera. This suggests the possibility of an opportunistic infection leading to transient bacteraemia. It is also possible that the two patients who died in our series might have developed septicaemia due to serum resistant *C. jejuni* infection. It has been noted that *C. jejuni* could survive the activity of sera from immunocompromised subjects but not from normal subjects (16).

In septicaemia due to *Shigella*, the severity of septicaemia has been inversely correlated with serum bactericidal activity against these organisms; the more severe the case, the more resistant the organism (22). Greater serum resistance of *C. jejuni* isolated from blood or extraintestinal tissues compared with those isolated from stool has been reported (16). Unfortunately, isolates from the two children who died were not available for testing. Other factors that could have contributed to death are inadequate therapy and severe mainutrition. For the first 6 days these two children received ampicillin to which the *C. jejuni* isolates were resistant.

Because the aetiological role of these organisms in diarrhoea in Bangladesh has been questioned due to similar isolation rates from children with diarrhoea and healthy controls in previous studies (17,18), the possibility of *C. jejuni* bacteraemia might have been overlooked. The ability of *C. jejuni* to cause bacteraemia suggests that these organisms may be responsible for diarrhoeal illness, especially in the young, malnourished children of Bangladesh. An early attempt to detect *Campylobacter* and initiate effective antimicrobial therapy is indicated in this group of patients with diarrhoeal diseases.

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