BACTERIAL ENTERIC PATHOGENS IN CHILDREN WITH ACUTE DYSENTERY IN THAILAND: INCREASING IMPORTANCE OF QUINOLONE-RESISTANT *CAMPYLOBACTER*

L Bodhidatta[1], N Vithayasai[2], B Eimpokalarp[3], C Pitarangsi[1], O Serichantalergs[1] and DW Isenbarger[4]

Armed Forces Research Institute of Medical Sciences, Bangkok; [2]Queen Sirikit National Institute of Child Health, Bangkok; [3]Bamrasnaradura Infectious Diseases Hospital, Nonthaburi, Thailand; [4]Walter Reed Army Institute of Research, Washington DC, USA

Abstract. Current data on pathogen prevalence and drug resistance patterns are important for treatment and vaccine-development strategies. An etiologic study of acute bacterial dysentery was conducted in children up to 12 years of age in 2 major hospitals in and around Bangkok. Stool samples or rectal swabs and clinical data were collected. Standard microbiological methods were used to detect *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio*, *Aeromonas* and *Plesiomonas*. Pathogenic *E. coli* (ETEC, EIEC, STEC) was identified by digoxigenin-labeled probes. A total of 623 cases were enrolled: median age 11.0 months (range 1 month-12 years). At least one bacterial pathogen was isolated in 55% of cases. *Campylobacter* was the most common pathogen found (28%), whereas *Salmonella*, *Shigella* and ETEC were isolated from 18%, 9% and 6% respectively. EIEC, *Vibrio* and *Plesiomonas* were isolated from <1% and no STEC was detected. *C. jejuni* serotypes 36, 4 and 11 were the most common. The mean age of cases with *Campylobacter* was significantly lower than with *Shigella* (17.9 *vs* 52.8 months, p<0.001). Clinical presentations of *Campylobacter* and *Shigella* infections were compared: fever (28% *vs* 37%), abdominal colic (62% *vs* 80%, p<0.05), vomiting (38% *vs* 70%, p<0.001) and bloody stools (52% *vs* 48%). The *Campylobacter* isolates (80% *C. jejuni*, 20% *C. coli*) were 90% resistant to ciprofloxacin but sensitive to macrolides. All the *Shigella* isolates (70% *S. sonnei*) were sensitive to quinolones. Our study illustrates the increasing importance of quinolone-resistant *Campylobacter* and the decline of *Shigella* in the etiology of dysentery in Thailand. The clinical presentation of campylobacteriosis is similar to that of shigellosis, except that the patients may be younger and there may be less association with colic and vomiting; having fecal leukocytes will be >10/HPF. The use of macrolide antibiotics rather than quinolones would be reasonable in children <24 months of age; fluoroquinolones will be ineffective in at least half of culture-positive cases.

INTRODUCTION

Despite the remarkable improvement of sanitation and education during the past decade, diarrheal disease is still one of the most important causes of morbidity and mortality among children in developing world (Townes *et al*, 1997; Bhan, 2000). Knowledge of the etiology of diarrheal disease and the drug resistance patterns of pathogens are important steps toward the implementation of effective disease control. In Thailand, the use of routine stool cultures and drug susceptibility testing as treatment guidelines for diarrheal disease are often limited by cost and laboratory capabilities. Antibiotic prescriptions are usually based on clinical signs and symptoms without culture results.

Our previous studies of pediatric diarrheal disease in Thailand conducted during 1984-1994 consistently demonstrated *Shigella*, *Campylobacter* and Enterotoxigenic *E. coli* as major bacterial enteric pathogens (Echeverria

Correspondence: Dr Ladaporn Bodhidatta, Department of Enteric Diseases, Armed Forces Research Institute of Medical Sciences (AFRIMS), 315/6 Rajvithi Road, Phyathai, Bangkok 10400, Thailand.
Tel: ++66 (0) 2644-6125; Fax: ++66 (0) 2644-4980
Email: ladapornB@Thai.amedd.army.mil

et al, 1989; Varavithaya *et al*, 1990; Echeverria *et al*, 1994). Approximately 20% of all children with diarrhea had bloody diarrhea and *Shigella* was a leading pathogen among cases of dysentery identified in 23-50% (Taylor *et al*, 1986; 1988a; 1991). Dysentery is usually a sign of invasive enteric infection that carries a substantial risk of serious morbidity and death. Compared with watery diarrhea, bloody diarrhea generally lasts longer, is associated with more complications, is more likely to adversely affect a child's growth, and has a higher case fatality rate (Black *et al*, 1982; Briend *et al*, 1989).

This study was conducted in order to determine the etiology and drug resistance pattern of enteric pathogens in children with mucous bloody diarrhea in Thailand during 1998-2000. Since etiologic agents and drug resistance patterns vary from place to place and change over time, current local data about pathogen prevalence will lead to more rational treatment and vaccine strategies.

MATERIALS AND METHODS

Case enrollment

Children less than 12 years old with mucous bloody diarrhea who presented to the Queen Sirikit National Institute of Child Health, Bangkok, and Bamrasnaradura Infectious Diseases Hospital, Nonthaburi, were enrolled in the study during the period August 1998-May 2000. Dysentery was defined as 3 or more loose stools in 24 hours with any of these stool containing visible mucous and/or blood, as reported by a parent or adult guardian in association with at least one constitutional symptom (fever, abdominal colic, nausea and vomiting). After obtaining written informed consent, data on clinical history, demographic information, symptoms and previous medication were recorded on questionnaires by research nurses.

This study was approved by the Human Use Review Committee, Walter Reed Army Institute of Research; the Human Subjects Research Review Board, US Army Medical Research and Material Command and the Ethical Review Committee, Ministry of Public Health, Thailand.

Collection of specimens

Stool specimens or 4 rectal swabs in Cary-Blair transport media were collected from cases, transported in a Styrofoam box with ice packs, and processed at AFRIMS within 3 hours of collection.

Examination of specimens

Bacterial enteric pathogens were isolated and identified by standard bacteriological methods as previously described (Echeverria *et al*, 1989). *Shigella* was serotyped by Denka-Seiken antisera and monoclonal antibodies. *Campylobacter* was bio-typed according to hippurate hydrolysis and serotyped with the Lior serotyping system. Up to 5 colonies of lactose-fermenting *E. coli* and non-lactose-fermenting *E. coli* were processed and tested for invasiveness and toxin production; heat-labile (LT), heat-stable (ST) and Shiga toxin (STx) by DNA hybridization technique using digoxigenin-labeled probes (Valentiner-Branth *et al*, 1999). Only 25% of cases were randomly selected for testing by DNA hybridization.

All the bacterial enteric pathogens isolated were tested for antimicrobial susceptibility by the standard disk diffusion method, using commercially prepared antibiotic disks containing chloramphenicol, ampicillin, azithromycin, colistin, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, neomycin, streptomycin, sulfisoxazole, tetracycline, trimethoprim/sulfamethoxazole. *Campylobacter* spp were tested for antimicrobial susceptibility by the agar dilution method.

RESULTS

A total of 623 cases with mucous bloody diarrhea were enrolled in the study. The median age of the patients was 11.0 months (range 1 month-12 years) and 39% were girls. At least one bacterial pathogen was identified from 341

(55%) cases; thirty-one (5%) cases yielded more than one pathogen. The bacterial enteric pathogens identified in this study are shown in Table 1. *Campylobacter* was the commonest pathogen found (28% of cases); *C. jejuni* accounted for 80% of these isolates. Most of the *C. jejuni* isolates belonged to Lior serotype 36 (21%), serotype 4 (9%) and serotype 11 (6%).

Table 1
Isolation of bacterial enteric pathogens from children with dysentery in Thailand (N=623).

	Number (%)
Campylobacter spp (Total)	174 (28)
C. jejuni	138
C. coli	36
Shigella spp (Total)	56 (9)
S. flexneri	16
S. flexneri 1b	1
S. flexneri 2a	9
S. flexneri 3a	3
S. flexneri 4	1
S. flexneri 6	2
S. sonnei	40
Non-typhoidal *Salmonella*	110 (18)
Enterotoxigenic *E. coli* (ETEC)[a]	40 (6)
Enteroinvasive *E. coli* (EIEC)[a]	4 (0.6)
Shiga toxin-producing *E. coli* (STEC)[a]	0
Vibrio spp	4 (0.6)
Plesiomonas shigelloides	6 (1)

[a]DNA hybridization was randomly performed in 25% of samples. Number presented in this table was calculated by actual number x 4.

Salmonella, *Shigella* and ETEC were isolated from 18%, 9% and 6% of patients respectively. Approximately 70% of the *Shigella* isolates were *S. sonnei*. No *S. dysenteriae* was identified. *Salmonella* group B and group C were the most common serogroups identified. Forty percent of ETEC isolates produced heat-labile toxin (LT), 20% produced heat-stable toxin (ST) and 40% produced both. EIEC, *Vibrio* and *Plesiomonas* were isolated from fewer than 1% of the patients and no Shiga toxin-producing *E. coli* was detected in this study.

The age specific isolation rates of *Campylobacter*, *Salmonella* and *Shigella* were shown in Table 2. Overall, children with dysentery who were infected with *Campylobacter* and *Salmonella* species were younger than those infected with *Shigella*. The rate of isolation of *Campylobacter* in children under 24 months of age was significantly higher than in older age groups (138/443 *vs* 36/180; p=0.006). On the other hand, the rate of isolation of *Shigella* in children over 24 months of age was significantly higher than in the younger age groups (46/180 *vs* 10/443; p< 0.00001).

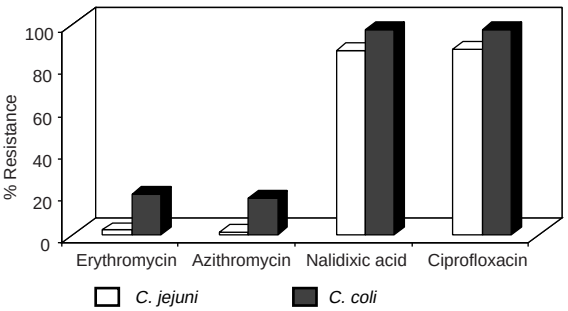
Characteristics and clinical manifestations of patients infected with *Campylobacter* or *Shigella* as a single pathogen are compared in Table 3. The mean age of patients with *Campylobacter* was significantly lower than patients with *Shigella* (17.9 *vs* 52.8 months; p<0.00001). No significantly difference was detected between the percentage of patients with

Table 2
Age specific isolation rate of *Campylobacter*, *Salmonella* and *Shigella* in children with dysentery.

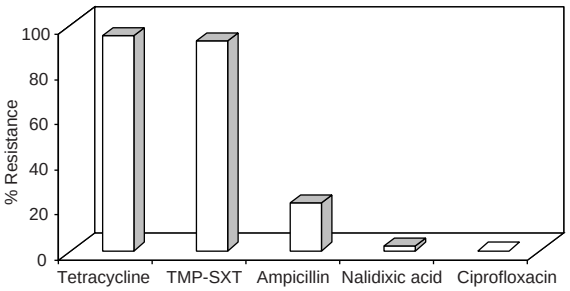
Age group (months)	Number (N=623)	No. (%) isolation of *Campylobacter* (N=174)	No. (%) isolation of *Shigella* (N=56)	No. (%) isolation of *Salmonella* (N=110)
0-6	163	39 (24)	0	50 (31)
7-12	176	63 (36)	3 (2)	36 (20)
13-24	104	36 (35)	7 (7)	10 (10)
25-36	44	14 (32)	7 (16)	4 (9)
37-48	39	12 (31)	15 (38)	2 (5)
49-60	26	2 (8)	5 (19)	1 (4)
> 60	71	8 (11)	19 (27)	7 (10)

Table 3
Comparison of characteristics and clinical manifestations of dysentery patients infected with *Campylobacter* and *Shigella* as a single pathogen.

	*Campylobacter*iosis N=138	Shigellosis N=46	p-value
Mean age (months)	17.9	52.8	p<0.00001
% of receiving prior antibiotics	31	22	NS
% of history of visible blood in stool	43	28	NS
% of observed bloody stool	52	48	NS
% of fever ≥38ºC	28	37	NS
% of abdominal colic	62	80	p=0.02
% of vomiting	38	70	p=0.0002
% of having fecal WBC >10/HPF	64	83	p=0.02



Antibiotic resistance pattern of *Campylobacter* isolates



Antibiotic resistance pattern of *Shigella* isolates

Fig 1–*In vitro* susceptibility of *Campylobacter* and *Shigella* to commonly used antibiotics.

fever, prior antibiotic usage, and visible blood in stool either by history or by observation. Shigellosis patients were significantly more likely to have abdominal colic, vomiting, and fecal leukocyte more than 10/ HPF.

The *in vitro* susceptibility of *Campylobacter* and *Shigella* is illustrated in Fig 1. *C. jejuni* and *C. coli* isolates were resistant to ciprofloxacin in 88% and 97% of cases respectively, whereas only 1% and 17% were resistant to azithromycin. *Shigella* isolates were uniformly sensitive to ciprofloxacin but highly resistant to tetracycline and TMP/SXT (95% and 93%). Ampicillin resistant *Shigella* spp were detected in 21% of cases.

DISCUSSION

The etiologic pattern of dysentery in Thailand has changed since it was described in previous studies (Taylor *et al*, 1986; 1988a; 1991; Murphy *et al*, 1993). *Campylobacter* has become the leading pathogen, while the isolation rate of *Shigella* has decreased. *E. coli* producing heat-labile or heat-stable toxins accounted for 6% of cases; no Shiga toxin-producing *E. coli* was detected. No etiologic agent was identified in 45% of patients with dysentery in this study: this finding may be explained by the fact that no effort was made to detect parasites or diarrhea-related viruses and individuals who had been treated with antibiotics prior to enrollment were not excluded from participation.

The *C. jejuni* and *C. coli* isolates in this study were highly resistant to ciprofloxacin (88% and 97%). This is in contrast to other

countries in this region, *eg* Vietnam, where ciprofloxacin-resistant *Campylobacter* was detected in only 7% of patients (Isenbarger *et al*, 2002). This is probably due to the widespread use of fluoroquinolones in Thailand since 1990. Most of the *Campylobacter* isolates were sensitive to macrolide antibiotics: *C. coli* strains were found to be more resistant than *C. jejuni*.

Unfortunately, the range of antimicrobials for the treatment of shigellosis has narrowed considerably in recent years as bacterial resistance has increased (Hoge *et al*, 1998). Resistance to ampicillin and cotrimoxazole is widespread and resistance to nalidixic acid is developing. The fluoroquinolones, which are related to nalidixic acid, are now the drugs of choice in many areas. In this study, *Shigella* isolates were uniformly sensitive to fluoroquinolones. Interestingly, ampicillin-resistant *Shigella* spp continue to decline after the overall reduction of ampicillin usage in Thailand.

Our previous study on the epidemiology of *Campylobacter* infection in Thailand demonstrated that *Campylobacter* infection was often associated with mild illness or asymptomatic infection and was less often associated with bloody diarrhea (Taylor *et al*, 1988b). However, this study suggests that *Campylobacter* has become the leading pathogen among children with mucous bloody diarrhea and its clinical features are comparable to those of shigellosis. Although a number of studies did not show that treatment with erythromycin significantly altered the clinical course of *Campylobacter* infection (Karmali and Fleming, 1979; Pai *et al*, 1983; Taylor *et al*, 1987), treatment could shorten the duration of convalescent excretion. In developing countries, where asymptomatic carriers may contribute to the transmission of disease, treatment could play a role in reducing transmission. However, the prevalence of *Campylobacter* in asymptomatic children should be the subject of further study in order to determine the background rate of carriage.

The World Health Organization currently recommends oral rehydration therapy plus continued breast feeding for children with diarrhea, and antibiotics for dysentery and associated symptoms. (Bhan, 2000) In Thailand, fluoroquinolones have been used as the first-line antibiotics for the treatment of diarrheal disease, while erythromycin is not often recommended. Unfortunately, this study has illustrated the increasing importance of quinolone-resistant *Campylobacter* as an etiologic agent of dysentery, especially in children under 2 years of age. Use of macrolide antibiotics as an empiric treatment would appear to be reasonable when antibiotic treatment is indicated for children of less than 2 years of age; fluoroquinolones will be ineffective in at least half of culture-positive cases. Monitoring of the antimicrobial resistance of enteric pathogens is an essential part of any diarrheal disease control program that advocates empiric treatment.

Although public health interventions can partly contribute to the control of diarrheal disease, the most promising possibility for specific prevention is the use of vaccines directed at important and prevalent pathogens. Changes in the etiology of dysentery and drug resistance patterns will have a marked effect on the treatment and prevention of dysentery in Thailand.

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REFERENCES

Bhan MK. Current and future management of childhood diarrhea. *Int J Antimicrob Agents* 2000; 14:

71-3.

Black RE, Brown KH, Becker S, Abdul Alim AR, Huq I. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. II Incidence of diarrhea and association with known pathogens. *Am J Epidemiol* 1982; 115: 315-24.

Briend A, Hasan KZ, Aziz KMA, Hoque BA. Are diarrhoea control programmes likely to reduce childhood malnutrition? Observation from rural Bangladesh. *Lancet* 1989; 2: 319-22.

Echeverria P, Taylor DN, Lexsomboon U, *et al*. Case-control study of endemic diarrheal disease in Thai children. *J Infect Dis* 1989; 159: 543-8.

Echeverria P, Hoge CW, Bodhidatta L, *et al*. Etiology of diarrhea in a rural community in Western Thailand: Importance of enteric viruses and enterovirulent *E. coli*. *J Infect Dis* 1994; 169: 916-9.

Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrhea pathogens in Thailand over 15 years. *Clin Infect Dis* 1998; 26: 341-5.

Isenberger DW, Hoge CW, Srijan A, *et al*. Comparison of antibiotic resistance of diarrheal pathogens from Vietnam and Thailand,1996-1999. *Emerg Infect Dis* 2002; 8: 175-80.

Karmali MA, Fleming PC. *Campylobacter* enteritis in children. *J Pediatr* 1979; 94: 527-33.

Murphy GS, Bodhidatta L, Echeverria PE, *et al*. Ciprofloxacin and loperamide in the treatment of bacillary dysentery. *Ann Int Med* 1993; 118: 582-5.

Pai CH, Gillis F, Tuomanen E, Marks MI. Erythromycin in treatment of *Campylobacter* enteritis in children. *Am J Dis Child* 1983; 137: 286-8.

Taylor DN, Echeverria P, Pal T, *et al*. The role of *Shigella* spp, enteroinvasive *Escherichia coli*, and other enteropathogens as causes of childhood dysentery in Thailand. *J Infect Dis* 1986; 153: 1132-8.

Taylor DN, Blaser MJ, Echeverria P, Pitarangsi C, Bodhidatta L, Wang WL. Erythromycin-resistant *Campylobacter* infections in Thailand. *Antimicrob Chemother* 1987; 31: 438-42.

Taylor DN, Echeverria P, Sethabutr O, *et al*. Clinical and microbiologic features of *Shigella* and enteroinvasive *Escherichia coli* infections detected by DNA hybridization. *J Clin Microbiol* 1988a; 26: 1362-6.

Taylor DN, Echeverria P, Pitarangsi P, Seriwatana J, Bodhidatta L, Blaser MJ. Influence of strains characteristics and immunity on the epidemiology of *Campylobacter* infections in Thailand. *J Clin Microbiol* 1988b; 26: 863-8.

Taylor DN, Bodhidatta L, Echeverria P. Epidemiologic aspects of shigellosis and other causes of dysentery in Thailand. *Rev Infect Dis* 1991; 3(S4): S226-30.

Townes JM, Quick R, Gonzales OY, *et al*. Etiology of bloody diarrhea in Bolivian children: implication for empiric therapy. *J Infect Dis* 1997; 175: 1527-30.

Valentiner-Branth P, Steinsl H, Gjessing H, *et al*. Community based randomized controlled trial of reduced osmolarity oral rehydration solution in acute childhood diarrhea. *Ped Infect Dis J* 1999; 18: 789-95.

Varavithaya W, Vathanophas K, Bodhidatta L, *et al*. Importance of *Salmonella*e and *Campylobacter jejuni* in the etiology of diarrheal disease among children less than 5 years of age in a community in Bangkok, Thailand. *J Clin Microbiol* 1990; 28: 2507-10.