A Prospective Study to Determine the Prevalence of Acute Diarrhea Caused by *Campylobacter* species in Adults Attended at King Chulalongkorn Memorial Hospital, Thailand

Chanon Maharak, M.D.¹,  
Sumate Hutpattanasilp, M.D.¹,  
Jakrapan Pupaibool, M.D.¹,  
Kanchalee Lertpocasombat, M.Sc.²,  
Sivanee Joy-bai, B.Sc.²,  
Chusana Suankratay, M.D., Ph.D.¹

**ABSTRACT**

**Background:** There have been no prospective studies to determine the prevalence of acute diarrhea caused by *Campylobacter* species in adult patients in Thailand.

**Objectives:** To determine the isolation rate of *Campylobacter* species from the stool of adult patients presented with acute diarrhea attended at King Chulalongkorn Memorial Hospital, Bangkok, Thailand.

**Patients and Methods:** This prospective study was carried out from October 2006 to February 2009. Apart from routine cultures for pathogenic bacteria, the stool specimens were also examined for *Campylobacter* species by direct Gram stain with a 1:20 dilution of carbol-fuchsin used as a counterstain and cultured using two methods including a direct plating method onto a charcoal-based selective medium as well as a membrane filtration method onto a *Brucella* blood agar, and incubated for 72 hours at 42°C and 37°C in a microaerophilic atmosphere. Demography, clinical features, and microbiologic results were also collected and analyzed.

**Results:** A total of 100 patients were included in the study. There were 50 males and 50 females with the mean age of 50 years (range: 16-98 years). The average duration of diarrhea was 42 hours (range: 1-168 hours). Inflammatory and non-inflammatory diarrhea were noted in 63 and 37 patients, respectively. The causative pathogens were identified in 32 (32%) patients. *Salmonella* species was the most common causative pathogen (16 patients, 50%), followed by *Vibrio* species (7, 21.9%), *Clostridium difficile* (3, 1%)
9.4%), Aeromonas species (2, 6.3%), Plesiomonas species (2, 6.3%), and Entamoeba histolytica (1, 3.1%). Campylobacter jejuni was isolated from only one male patient with non-Hodgkin’s lymphoma who presented with acute watery diarrhea. The diagnosis was confirmed by direct Gram stain and the two methods of isolation. He gradually improved after three days of treatment with ceftriaxone.

Conclusions: To the best of our knowledge, this is the first prospective study to determine the prevalence of acute diarrhea caused by Campylobacter species in adults in Thailand. Due to the very low isolation rate, we do not recommend the routine culture for Campylobacter species in adult patients presented with acute diarrhea in our institute. (J Infect Dis Antimicrob Agents 2009;26:35-42.)

INTRODUCTION

Campylobacter species are one of the major causes of acute bacterial gastroenteritis worldwide.1-4 They can cause both inflammatory and non-inflammatory diarrhea as well as other non-diarrheal diseases including Guillain-Barre’ syndrome, reactive arthritis, Reiter’s syndrome, and hemolytic uremic syndrome.2-4 The frequency of acute diarrhea caused by Campylobacter species was reported to be higher than that caused by other bacteria in many studies in the United States and other developed countries.1,5,6 Campylobacter diarrhea commonly causes substantial mortality and morbidity in pediatric patients in developing countries.9 They are also among the most common causative agents of traveler’s diarrhea in many developing countries including Thailand.10-14

To date, there have been no prospective studies to determine the prevalence of acute diarrhea caused by Campylobacter species in adult patients in Thailand. All previous studies were carried out to determine the occurrence of Campylobacter diarrhea in pediatric patients15-19 or travelers from developed countries.10-14 The isolation rates of Campylobacter species in the stool of Thai children with acute diarrhea ranged from 2.4 percent to 28 percent.15-19 Some studies were carried out in healthy adults19, food animals19-22, and dietary product.21

The present study was thus aimed to determine the isolation rate of Campylobacter species from the stool of adult patients presented with acute diarrhea attended at King Chulalongkorn Memorial Hospital (KCMH), a medical university, Bangkok, Thailand.

PATIENTS AND METHODS

Study design

A prospective study was carried out from October 2006 to February 2009. A written informed consent was obtained from all patients, and the present study was approved by the ethical committee of KCMH.

Patients

The inclusion criteria included adult patients older than 15 years old with a documented episode of acute diarrhea. The exclusion criteria included 1) previous use of antimicrobials before enrollment and 2) inability to obtain the stool specimen.

Microbiological methods

All stool specimens were obtained from inpatient, outpatient, and emergency departments. The stool specimen and rectal swab were collected in a clean container and inoculated into Cary-Blair media, respectively, and both were transported to the microbiology laboratory within two hours. The fresh specimen was examined as a wet mount under
microscopy for white and red blood cells and parasites by spreading a fleck of stool mixed with a drop of saline solution over a glass slide and covering with a coverslip. In addition, the stool specimen was examined for *Campylobacter* species by direct Gram stain microscopy as previously described by Wang and colleagues. A few drops of stool specimen were placed on a glass slide, air-dried, and then stained by the conventional Gram stain method with a 1:20 dilution of carbol-fuchsin used as a counterstain. All smears were examined by one of the authors, and the positive result for *Campylobacter* species was determined by the presence of small, gull wings, S-shaped or spirochetal form of Gram-negative bacilli.

The routine stool culture for bacterial pathogens was performed as previously described elsewhere. The specimens were both immediately inoculated onto *Salmonella Shigella* (Oxoid, London, UK), thiosulfate citrate bile salt sucrose (Oxoid, London, UK), and MacConkey (Oxoid, London, UK) agar plates and enriched in tetrathionate (Oxoid, London, UK) broth, and incubated at 37°C for 18 hours. Enteric pathogens were identified by standard bacteriologic, biochemical, and serologic methods.

An isolation of *Campylobacter* species was performed by the two methods including a direct plating method onto a charcoal-based selective medium (Oxoid, London, UK) and a membrane filtration method [Gelman cellulose triacetate (Gelman Instrument Co, Ann Arbor, US)] before plating onto a *Brucella* blood agar (Oxoid, London, UK) as previously described. All cultures were incubated at 42°C and 37°C in a microaerophilic condition (10% CO₂, 5% O₂, and 85% N₂) for 72 hours.

**Definitions**

Acute diarrhea was defined as the occurrence of equal or more than 3 loose stools or bloody and/or mucous stool of at least one time with a duration of less than 14 days. An inflammatory diarrhea was defined as an acute diarrhea with the presence of fever (oral temperature of more than 37.8°C) or fecal white blood cells and/or red blood cells of more than 5 cells per high-power field under microscopy.

**Statistical analysis**

The present study was designed to determine the isolation rate of *Campylobacter* species from the stool of adult patients presented with acute diarrhea. Assuming that 10 percent of acute diarrhea would be caused by *Campylobacter* species in our institute (unpublished data), a sample size of 115 patients was calculated with 95-percent confident interval. The SPSS software version 12 was used for the analysis. The values were presented with the percentage for the categorical variables.

**RESULTS**

**Demographic data**

A total of 100 patients were included during the study period from October 2006 to February 2009. There were 50 (50%) males and 50 (50%) females with the mean age of 50 years (range: 16-98 years). Comorbidities were noted in 68 (68%) patients (Table 1). Of these comorbidities, hypertension was the most common disease (21%), followed by solid organ tumor (16%), diabetes mellitus (14%), old cerebral infarction (9%), cirrhosis (5%), HIV infection (5%), coronary arteries disease (5%), and systemic lupus erythematosus (4%).

**Clinical data**

There were 38 (38%) and 62 (62%) inpatients and outpatients, respectively. The most common clinical presentation was watery diarrhea (85%), followed by abdominal pain (56%), nausea/vomiting (41%), fever
(31%), mucous diarrhea (23%), bloody diarrhea (6%), and tenesmus (6%). The average duration of diarrhea was 42 hours (range: 1-168 hours).

The physical sign most frequently noted was dry mucosa (75%), followed by fever (31%), abdominal tenderness (24%), and hypotension (4%).

**Laboratory data**

There were 63 and 37 episodes of inflammatory and non-inflammatory diarrhea, respectively. The causative pathogens were identified in 32 (32%) specimens (Table 2). *Salmonella* species was the most common causative pathogen (16 patients, 50%), followed by *Vibrio* species (7, 21.9%), *Clostridium difficile* (3, 9.4%), *Aeromonas* species (2, 6.3%), *Plesiomonas* species (2, 6.3%), and *Entamoeba histolytica* (1, 3.1%). *Campylobacter jejuni* was isolated from only one (3.1%) patient.

**A case with Campylobacter diarrhea**

A 51-year-old man with relapsed non-Hodgkin’s lymphoma was hospitalized due to acute watery diarrhea in association with nausea/vomiting and abdominal pain eight days after receiving the fourth course of chemotherapy (rituximab, cyclophosphamide, vincristine, and prednisolone). Physical examination revealed a non-febrile patient with mild dehydration. A complete blood count
showed white blood cell count of 23,530 cells/mm$^3$ (82% neutrophils, 17% lymphocytes, and 1% monocytes). Direct Gram stain of the fresh stool specimen was positive for *Campylobacter* species with the presence of many small, gull wings, S-shaped Gram-negative bacilli. *Campylobacter jejuni* was isolated from the stool specimens by both methods of isolation. He gradually improved after three days of treatment with ceftriaxone 2 g once daily, and discharged home with a full clinical recovery.

**DISCUSSION**

To the best of our knowledge, the present study is the first prospective study to determine the prevalence of acute diarrhea caused by *Campylobacter* species in adult patients in Thailand. In contrast to most previous studies carried out in Thailand and other industrialized countries$^{1-6}$, the isolation rate was very low in the present study. Factors regarding epidemiology, clinical features, and microbiological identification including the age group of patients (children or adults), the types of patients (inpatients or outpatients), the travel status of patients (traveler or native individual), the diarrheal setting (outbreak or non-outbreak setting), the location of hospital enrolled in the study (urban or rural area), the seasonality, the types of diarrhea (inflammatory or non-inflammatory diarrhea), and the methods used to detect the organism, could affect the isolation rate of *Campylobacter* species. In general, the higher prevalence of *Campylobacter* diarrheal diseases is observed in children of less than one year of age and young adults, in comparison with the older age group.$^{2-4,28}$ Previous studies in Thailand showed the recovery rates of *Campylobacter* species in pediatric patients$^{15-19}$ ranged from of 2.4 percent to 28 percent; these were higher than that of the present study which was carried out in adult patients, with most of them being in the old age group.

Patients with *Campylobacter* diarrheal disease are more likely to have symptoms and signs of inflammatory response as well as extra-intestinal
complications\(^2-^4,^2^8\), and hence are likely to be hospitalized than the control group. Most previous studies were carried out in hospitalized patients, and hence over-reporting of the prevalence may occur. The present study included both inpatient and outpatient groups, and probably reflects the true prevalence of *Campylobacter* diarrhea.

*Campylobacter* species are among the major causes of diarrhea in travelers from developed countries. All previous studies in adult patients in Thailand were performed in travelers especially in American military personnel.\(^1^0-^1^4\) The diarrheal diseases in this setting are most likely caused by the consumption of common contaminated food or water, indicating a small outbreak over a short period of study and hence over-reporting the prevalence of the diseases. The present study enrolled the patients in the longer period of more than two years, and hence the results represented a non-outbreak setting of the diseases. In addition, traveler’s diarrhea is categorized in the special group of acute diarrhea since it occurs in non-immuned hosts, compared with that in native individuals.\(^2^7\) Therefore, the recovery rate of *Campylobacter* species should be higher in traveler’s diarrhea than that in the present study.

The important route of *Campylobacter* infections is the consumption of contaminated poultry products or unpasteurized milk, and hence the high prevalence was frequently reported in rural areas than that in urban areas of developing countries due to poor hygiene and sanitation.\(^2-^4\) The present study was carried out in Bangkok where most patients lived in metropolitan area and might have better sanitation.

*Campylobacter* infections usually occur during the summer season.\(^2-^4,^2^8\) All previous studies in Thailand were carried out in a shorter period of less than one year.\(^1^5-^1^9\) The present study was carried out for a longer period of more than two years, and hence our results may represent the true prevalence of the disease in Thailand.

Most patients with *Campylobacter* infection have non-inflammatory diarrhea.\(^2-^4,^2^8\) In the present study, the very low rate of isolation is probably due to the inclusion of approximately two-thirds of the patients with inflammatory diarrhea.

In Thailand, a few hospitals are capable to isolate *Campylobacter* species from the stool specimens. At our institute, an isolation of *Campylobacter* species is performed only upon request by a caring clinician. An isolation of *Campylobacter* species from the stool requires complicated techniques including selective culture media, specialized membrane filtration system, and incubation at 42°C under a microaerophilic environment. Different methods have different sensitivity and specificity for the detection of *Campylobacter* species in the stool specimens.\(^2^3,^2^5,^2^6\) Most previous studies in Thailand were carried out using only one method for the detection.\(^1^0-^1^9\) Under-reporting of the prevalence of *Campylobacter* diarrhea in the present study is less likely since all three methods were used to determine the presence of organism.

There are some limitations in the present study. The study was carried out in only one hospital in Bangkok, and hence the results may not be generalized to other hospitals especially located in rural areas. In addition, a passive surveillance in the present study usually identifies the patients with severe infection who seek medical attention, and hence may underestimate the true prevalence of the diseases in the community. However, our results provide the first data of the burden of *Campylobacter* diarrheal diseases in adult patients in Thailand.

In conclusion, to the best of our knowledge, this is the first prospective study to determine the prevalence
of acute diarrhea caused by *Campylobacter* species in adults in Thailand. Due to the very low isolation rate, we do not recommend the routine culture for *Campylobacter* species in adult patients presented with acute diarrhea in our institute.

**Potential conflicts of interest**

All authors have no conflict of interest.

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