

## INFECTIOUS DISEASES

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### ***Campylobacter jejuni* Among Patients with Gastroenteritis: Incidence at a Reference Microbiology Laboratory in San Juan, Puerto Rico**

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**Objective.** A study designed to evaluate the incidence of *Campylobacter jejuni* infection among patients with gastroenteritis referred to a Microbiology Laboratory in San Juan was conducted from December 1998 through May 1999.

**Background.** *Campylobacter jejuni* is recognized around the world as one of the principal causes of gastroenteritis. A specific serotype of this organism has been recently mentioned as a major pathogen preceding Guillain-Barré syndrome. Establishing incidence of *Campylobacter jejuni* infection is important in order to develop uniform guidelines for clinical laboratories; whether to attempt isolation of this pathogen from all stool samples, or to perform the special procedures only when it is specifically requested by the attending physician.

**Methods.** In this study, stool samples received at the Microbiology Department of a Reference Laboratory in San Juan, with clear signs of gastroenteritis (i.e. bloody and/or liquid stools) were selected for *Campylobacter* culture. Samples were fresh rectal swabs, liquid stools or previously inoculated

*Campylobacter* thioglycollate broth. Stools were inoculated directly to the agar, either by using a swab or alternately 1-2 drops of liquid stools, or of the previously inoculated *Campylobacter* thioglycollate broth. Plates were incubated for 72 hrs. at 42 °C in a microaerophilic atmosphere (Beckton Dickinson, BBL Campy Pouch).

**Results.** *Campylobacter jejuni* was isolated from 3/98 (3.0%) of the samples tested; two of which were cultured as specifically requested by the patient's physician. One isolate was obtained from a sample with no specific request for *Campylobacter* culture. Enteropathogens other than *Campylobacter jejuni* were isolated from 18/380 (4.7%) of routinely cultured samples.

**Conclusions.** The study showed a small increment in *Campylobacter jejuni* isolation when testing samples with no specific request for *Campylobacter* culture. *Campylobacter jejuni* incidence was second to *Salmonella* species. **Key words:** Diarrhea, *Campylobacter* infection, Microbiology

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**D**iarrheal diseases represent one of the major causes of morbidity and mortality throughout the world, both in the developed as well as in the underdeveloped countries (1-4). Diarrheal illness can be classified as either inflammatory or non-inflammatory. The inflammatory diarrheas tend to be more serious and often need to be followed by more extensive diagnostic studies and culturing procedures. It has been estimated

that more than four million children of less than one year of age, die every year due to infectious diarrhea (2). *Campylobacter jejuni* is one of the most common causes of inflammatory gastroenteritis (4). Associated symptoms are diarrhea, severe abdominal pain, fever, fecal leukocytes, and bloody feces.

The diagnosis of *Campylobacter jejuni* gastroenteritis is important due to the serious complications that can be associated with this infection such as: acute colitis similar to Crohn's disease (5), Fisher syndrome (6), and Guillain-Barré syndrome (7-13). There is at least one case of lethal maternal sepsis (14) associated with this bacteria. Cases of toxic megacolon, pseudomembranous colitis, and massive lower gastrointestinal hemorrhage have also been described (15). Recognition of the role of this disease is

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difficult, since the microorganism has relatively fastidious growth requirements. Some laboratories perform the special culture process only when specifically requested by the patient's physician and others prefer to try *Campylobacter* isolation on every stool received with requisition for microbiological culture. Health authorities on the island have not established clear laboratory guidelines as of this moment. The incidence of this infection in Puerto Rico is not known and was therefore investigated among samples from patients with gastroenteritis received at a reference microbiology laboratory in San Juan.

### Materials and Methods

**Samples.** Samples were selected for *Campylobacter* culture using different criteria. A questionnaire was designed to determine if the sample was appropriate for culture in terms of date of collection, consistency of stool, and transport media. Only fresh samples with liquid or aqueous consistency, and adequately transported samples were selected. Results of occult blood and fecal leukocyte tests were also recorded when available. Samples were fresh rectal swabs or liquid stools and samples previously inoculated in *Campylobacter* thioglycollate broth.

**Media.** The basal media used for the isolation of *Campylobacter jejuni* was *Campylobacter* agar, CVA (PML Microbiologicals) consisting of: nutritious base media containing 15.0 g of proteose peptone, 2.5 g liver digest, 2.5 g yeast extract, 5.0 g sodium chloride, 12.0 g agar, 50.0 ml sheep blood, 20.0 mg of Cefoperazone, 10.0 mg Vancomycin, and 2.0 mg of Amphotericin B. Final pH of the media was  $7.4 \pm 0.2$  at 25 °C. For the transportation of samples Cary Blair transport media (Difco swabs) or *Campylobacter* thioglycollate broth (PML Microbiologicals) was used.

**Inoculation of samples.** Stools were inoculated directly to the agar at room temperature, either by using a swab or alternately 1-2 drops of liquid stools, or of the previously inoculated *Campylobacter* thioglycollate broth.

**Incubation.** Plates were incubated for 72 hours at 42 °C in a microaerophilic atmosphere (BBL Campy Pouch). In this system, microaerophilic environment is generated by a combination of Hydrogen plus CO<sub>2</sub> with integral palladium catalyst. Produced atmosphere is 5-15% CO<sub>2</sub> with a residual atmosphere of approximately 5-10% O<sub>2</sub>.

**Identification.** Isolated colonies were presumptively identified as *Campylobacter jejuni* using colony morphology, gram staining (Difco) to confirm bacterial morphology, Oxidase test reagent (Difco), sensitivity to Nalidixic acid (30 mcg disk), resistance to Cephalothin (30 mcg disk), and hippurate hydrolysis. Quality control

for media and reagents were *Campylobacter jejuni* ATCC 33291 and ATCC 29428 (BBL). Other organisms used as negative controls were *Escherichia coli* ATCC 25922, and *Candida albicans* (recently isolated strain).

### Results

A total of 380 samples were tested for enteropathogens other than *Campylobacter jejuni*. Results were positive in 18/380 (4.7%) of the samples. Table 1 shows the results obtained during each month of the study. Three isolates of *Campylobacter jejuni* were obtained from samples

**Table 1.** Enteropathogens Isolated from December 1998 to May 1999\*

Month	Samples tested †	Number of isolates ‡	Enteropathogen isolated §
December	69	7	<i>Salmonella</i> species
January	68	1	<i>Bacillus cereus</i>
February	75	2	<i>Staphylococcus aureus</i> **
		1	<i>Salmonella</i> O group C <sub>1</sub>
March	63	1	<i>Yeast</i> species ††
April	59	1	<i>Salmonella</i> O group B
		1	<i>Salmonella</i> O group C <sub>1</sub>
		1	<i>Salmonella</i> O group D
		1	<i>Salmonella</i> O group C <sub>1</sub>
May	46	1	<i>Shigella sonnei</i>
		1	<i>Salmonella</i> species.

\* Not including *Yersinia* species, ova & parasites, or viral tests.

† Total stool samples routinely tested.

‡ Isolates other than *Campylobacter jejuni*.

§ Serology tested with Difco Reagents.

\*\* Abundant growth, with scanty growth of normal enteric flora.

†† Pure growth, sample with no normal enteric flora.

selected for *Campylobacter* culture. Of these samples, two had requests for *Campylobacter* culture, and one had no special culture request. None of these three patients were positive for other enteropathogens. In addition, all three patients had positive findings in fecal leukocyte tests and two were positive for occult blood. Table 2 summarizes these results.

Of all the samples received, 282 (74%) were rejected because the questionnaire was incomplete or not available, transport media was not adequate or samples were not fresh (i.e. received 8 hours or more after collection). The remaining 98/380 (26%) was inoculated as described in the materials and methods section.

Results obtained showed that the incidence of

**Table 2.** *Campylobacter jejuni* isolates\*

Month	Total samples with request for <i>campylobacter</i> culture / total samples tested †	Total <i>Campylobacter</i> isolates
December	1/16	0
January	1/18	1
February	2/13	
March	6/18	1
April	4/19	1
May	0/14	0

\* Selected for *Campylobacter jejuni* culture.

† Total of samples with *Campylobacter* culture requisition / total of samples tested.

**Table 3.** Enteropathogen incidence\* December 1998-May 1999

Enteropathogen	Incidence † (%)
Salmonella species	14/380 (3.7%)
<i>Campylobacter jejuni</i>	3/98 (3.0%)
<i>Bacillus cereus</i>	1/380 (0.3%)
<i>Staphylococcus aureus</i>	1/380 (0.3%)
Yeast species	1/380 (0.3%)
<i>Shigella sonnei</i>	1/380 (0.3%)

\* Not including culture for *Yersinia* species, viral tests or parasite preparations.

† In total patients with gastroenteritis tested.

*Campylobacter jejuni* was lower than the incidence of *Salmonella* species. The results are shown in table 3.

## Discussion

The isolation of *Campylobacter jejuni* relies on the quality of the sample and the culture techniques employed. It is important to perform cultures in optimal conditions (15-20), following three critical steps for the isolation of enteric *Campylobacter* from stools: specimen should be promptly plated or refrigerated in an appropriate transport medium; plating medium should be enriched to support growth and also selectively inhibit growth of normal stool flora; and the atmosphere should be microaerophilic.

Bolton et al report good performance for CampyPak Plus system when isolating *Campylobacter* species (21). Some reports suggest that 72 hours of incubation instead of 48 hours will lead to a significant increment in isolation rate and others suggest that filtering techniques do not assure important increment in percent of recovery (22). Therefore, we decided to use CampyPaK Plus, incubate

for 72 hours instead of 48 hours, and not use filtering techniques. In addition, species identification can be affected by inoculum size and hence, we used a standard inoculum of 10<sup>6</sup>CFU/ml as recommended by On et al (23). Although all these aspects were thoroughly controlled during this study, false negative results cannot be completely overruled; consequently, incidence rates could be higher.

## Conclusions

Diagnosis of *Campylobacter jejuni* enteritis is important; nonetheless, time expenditure and use of special culture techniques for specimens in which there is little chance to recover significant pathogens should not be considered cost-effective procedures. Physicians need to be clearly informed about the scope of the bacterial culture; laboratory reports must specify which enteropathogens are or are not detected with the techniques employed. Public health authorities should establish specific guidelines and educational programs in accordance with incidence of this disease in our population and the prevalence of this infection in pets.

When *Campylobacter jejuni* is suspected, the laboratory should take all measures needed in order to assure optimal conditions for isolation. This includes having a well-designed quality assurance program and educating both physicians and medical technologists about the special requirements for accurate *Campylobacter jejuni* culture isolation.

The sequelae to foodborne disease can have long-term impact. First, it means health and psychological impact on patients (e.g. Guillain Barré syndrome). Second, it means an economic impact on patient and health authorities as well. Some studies calculate costs were up to \$1.5 to \$8.0 billion in United States for the year 1995 (24).

This study strongly indicates that further investigation is needed in Puerto Rico to evaluate: which are the main risk factors for *Campylobacter* infection; what is the incidence of this microorganism in food (e.g. chicken industry); what, if any, is the relation between this disease and Guillain-Barré syndrome patients; and what recommendations or guidelines for clinical laboratories are needed for the optimal recovery of this bacteria in stool cultures.

## Resumen

Para determinar la incidencia de *Campylobacter jejuni* entre pacientes con gastroenteritis, se estudiaron 98 muestras de excreta recibidas durante los meses de

diciembre del 1998 a mayo del 1999, en un laboratorio de referencia en San Juan, Puerto Rico. Se utilizaron muestras en hisopos con medio de transporte, muestras líquidas o previamente inoculadas en caldo Campyloglicolato. Las muestras fueron evaluadas para presencia de *Campylobacter jejuni* usando métodos de cultivo en medio selectivo, incubadas a 42 °C y en atmósfera microaerofílica durante 72 horas. Un 3% del total de muestras arrojó cultivo positivo, de los cuales dos tenían requisición para cultivo de *Campylobacter* y uno fue aislado de una muestra sin requisición para ese análisis en particular. En resumen se encontró un ligero aumento en el aislamiento de *Campylobacter jejuni* en muestras sin requisición específica para cultivo de *Campylobacter*. La incidencia resultó más baja que la de *Salmonella* species.

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1 Not including *Yersinia* species, ova & parasites, or viral tests.