

Incidence, isolation and characterization of *Campylobacter* species in Osogbo

*OC Adekunle¹, AO Coker², DO Kolawole³

¹Department of Medical Microbiology & Parasitology, Ladoké Akintola University of Technology, Osogbo, Nigeria.

²Department of Medical Microbiology & Parasitology, University of Lagos, Lagos, Nigeria.

³Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

*Corresponding Author: toyintoro@yahoo.com
P.O. Box 14066, U.I. Ibadan, Oyo State, Nigeria.

Abstract

This study was conducted to establish *Campylobacter* as a bacterial agent of diarrhea in Osogbo, Osun State in Nigeria. Stool samples of six hundred and two (602) children aged between 0 and 36 months presenting with diarrhea and one hundred children (100) children age-matched controls without diarrhea were collected and cultured using Butzler type media. The sample collection was done for over a period of 2 years. Three (3) samples out of 602 samples were positive for *Campylobacter coli*, giving an occurrence of approximately 0.5% in Osogbo. The isolation of *Campylobacter coli* was an indication that *C. coli* is a causative organism of gastroenteritis in this environment.

Keywords: *Campylobacter*, diarrhea, isolation, strains, causative organism, bacteria, occurrence, stool sample.

Introduction

Campylobacters are curved rods that were classified as vibrios for many years. (Marcus et al., 1985). *Campylobacters* are found to be associated with animal and human diseases (Uaboi-Egbenni, 2008)). The most common human disease caused by *campylobacters* is acute gastroenteritis (Smibert, 1978).

This infection occurs primarily in infants, elderly people, and patients with underlying disease. The disease is accompanied by fever, bloody diarrhea, headache and abdominal pain. A collective name for infectious diseases caused by members of these bacteria is called *Campylobacteriosis* (Coker et al., 2002). *Campylobacteriosis* is a self-limited disease and antimicrobial therapy is not generally indicated. However, treatment can reduce the duration and the severity of illness if it is initiated early in the cause of infection. There is no information in literature on the occurrence of *campylobacter* in stool specimens in Osogbo. This work is therefore an original explorative research into the involvement of these agents in cases of

gastroenteritis in this environment. The work is designed to carry out prevalence studies on *campylobacter* species causing gastroenteritis.

Materials and Methods

This study was carried out prospectively at the General Hospital Asubiaro, Ladoké Akintola University Teaching Hospital, Jaleyemi Private Hospital, all in Osogbo between March 2006 and June 2008. Subjects were patients between the ages of 0 and 36 months who presented with foul-smelling diarrhea with or without mucus, fever and abdominal pain at the Pediatrics Units of the Hospitals.

A total of 602 patients with diarrhea and 100 children without diarrhea for the past 2 weeks were recruited. Stool samples were collected and rectal swab were collected. Butzler –type – medium was used to inoculate the specimen and were incubated at 42°C for 72 hours. All the distinct colonies found were gram- stained, and different biochemical tests such as catalase test, oxidase test, and turbidity standard test were performed. Biotyping was carried out using Lior biotyping scheme.

Biotyping scheme for *C. jejuni*, *C. coli* & *C. lariidis*.

Biotype	<i>C. jejuni</i>				<i>C. coli</i>		<i>C. lariidis</i>	
	I	II	III	IV	I	II	I	II
Hippurative hydrolysis	+	+	+	+	-	-	-	-
Rapid H ₂ S test	-	-	+	+	-	-	+	+
DNA hydrolysis	-	+	-	+	-	+	-	+

Confirmation of *Campylobacter* species using PCR

To confirm campylobacter isolates using PCR, DNA was isolated from a fresh culture of each strain of campylobacter and PCR primers - (A1) Pfla B-F, AAG GAT CCA CAC TTA AAG GCG CTA TGG CTG TGA TG; (B2) Pfla B-R, AAG GAT CCG ATG TTG GTG TTT ATC CTA AAA CC specific for *Campylobacter* were used for identification. Primers were obtained from Biomers.net, the biopolymer manufacturer in Germany.

Statistical analysis

To determine the significance of differences in infection rates, the SPSS software (version 10.1) was used. The differences were considered significant when the p-value was less than 0.05.

Results

Distribution of campylobacter-associated infection in the study population

Of 602 stool samples collected from patients with diarrhea, 234 were from females and 368 from males aged one month to 36 months with a median of 15 months. Of the 602 diarrhea, only 3 (0.5%, 3/602) were due to *Campylobacter* sp.. Of the 3 diarrhea patients infected by *Campylobacter* sp. all were males (100%; 3/3). This is an indication that the infection the infection rate higher is higher among males (0.82%; 3/368) than females (0%; 0/234) in Osogbo. The difference was

statistically significant ($p < 0.05$, 95% confidence interval). Table 1 indicates the distribution of the study population by sex and also by the infection rate of campylobacter spp. Table 3 indicates the distribution of the study population by age-group and also by the infection rate of *Campylobacter* sp..

A total of 702 subjects comprising 602 (86%) with diarrhea and 100 (14%) without diarrhea which served as control, were examined during the period of study. From the 602 subjects with diarrhea, 3 were positive for campylobacter species giving a prevalence rate of ~0.5% (Table 1). All the patients positive for *Campylobacter* specie were males. None of the control subjects was positive for campylobacter species (Table 2). From the age distribution of children with diarrhea, all the isolates were gotten from children between ages 0-36 months (Table 3).

The distribution of the *Campylobacter* sp. and other enterobactereace isolated in the various centres is shown in table 1. Three (3) i.e. (100%) *Campylobacter* species were isolated from samples collected from General Hospital, Asubiaro. Of the isolates of other enterobactereace, (87.14%) were gotten from samples collected from General Hospital, Asubiaro; (12.02%) were gotten from samples collected at Jaleyemi Private Hospital; while (0.84%) were gotten from samples collected at Pediatrics Unit of Ladoke Akintola University of Technology, Osogbo (Table 4).

Table 1: Distribution of patients with isolates (children with diarrhea).

Sex	No. examined	Campylobacter isolates	Isolates of other organisms
Male	368	3	365
Female	234	0	234
Total	602	3	599

Table 2: Control groups (children without diarrhea).

Sex	No. examined	Campylobacter isolates
Male	50	0
Female	50	0
Total	100	0

Table 3: Age distribution of patients with the isolates.

Age (months)	No. examined	Campylobacter isolates
0 – 6	110	0
7 – 12	179	0
13 – 18	68	2
19 – 24	49	1
25 – 30	125	0
31 – 36	71	0
Total	602	3

Table 4: Distribution of bacteria associated with gastroenteritis in various centres.

<u>Centres</u>	<u>No. examined</u>	<u>Campylobacter isolates</u>	<u>Other isolates</u>
Gen Hos Asubiaro	525	3	522
Jaleyemi/ Private Hospital	72	0	72
Lautech tech Hospital	5	0	5
Total	602	3	599

No campylobacter isolated was gotten from the control group.

The results of biochemical tests and PCR showed that all the isolates were *Campylobacter coli*.

Discussion

Occurrence of enteritis caused by *Campylobacter* sp. has been recorded virtually all over the world. In this study, the prevalence rate of campylobacter enteritis in Osogbo was appropriately 0.5%. When compared with reports of previous studies in other centres, it was observed to show least prevalence. For instance, Coker et al. (1994), in Lagos reported prevalence of 12.4% and in 2002 at Ile-Ife, Aboderin et al. reported 19.1%. Also, Samuel et al. (2002) at Ilorin reported 8.2%. However, in this study in Osogbo, the small number of *Campylobacter* sp. observed may be an indication that campylobacteriosis have very low prevalence rate in Osogbo. It is very important to note that occurrence of enteritis caused by other bacteria was higher when compared with incidence of campylobacteriosis. This is in contrast with reports from Quebec where reports showed that prevalence of campylobacteriosis is more than the combined total caused by other bacteria such as *Salmonella*, *Shigella*, *E. coli*, etc.

In this study, *Campylobacter* sp. was not isolated from the control group. Although, this observation is in agreement with the reports of Coker et al., (1985 and 1984) which showed that *Campylobacter jejuni* or *coli* is pathogenic, however, reports from other developing countries have shown recovery of *Campylobacter* sp. from children without diarrhea (Megraud et al., 1990). Value as high as 14.9% has been reported (Megraud et al., 1990). Acquisition of the pathogen because of poor sanitation and contact with animals early in life may explain the isolation from healthy children. The report flight incidence recovery from asymptomatic persons in developing countries is in contrast with reports of low incidence of recovery from developed countries such as Netherlands i.e. 0.5% (de Wit et al., 2001). Reports have shown that prevalence rate of enteritis due to

Campylobacter sp. in developing countries (5-20%) is higher when compared with incidence in developed countries (5.1-7.1%) (Coker et al., 2002), and this may be due to poor sanitation. Poor hygiene and sanitation and close proximity to animals in developing countries all contribute to easy and frequent acquisition of any enteric pathogen, including *Campylobacter*.

In this study, the clinical spectrum of campylobacter enteritis ranges from a watery, mucoid, non-bloody diarrhea to abdominal pain and fever. This is in agreement with earlier reports by Oberhelmen (2000) and Taylor (1992), which indicated that the disease is less severe in developing countries. In developed countries, disease is characterized by bloody stool, fever and abdominal pain, Rao et al. (2001) and Coker et al. (1985) reported clinical features such as watery stool, fever, abdominal pain, vomiting, and dehydration in patients with diarrhea in developing countries. It is noteworthy to report that all children in this study who were suffering from campylobacteriosis were all above 6 months. Although campylobacter infections occur mostly below the age of 2 years, however, the frequency of infection is reduced in children below 6 months of age. This finding may be connected with strong baby-friendly initiative in this environment. This programme encourages exclusive breastfeeding of infants for the first 6 months before other foods are introduced. The immunity acquired from mothers' breast milk will limit the effect of campylobacter in these children. Furthermore, due to exclusive breastfeeding, exposure to adults foods that may be contaminated and eventually serve as sources of campylobacteriosis will be greatly reduced thus limiting prevalence rate in children below 6 months.

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