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## CAMPYLOBACTER SPP. EPIDEMIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY IN A DEVELOPING COUNTRY, BURKINA FASO (WEST AFRICA)

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RUNNING TITLE: CAMPYLOBACTER IN BURKINA FASO

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### ABSTRACT

Data on campylobacteriosis are almost nonexistent in Burkina Faso. In this study conducted from 2006 to 2008 in Ouagadougou, stool specimens and sociodemographic data were collected from 1 246 patients attending the university teaching hospital for enteritis. Stool samples were analyzed for the presence of *Campylobacter* by the direct culture method on selective mCCDA agar followed by antibiotic susceptibility testing on the isolated strains. The isolation rate of *Campylobacter* was 2.3%, comprising of the following species *C. jejuni* (51.8%), *C. coli* (13.8%), and *C. upsaliensis* (3.5%). However, 30.9% of the isolates were unidentified. No resistant strain was found to gentamicin. The resistance to amoxicillin+clavulanic acid (3.4%) was lower than those (10.3-34.5%) to the other antibiotics: erythromycin (10.3%), tetracycline (10.3%), ciprofloxacin (13.8%), amoxicillin (24.1%) and ceftriaxone (34.5%), nalidixic acid (34.5%). Significant associations were found between *Campylobacter* enteritis and contact with animals ( $P=0.03$ ), and HIV infection ( $P<0.0001$ ), in contrast to other sociodemographic and seasonal factors. From the data obtained Amoxicillin+clavulanic acid appear to be the first choice for treatment. The implementation of a national program may be helpful in controlling the spread of the disease and the increase of resistance to antibiotics.

Keywords: *Campylobacter*, epidemiology, HIV, drug resistance, Burkina Faso.

### INTRODUCTION

*Campylobacter* gastroenteritis have a major public health importance worldwide, and their rate has been increasing (1, 2). *Campylobacter* is the most common cause of culture proven bacterial gastroenteritis in developed and developing countries, responsible for 400-500 million cases of diarrhea each year (3). However, the clinical, laboratory and epidemiological data available are mostly from developed countries (1, 2, 4, 5). In these countries, the highest rates are found among children less than five years old and young adults, mostly men aged from 20 to 29. In developing countries where studies have been conducted, the data showed that *Campylobacter* enteritis is particularly associated with young children (1, 2). These last years, the diarrhea mortality has declined in the world due to increased use of therapies, improved nutrition feeding, hygiene and sanitation. More recent data showed that the rates of campylobacteriosis have been decreasing in numerous developing countries (3, 6, 7, 8).

*Campylobacter* infection is a zoonotic disease that can be hyper endemic, linked to outbreaks and sporadic infections. Known risk factors for the disease include ingestion of undercooked poultry and other meat, contaminated food and water or unpasteurized milk and dairy products, direct contact with pets, farm animals, small children, and swimming in lakes, but also travel abroad. Direct

person-to-person transmission between adults appears to be uncommon. In some developed countries, seasonal patterns in pets and in human infections as well as travel-related infections have been observed (2, 4, 9, 10, 11). Several hypotheses have been advocated to explain the seasonality of *Campylobacter* infections, including the controversial role of flies as vectors (12, 13); however much remains to be done about the epidemiology of these pathogens. Clinically, the disease is often more severe and longer in developed countries, with bloody stools, than cases in developing countries where it is shorter and stools are more liquid and can contain leukocytes (1, 13). *Campylobacter* enteritis can be asymptomatic and self-limiting; in severe cases effective antibiotic therapies are available. However, acquired resistance to macrolides, fluoroquinolones and other antibiotics used most widely gives rise to a challenge in campylobacteriosis control worldwide (3, 14, 15, 16). Data on campylobacteriosis in developing countries including most of the sub-Saharan Africa are few (1, 17, 18, 19). In Burkina Faso, no data of autochthon cases are available for animals, foods, environment or humans. The only scientific report related to *Campylobacter* enteritis acquired in Burkina Faso is about cases from Belgian (8) and Swedish (9) international travellers who had stayed in the country between 1994 and 2006.

The aim of this study was to present bacteriological and epidemiological data on *Campylobacter* enteritis among autochthon patients with diarrhea in Burkina Faso.

## MATERIALS AND METHODS

### Patients and Sample collection

A series of 1246 consecutive specimens of stool were obtained from patients attending clinics at the University Hospital CHUO for acute gastroenteritis, between November 2006 and February 2008. No patient received antibiotic therapy up to 72h prior to the sample collection which was carried out using sterile flasks. Sociodemographic data and macroscopic aspects of stool specimens were recorded using a structured questionnaire. The informed consent of each patient and the approval of the institution ethical committee had been obtained. All stool specimens were collected at the University Hospital and were analysed within 1h after arrival.

### Bacteriological investigations and drug susceptibility testing

The presence of leukocytes and erythrocytes in stools was determined microscopically. For *Campylobacter* isolation, each sample was streaked onto a modified charcoal cefoperazone desoxycholate agar, mCCDA (Oxoid, United Kingdom). The CCDA comprised of *Campylobacter* blood-free selective agar base with *Campylobacter* selective supplement (Oxoid) and *Campylobacter* growth supplement (Oxoid). The plates were incubated for 48 h at 37°C under microaerophilic conditions (5% O<sub>2</sub>, 5% CO<sub>2</sub>, 2% H<sub>2</sub>, and 88% N<sub>2</sub> by volume) generated by Campy pack plus (Becton Dickinson) or GENbox Microaer (bioMérieux SA; France). Colonies resembling those of *Campylobacter* (gray, flat, and spreading) were further evaluated by Gram's stain, oxidase and catalase activities, and by growth on duplicate plates of Columbia Blood Agar with 5% (v/v) defibrinated horse blood (bioMérieux SA). The two plates were incubated at 37°C for 48h, under different conditions: one aerobically and one under microaerophilic conditions. Oxidase- and catalase-positive colonies exhibiting a characteristic Gram stain appearance (Gram-negative S-shaped rods) and growing only under microaerophilic conditions were reported to identify the genus of *Campylobacter*. Suspensions of these colonies to a turbidity equivalent to 6 McFarland were inoculated to API® Campy gallery system (bioMérieux) to differentiate the *Campylobacter* strains into species.

Drug susceptibility tests of the isolates were conducted by disc diffusion method: all laboratory-confirmed *Campylobacter* isolates were inoculated onto Mueller-Hinton agar plates supplemented with 5% sheep's blood (bioMérieux) and incubated at

37°C for 24h. Amoxicillin (25µg), amoxicillin/clavulanic acid (10µg), ceftriaxone (30µg), gentamicin (10µg), erythromycin (15µg), tetracycline (30µg), nalidixic acid (30µg) and ciprofloxacin (5µg) disks (bioMérieux) were tested. The disc diffusion method was used, according to the guidelines of the French Society for Microbiology (20). In this study any strain with an intermediate sensitivity was considered resistant. *C. jejuni* ATCC 25936 and *C. coli* ATCC 33559 strains were used as controls throughout the testing period.

### Statistical analysis

The Epi-Info 3.3.2 version 2004 software was used to record all data. Comparisons between qualitative variables were done using the Chi-2 test (Likelihood Ratio or Linear-by-Linear Association or Mantel-Haenszel method). Statistical significance between differences was set at  $P < 0.05$ .

## RESULTS

### Patient characteristics

Among the 1246 patients, 638 (51.2%) were male. Their mean age was 21 years [ranges: 0-80years]; the most important age ranges were 0-5 years (n=494; 39.7%), 21-25 years (n=174; 11.9%) and 26-30 years (n=158; 10.9%). Patients living in Ouagadougou (n=1188; 95.3%) were more represented than those coming from other urban and rural areas (n=58; 4.7%). More than half (n=670; 53.8%) of overall patients were educated and those hospitalized (n=640; 51.4%) were more represented than outpatients (n=606; 48.6%). Seven (0.6%) patients were HIV-1 positive.

### Macroscopic aspects of stool specimens and faecal leukocytes

Four types of stools were reported: pasty (65%), liquid (18.9%), mucous (11.1%) and formed (5%). Pasty stool specimens were more frequent than other types. Leukocytes were found in 164 (13.2%) fresh stool specimens after microscopic examination.

### Bacteriological data

In this study in Burkina Faso, the isolation rate of *Campylobacter* was 2.3% (29/1246). *C. jejuni* (51.8%, comprising 38% of *C. jejuni jejuni* and 13.8% of *C. jejuni doylei*) and *C. coli* (13.8%) were the most prevalent species. However, 30.9% (n=9) of strains had *Campylobacter*'s characteristics, even if they could not be identified using Api® Campy (bioMérieux SA).

The results of drug susceptibility tests are reported in Table 1. They showed that only gentamicin was effective against all strains. Ceftriaxone- and amoxicillin-resistant strains represented 34.5% and 24.1%, respectively. The resistant rate was moderate to erythromycin (10.3%) and tetracycline (10.3%), and low to amoxicillin/clavulanic acid (3.4%).

TABLE 1: SUSCEPTIBILITY OF *CAMPYLOBACTER* STRAINS TO ANTIBIOTICS

Antibiotic tested	Sensitive (%)	Resistant (%)
Ampicillin (AM)	22 (75.9)	7 (24.1)
Amoxicillin+clavulanic acid (AMC)	28 (96.6)	1 (3.4)
Ceftriaxone (CRO)	19 (65.5)	10 (34.5)
Gentamicin (GM)	29 (100)	0
Erythromycin (E)	26 (89.7)	3 (10.3)
Tetracycline (TE)	26 (89.7)	3 (10.3)
Nalidixic acid (NA)	19 (65.9)	10 (34.1)
Ciprofloxacin (CIP)	25 (86.2)	4 (13.8)

#### Clinical illness of patients infected by *Campylobacter*

Clinical signs of the patients on presentation were as follow: diarrhea (62.1%), abdominal pains (62.1%), fever (51.7%), asthenia (44.8%) and vomiting (31%) were the most common symptoms. A single (3.4%) infected patient had none of these signs. Three of 7 (43%) of HIV-positive patients had *Campylobacter*, but only 26 (2%) among the other 1239 patients.

#### Epidemiological characteristics of *Campylobacter* enteritis

The patient variables studied are reported in Table 2. The number of *Campylobacter* strains isolated from females was more prominent, but the difference was not statistically significant ( $P=0.15$ ; Odds Ratio: 0.575; 95%CI: 0.26-1.22). No statistically significant difference was found between all age ranges of *Campylobacter* infected patients ( $P=0.14$ ) except those of 0-5 and 21-40 years ( $P=0.62$ ; OR: 0.823; 95%CI: 0.37-1.79) which showed a higher infection frequency. Attendance at school or not was not a risk factor for *Campylobacter* enteritis ( $P=0.69$ ; OR: 1.158; 95%CI: 0.55-2.42); no difference was associated with the level of education. All the cases of *Campylobacter* infections were found in patients from Ouagadougou. The patients who were in touch with pets (3.4%) were more frequently infected than those who had no contact (2%) with animals ( $P=0.03$ ; OR: 0.423; 95%CI: 0.19-0.93).

The frequencies of isolation of *Campylobacter* were more common in January, June, July, October and November than other months in 2007 (Figure 2). The complete data for the year 2007 did not show any statistically significant difference in the monthly rates of infection ( $P=0.20$ ). There was no difference between the rates of infection during the dry season and the rainy season ( $P=0.85$ ; OR: 0.930; 95%CI: 0.42-2.02): indicating that, there was no seasonality in the pattern of the disease.

#### DISCUSSION

The rate of *Campylobacter* enteritis in the studied population was 2.3%. Such a rate in urban sites of Africa is uncommon: the rates reported in most of

other developing countries are usually higher (1, 3, 19). However, a comparable rate of 2.4% was reported in the Northeast of Brazil (21) and in Teheran (22). *C. jejuni* and *C. coli* were the main species identified in Burkina Faso: this result was comparable to those found as well in other developing countries where enteritis due to *Campylobacter* have been studied as in developed countries (1, 3, 5, 6, 19, 23, 24).

More than 30% of the strains in Burkina Faso were not identified by the gallery Api<sup>®</sup>Campy: this limitation has also been reported in was reported by other studies using this gallery. The authors reported misidentifications of *C. concisus* for *C. mucosalis*, and they reported additional problems in identifying certain *C. coli* and *C. lari* strains (4, 25). The use of molecular techniques will allow these strains to be typed and possibly identify new pathogenic species of *Campylobacter*.

The infection rate in females was higher than in males, although this difference was not statistically significant. In some developed countries, it has been demonstrated that the rate of *Campylobacter* enteritis was higher in males than in females (6, 23, 26). The association between gender and campylobacteriosis may vary according to the behaviour, geographical area and the population, and it can be different within the same country. In Burkina Faso, children of 0-5years old and young adults of 21-40 years old were the most infected. The high recovery rate of *Campylobacter* species in children is common worldwide, and Burkina Faso is no exception. However, a high rate in young adults comparable to that in children can be associated with a common source of contamination: indeed, these young adults in Burkina Faso are workers who eat in the street, outside of their house. Neither schooling, nor the level of education were risk factors to enteritis due to *Campylobacter*, contrary to data reported in a study in Jordan where education was a risk factor that correlated significantly with diarrhea due to *Campylobacter* enteritis (27). The education of the consumers of vector products of campylobacteriosis is important in the implementation of national programs of surveillance and fight against *Campylobacter*. More

TABLE 2: SOCIODEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS

Characteristics	<i>Campylobacter</i>		Total (%)	<i>P</i> value	OR	95%CI
	Positive	Negative				
<b>Gender</b>						
Male	11	627	638	0.153	0.575	[0.26-1.22]
Female	18	590	608			
<i>Total</i>	29	1217	1246			
<b>Age (year)</b>						
0-5	12	482	494	0.148		
6-20	1	121	122			
21-40	14	463	477			
41-50	2	75	77			
>50	0	76	76			
<i>Total</i>	29	1217	1246			
<b>Education level</b>						
Not schooled	13	563	576	0.698	1.158	[0.55-2.42]
All schooled	16	654	670			
<i>Primary school</i>	3	159	162	0.751		
<i>Secondary school</i>	7	262	269			
<i>High school</i>	6	233	239			
<i>Total</i>	29	1217	1246			
<b>Residence in Burkina Faso</b>						
Ouagadougou	29	1159	1188			
Other localities	0	58	58			
<i>Total</i>	29	1217	1246			
<b>Contact with animal</b>						
Yes	9	255	264	0.034	0.423	[0.19-0.93]
No	20	962	982			
<i>Total</i>	29	1217	1246			
<b>Hospitalization</b>						
Outpatients	17	623	640	0.28	0.664	[0.31-1.4]
All inpatients	12	594	606			
<i>Total</i>	29	1217	1246			
<b>HIV infection</b>						
Yes	3	4	7	0.000		
No	26	1213	1239			
<i>Total</i>	29 (2.3)	1217	1246			
<b>Stool consistency</b>						
Formed	0	62	62	0.140		
Pasty	18	792	810			
Liquid	9	226	235			
Mucous	2	137	139			
<i>Total</i>	29	1217	1246			
<b>Leukocytes in stools</b>						
Yes	18	146	164	0.265	1.54	[0.72-3.28]
No	11	1071	1082			
<i>Total</i>	29	1217	1246			

than 95% of the patients came from Ouagadougou and the others were also city-dwellers: these findings are comparable to those reported by Beatty

et al., (28) in Kenya. The close proximity to animals in developing countries contributes to easy and frequent acquisition of *Campylobacter* which can be

transmitted to humans directly by infected animals (1, 3, 4, 5). In Burkina Faso, the results showed that contact with live animals was a significant risk factor (OR: 0.423; 95%CI: 0.19-0.93;  $P=0.03$ ). The prevalence of HIV infection in Burkina Faso was 1.6% in 2007, with an estimated number of 130,000 [110,000-160,000] people of all ages living with HIV (29). The diarrheic diseases constitute an important part of the opportunist infections in AIDS-patients. Statistically significant associations between *Campylobacter* enteritis and AIDS have been reported (1, 30). The clinical manifestations and substantial mortality are more frequent in AIDS patients than HIV-negative patients (1, 24, 31). The results obtained in Burkina Faso confirmed these observations: HIV-positive patients were more infected by *Campylobacter* than HIV-negative patients ( $P<0.0001$ ). However, Awole et al., (32) have reported higher prevalence rates of *Campylobacter* species in HIV-negative patients (25%) than in HIV-positive (13.1%). Manfredi et al., (33) agree with such a finding in the era of highly active antiretroviral therapy (HAART): HIV-infected persons progressively feel better and clinically they look the same as other patients in the general population.

Numerous works reported that diarrheas due to *Campylobacter* are clinically less severe in the developing countries than in the developed countries. Watery, mucosal and/or even bloody stools associated with fever, abdominal pain, vomiting and presence of fecal leukocytes were described (1, 3, 5, 21, 22). In Burkina Faso, no bloody stools were found and mucosal stools were few (7%) in *Campylobacter* infected patients. The pasty stools (62.1%), abdominal pain (62.1%), diarrhea (51.7%), fever, asthenia (44.8%) and vomiting (31%) were the main clinical signs found in infected patients. The presence of leukocytes in 13.2% of stools could be associated with *Campylobacter*, but can also be associated with other invasive bacteria often found in co-infections. No seasonality was found with *Campylobacter* enteritis in Burkina Faso which is similar to reports from several developing countries (1, 2, 5); these findings were different from data in Ifakara (Tanzania), where Vargas et al., (34) found *Campylobacter* species in 2.5% of children less than five years of age, only in the dry season. However, the seasonality of campylobacteriosis is much debated (10).

Several studies have reported good correlations between disc diffusion and agar dilution methods for the drug susceptibility testing of *Campylobacter* in developed and in developing countries (4, 15, 35, 36). In developing countries, various patterns of *Campylobacter* susceptibility to antibiotics were described. The disc diffusion method was used in the study in Burkina Faso. The rate of resistance to amoxicillin (24.1%) was reduced to amoxicillin+clavulanic acid (3.4%), while it was with ceftriaxone. These antibiotics are commonly used against Gram-negative rods in developing countries. The amoxicillin and ampicillin activities

against bacteria are comparable usually. Recent data on the resistance of *Campylobacter* strains isolated from humans to amoxicillin are rare.

The rate of resistance to amoxicillin+clavulanic acid in the study in Burkina Faso was lower than that (44%) reported by Samie et al., (37) in South Africa. However, the resistance rate to ceftriaxon in Burkina Faso was higher than (8%) in South Africa while it was lower than those reported in other cities from developing countries as Ilorin in Nigeria (84%) or Teheran in Iran (47%) (23, 38). Gentamicin was the sole effective antibiotic against all the strains of *Campylobacter* in Burkina Faso as in Iran (38); resistance of 10.2% was reported from north India (39), 4 to 21% in Nigeria (23, 37) were found to this antibiotic. Erythromycin is widely used in both children and adults world wide. It has been reported that the resistance rates of *Campylobacter* to erythromycin is increasing and vary between 12 and 95% (28, 37, 40); however, the rate was lower in Burkina Faso (10.3%) and such lower rates were also found in north India, (6.1%) and in Sudan (1.7%) (15, 39). Besides, Serichantalergs et al., (16) reported a decrease in the *Campylobacter* resistance rates to erythromycin, from 1996 to 2000, in urban Bangkok (Thailand). None resistant strain was found in studies conducted in Kampala (Uganda) and in Ilorin (Nigeria) (23, 41). The rate of *Campylobacter* resistance to tetracycline was 10.3%: with this antibiotic also various resistance patterns are found in developing countries (15, 28, 37, 38, 39). All the studies in developing countries report resistant *Campylobacter* to nalidixic acid, at various rates, but always higher than those to ciprofloxacin. In a few countries, probably in limited populations, ciprofloxacin was reported as effective against all the strains of *Campylobacter* (23, 31). The rate may be lower than 10% in other developing countries, (41). However, the *Campylobacter* resistance to fluoroquinolones is high (11-95%) and continue to increase in developing countries, globally, particularly in Thailand (16, 28, 37).

The data reported in Burkina Faso (Table 2) is in keeping with this trend. The findings in the study in Burkina Faso showed that contact with infected animals and HIV infection are significant risk factors for acquiring *Campylobacter* enteritis. No seasonality was associated with the disease. The strain susceptibility testing to antibiotics showed various patterns: gentamicin was effective against all the strains, but amoxicillin+clavulanic acid, erythromycin and tetracycline showed satisfactory activities allowing for their use against *Campylobacter* enteritis in Burkina Faso. The implementation of a national program to fight against campylobacteriosis will allow for a better understanding of the epidemiology of these bacteria and to survey the evolution of their resistance to antibiotics. The use of molecular techniques will enhance the identification and estimation of the variety of the species of *Campylobacter* in the Burkina Faso.

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