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Burden and epidemiology of *Campylobacter* species in acute enteritis cases in Burkina Faso

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Abstract

Background *Campylobacter spp.* is a significant etiological agent of bacterial gastroenteritis globally. In Burkina Faso (BFA), the actual impact of this pathogen on gastroenteritis is considerably underestimated, primarily due to inadequate surveillance systems.

Objectives This study aimed to investigate the proportion of *Campylobacter* species responsible for acute gastroenteritis among patients of all ages in urban and rural areas of BFA, using molecular biology techniques.

Study design & methods Between 2018 and 2021, faecal specimens were obtained from 1,295 individuals presenting with acute gastroenteritis. These samples underwent screening for the *Campylobacter coli/jejuni/lari* complex utilizing real-time polymerase chain reaction (PCR) assays. Subsequently, positive samples were subjected to species-level differentiation through the application of species-specific primers.

Results *Campylobacter spp.* was detected in 25.0% (324/1,295) of the samples analysed. The majority of positive samples (95%, 308/324) were obtained from children under 5 years of age. Species identification was performed on a subset of 114 isolates, revealing 51 *Campylobacter jejuni*, 10 *Campylobacter coli*, and 53 *Campylobacter* isolates that remained unspeciated.

Conclusions This study reveals a significant prevalence of *Campylobacter* species among patients with acute gastroenteritis, with a particularly high incidence observed in children under 5 years of age. Based on these findings, the implementation of routine *Campylobacter* surveillance in public health laboratories is strongly recommended to better monitor and address this health concern.

Keywords Bacterial gastroenteritis, Zoonosis, *Campylobacter*, *Burkina Faso*, Sub-saharan Africa

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Background

Campylobacter is an emerging zoonotic bacterium that has been identified as the leading cause of bacterial gastrointestinal infections in high-income countries and a significant contributor to diarrheal diseases in children under five years old in low- and middle-income countries (LMICs) [1]. Annually, it is estimated to cause between 400 million and 500 million cases of diarrhea worldwide, affecting both developed countries and LMICs [2].

Campylobacter is a spiral-shaped and microaerophilic bacterium that thrives in low-oxygen environments [3, 4]. The *Campylobacter* genus comprises 18 species, but only a few are significant to human health. Notably, *Campylobacter coli* (*C. coli*) and *Campylobacter jejuni* (*C. jejuni*) are responsible for over 95% of human Campylobacteriosis cases [1]. *Campylobacter* spreads to humans primarily through zoonotic transmission, either by direct contact with animal faces or indirectly via contaminated food and water. While animals often carry the pathogen asymptomatically, it causes illness in humans upon ingestion, facilitated by its widespread presence in the environment and food chain [1].

Campylobacter infection is usually mild in adults but can be severe in young children, the elderly, pregnant women, and immunocompromised individuals (AIDS and Cancer), requiring antibiotic treatment [5, 6].

Stool culture is the primary diagnostic method for *Campylobacter* and antibacterial resistance in LMICs, but it takes 48–72 h, delaying timely antibiotic therapy. Remarkably, *Campylobacter* is a fastidious bacterium and can enter a Viable But Non-Culturable (VBNC) state under stress, evading detection by standard culture method [7]. Consequently, stool cultures often fail to detect this bacterium, likely underestimating its prevalence [8–10]. Due to culture's limitations as the gold standard for diagnosing these infections, alternative methods like PCR and Enzyme-Linked Immunosorbent Assays (ELISA) have emerged. Real-time PCR offers highly sensitive detection of *Campylobacter* species [7, 11].

Studies in West Africa have found *Campylobacter* prevalence rates between 2.3% and 20.3% among diarrheal outpatients using bacterial culture methods [8, 12, 13]. However, in Burkina Faso (BFA) and many other LMICs, the true incidence of *Campylobacter* infection in acute diarrhea cases remains undetermined. Unlike *Salmonella* and *Shigella*, which are actively monitored as part of surveillance programs for potentially epidemic diarrheal diseases, the national public health laboratories do not routinely test for or diagnose this zoonotic infection. Studies conducted between 2009 and 2019 on outpatients with acute diarrhea in Ouagadougou, BFA, reported prevalences ranging from 0.1 to 2% using culture, PCR, or both methods [8, 14, 15].

As part of the African Network for Improved Diagnosis, Epidemiology and Management of Common Infectious Agents (ANDEMIA) [16], we aimed to investigate the burden of *Campylobacter* species causing acute gastroenteritis in patients of all ages in urban and rural sentinel sites in BFA. We are also examining demographic factors that contributing to *Campylobacter* infection.

Methods

We conducted a cross-sectional study in Dano and Bobo-Dioulasso, BFA, between February 2018 to December 2021.

Study population

As part of ANDEMIA, this study examined patients of all ages presenting with acute gastroenteritis at urban and rural sentinel sites in BFA [16]. The primary symptom was acute diarrhea, characterized by the passage of three or more loose or watery stools within a 24-hour period. Patients were not eligible for inclusion if they had chronic diarrhea (persisting beyond 4 weeks) or had been hospitalized for more than 48 h. A total of 1,295 patients were enrolled at Souro Sanou University Hospital in Bobo Dioulasso in the "Hauts-Bassins" region and at Dano, Dissin and surrounding health centers in the "Sud-Ouest" region.

Biological analyses

Nucleic acids were extracted from stool samples or rectal swabs using IndiSpin Pathogen Kit (INDICAL BIOSCIENCE, Germany) according to the manufacturer´s instructions. The purified nucleic acid were then stored at -80 °C. Multiplex real-time PCR was performed to detect the *Campylobacter jejuni/coli/lari* complex using the FTLyo Bacterial gastroenteritis amplification kit (Siemens Health Care, Luxembourg) on an CFX 96 real-time PCR system (BIO-Rad Laboratories, USA) following the manufacturer's protocol. The screening PCR was unable to differentiate between the 3 species.

From the 324 *Campylobacter*-positive samples, a random selection of 114 specimens was chosen for typing. We carried out a multiplex PCR targeting the 16 S rDNA as a control for the *Campylobacter* genus. For the identification of *C. jejuni* and *C. coli*, we used specific *hipO* and *asp* primers, respectively, as detailed in Table 1 and previously described [17].The PCR was performed using the QIAGEN Multiplex PCR Kit (QIAGEN, Germany) in a 25 µL reaction volume containing 12.5 µL 2x Master Mix, 2.5 µL Q-Solution, 1.75 µL RNase-free water, primers (1 µL *hipO*, 2 µL *asp*, 0.25 µL 16 S rDNA; all 10 µM) and 5 µL template DNA. Cycling conditions were: 95 °C for 15 min; 35 cycles of 94 °C for 50 s, 57 °C for 90 s, 72 °C for 1 min; final extension at 72 °C for 3 min. PCR products were analyzed by 1.5% agarose gel electrophoresis.

Table 1 Oligonucleotide primers for *C. Coli* and *C. Jejuni* typing

Primer	Sequence	Expected length	Concentration	Reference
hipO-F	GACTTCGTGCAGATATGGATGCTT	344pb	0.2µM	[17]
hipO-R	GCTATAACTATCCGAAGAAGCCATCA			
asp-F (CC18F)	GGTATGATTTCTACAAAGCGAG	500pb	0.4µM	[17]
asp-R (CC519R)	ATAAAAGACTATCGTCGCGTG			
16 S-F	GGAGGCAGCAGTAGGGAATA	1062pb	0.5µM	[17]
16 S-R	TGACGGGCGGTGAGTACAAG			

Statistical analyses

Statistical analyses were conducted using Stata/MP 15.1 (StataCorp, Texas, USA). Proportions of *Campylobacter* positivity, defined as positive PCR result, were compared using the Pearson's Chi-square or Fisher's exact tests. Logistic regression models were used to investigate factors associated with *Campylobacter* positivity. Variables with p-values < 0.2 in the bivariable models were included in the full multivariable model. A top-down approach was then used to construct the final multivariable model. P-values < 0.05 were considered statistically significant.

Results

In this study, males comprised 55.3% (716/1,295) of the participants, while children under 5 years old accounted for 88.7% (1,148/1,295). The majority of participants (75.7%; 980/1,295) resided in rural areas. Out of 1,295 samples tested, 324 (25.0%) were positive for *Campylobacter spp.* Among these *Campylobacter* cases, 95% (308/324) were children under 5 years old, 59.2% (192/324) primarily drank well water and 88.2% (286/324) lived in rural areas (Table 2).

After conducting multivariable analysis, several factors remained statistically significant in their association with *Campylobacter* detection. These key factors included the patient's place of residence, age group, and whether they had a history of fever (Table 3). Patients from rural areas had twice (OR = 2; $CI_{95\%}$ = [1.25–3.19]; $p = 0.004$) the odds of having *Campylobacter* detected than those living in urban areas. In addition, children under 5 years old had nearly three times (OR = 2.96; $CI_{95\%}$ = [1.39–6.30]; $p = 0.005$) the odds of having *Campylobacter* detected compared to individuals aged 15 years old or older.

Of the 324 positive samples, 114 (35% = 114/324) was randomly selected for species identification. Among these, 106 were from children under 5 years old and 102 were from patients living in rural areas. Our analysis revealed 10 *C. coli* (8.8%), 51 *C. jejuni* (44.7%), and 53 non-typable samples (46.5%), with the latter requiring further investigation. All *C. coli* cases and 90.2% of *C. jejuni* cases were found in rural patients.

Discussion

This study identified the presence of *Campylobacter*, predominantly linked to human illness in both urban and rural areas of BFA. Throughout the study period, we observed a *Campylobacter spp.* infection prevalence of 25.0%. The age group most affected was children under five years old. This result reveals the importance of the circulation of this bacterium in BFA, especially among children under 5 years old. A systematic review and meta-analysis conducted in sub-Saharan Africa in 2021 reported a cumulative prevalence of *Campylobacter spp.* at 10.2% in patients suffering from diarrhoea with a high prevalence observed in children under 15 years. However, this finding was not statistically significant [18]. In our study, we reported a significant positive correlation between rural residence and *Campylobacter* positivity. This association may be attributed to several factors including the close proximity to livestock; the limited access to clean drinking water and food hygiene in these regions [19]. However, our data did not establish a link between positive *Campylobacter* results and the presence of domestic animals.

Previous studies carried out in BFA using culture as a detection technique, reported that the prevalences of *Campylobacter* infection ranged from 1 to 2% [8, 10]. In contrast, our study found a significantly higher prevalence of *Campylobacter* compared to these earlier findings in BFA. This could be attributed to the differences in the study populations and techniques used to identify *Campylobacter*. Sangaré et al. reported a prevalence of 2.3% among outpatients in urban areas from 2006 to 2008, using culture technique for the *Campylobacter* identification [8]. Also, a study carried out by Sawadogo et al., from February 5th to March 9th 2013, reported a prevalence of 1% from outpatients in Ouagadougou (urban area) using multiplex Real-Time PCR. However, it is important to note that there were differences in the detection kits utilized in their study compared to ours [14]. The application of molecular biology techniques for identifying *Campylobacter* allows for a more accurate assessment of infection prevalence due to its high sensitivity, as demonstrated in previous studies, when compared to traditional culture method [11, 20]. *C. jejuni* emerged as the most prevalent species identified, accounting for 51 out of 114 cases, followed by *C. coli*,

Table 2 Demographic and clinical characteristics of study participants according to *Campylobacter spp.* detection in stool samples

Symptomatic patients	Campylobacter's status		
	Positive *N* (%)	Negative *N* (%)	*p*-value*
Gender (*n* = 1,293)**			
Male	181 (25)	535 (75)	0.838
Female	143 (25)	434 (75)	
Age groups (*n* = 1,295)			
< 5 years	308 (27)	840 (73)	< 0.001*
5–15 years	6 (13)	41 (87)	
> 15 years	10 (10)	90 (90)	
Water type (*n* = 1,295)			
tap or mineral water	84 (24)	268 (76)	< 0.001*
Well water	192 (30.5)	437 (69.4)	
Other***	48 (15%)	266 (85)	
Residency (*n* = 1,295)			
Urban	38 (12)	277 (88)	< 0.001*
Rural	286 (29)	694 (71)	
Fever (*n* = 1,295)			
Yes	219 (22.5)	752 (77.4)	< 0.001*
No	105 (32.4)	219 (67.6)	
Nausea or vomiting (*n* = 1,290)**			
Yes	111 (20)	441 (80)	< 0.001*
No	212 (29)	526 (71)	
Diarrhea (*n* = 1,295)			
Yes, with blood (dysentery)	27 (26.4)	75 (73.5)	0.724
Yes, without blood	297 (25)	896 (75)	
Weight loss (*n* = 1,281)**			
Yes	156 (25)	462 (75)	0.691
No	161 (24)	502 (76)	
Comorbidities (*n* = 1,282)**			
Yes	0 (0)	15 (100)	0.024*
No	323 (25.5)	944 (74.5)	
Rapid malaria test (*n* = 1,180)**			
Negative	255 (25)	758 (75)	0.074
Positive	53 (32)	114 (68)	
Current hospitalisation (*n* = 1,291)**			
Yes	134 (20)	541 (80)	< 0.001*
No	189 (31)	427 (69)	
Contact with animal (*n* = 1,295)			
Yes	133 (26)	372 (74)	0.382
No	191 (24)	599 (76)	

*p-value < 0.05 (Pearson χ2 test); **less than 1,295 mean that for some patients there were no information; ***all types of water, including river water and rainwater; Percentage (%) are calculated per row.

which was identified in 10 out of 114 cases. These findings are consistent with previous studies conducted in humans [6, 8, 21, 22]. Nevertheless, 53 out of 114 *Campylobacter* species (46%) remained unidentified. This finding is consistent with the results of Sangaré et al., who, after identifying the species *C. jejuni*, *C. coli*, and *C. upsaliensis*, reported that 30.9% of their samples were also unidentified [8].

Considering the zoonotic nature of *Campylobacter* infections and the significantly higher prevalence observed in our study compared to existing literature from BFA, it is crucial to implement monitoring measures for this bacterium and its antibiotic resistance. In many regions globally where monitoring of *Campylobacter* resistance to antibiotics has been implemented, significant resistance levels to erythromycin and fluoroquinolones, common antibiotics for treating Campylobacteriosis have been observed [20, 22]. However, in BFA, despite the widespread consumption of poultry, laboratories do not routinely test for *Campylobacter* in cases of diarrhea, which could hinder effective monitoring and treatment strategies.

Table 3 Sociodemographic and clinical factors associated with *Campylobacter* infections

Characteristics	Bivariable			Multivariable		
	OR	95% CI	P-value*	aOR	95% CI	p-value*
Residence						
Urbain	Reference			Reference		
Rural	3	2.08–4.33	< 0.001*	2.00	1.25–3.19	0.004*
Gender						
Male	Reference					
Female	0.97	0.75–1.25	0.838			
Age (years)						
≥ 15	Reference			Reference		
5–15	1.31	0.44–3.86	0.616	1.11	0.33–3.72	0.859
< 5	3.3	1.69–6.42	< 0.001*	2.96	1.39–6.30	0.005*
Water type						
tap or mineral water	Reference			Reference		
Well water	1.4	1.04–1.88	0.02	1.25	0.88–1.77	0.209
Other	0.57	0.38–0.85	0.006	0.90	0.56–1.45	0.684
Ongoing fever						
No	Reference					
Yes	0.93	0.66–1.31	0.696			
History of fever						
No	Reference			Reference		
Yes	0.61	0.46–0.80	< 0.001*	0.68	0.49– 0.96	0.030*
Abdominal pain						
No	Reference					
Yes	0.94	0.70–1.25	0.681			
Nausea/vomiting						
No	Reference			Reference		
Yes	0.62	0.48–0.81	< 0.001*	0.81	0.60–1.09	0.181
Diarrhea						
Without blood	Reference					
With blood	1.08	0.68–1.71	0.724			
Weight loss						
No	Reference					
Yes	1.05	0.81–1.35	0.691			
Rapid malaria test						
Negative	Reference			Reference		
Positive	1.38	0.96–1.97	0.074	1.15	0.79–1.68	0.456
Current hospitalization						
No	Reference			Reference		
Yes	0.55	0.43–0.72	< 0.001*	0.97	0.69–1.38	0.903
Contact with animal						
No	Reference			Reference		
Yes	1.12	0.86–1.44	0.382			

*p-value < 0.05 (Wald's test); OR: Odds Ratio; aOR: adjusted Odds Ratio; CI: Confidence Interval

The limitation of relying exclusively on molecular detection methods is that they do not offer a comprehensive profile of antibiotic resistance. However, the rapid identification of the bacterium can significantly assist clinicians by enabling them to initiate targeted treatment with a specific antibiotic. This approach not only helps prevent the spread of the bacterium but also reduces the unnecessary use of antibiotics.

Conclusion

Acute diarrhoea is a significant public health concern, with *Campylobacter* being one of the most prevalent bacterial causes of this condition globally. Our research highlights the epidemiological burden of *Campylobacter* in Burkina Faso, a region with limited existing data. The findings indicate that *Campylobacter* infections are particularly prevalent among children and in rural areas. Further investigation is required to explore the specific

rural conditions that promote infection, particularly regarding interactions with animal reservoirs. Although molecular techniques are effective for diagnosing *Campylobacter* infections, comprehensive studies, including high-throughput sequencing, are essential for characterizing non-typable strains and evaluating their antimicrobial resistance profiles.These findings point out the critical importance of incorporating both phenotypic and molecular detection methods for *Campylobacter* into the national infectious disease surveillance system. Additionally, given the widespread use of antibiotics in various animal production systems, it is essential to remain vigilant about the potential for *Campylobacter* to develop resistance to these antibiotics.

Abbreviations

16S-F	16 S-forward
16S-R	16 S-Reverse
ANDEMIA	African Network for the Improvement of Diagnosis, Epidemiology and Management of Common Infectious Agents
asp-F	*asp*-forward
asp-R	*asp*-Reverse
BFA	Burkina Faso
bp	Base Pair
C. coli	*Campylobacter coli*
C. jejuni	*Campylobacter jejuni*
C. upsaliensis	*Campylobacter upsaliensis*
CHUSS	University Hospital of Souro Sanou
CI	Confidence Interval
ELISA	Enzyme-Linked Immunosorbent Assays
FTD	Fast Track Diagnostics
GI	Gastrointestinal Infection
hipO-F	*hipO*-forward
hipO-R	*hipO*-Reverse
LMIC	Low and Middle Income Countries
OR	Odd Ratio
p	p-value
PCR	Polymerase Chain Reaction
VBNC	Viable But Non-Culturable

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Author contributions
Conceptualization: SO, GS, TE, FHL, ASO, AP; Methodology: AORB, AZ, SO, GS, TE, FHL, ASO, EB; Formal analysis: AORB, AZ, GK, EB; Investigation: AORB, AZ, NFK, EB; Resources: AP, SAS, AO, MM, ASO; Data curation: AORB, NFK, EB; Writing – Original Draft: AORB, NFK, AZ, ASO; Writing – Review & Editing: AORB, NFK, AZ, GS, TE, AO, GK, MM, EB, ASO; Visualisation: AORB, NFK, AZ, GS, TE, EB, ASO; Supervision: AZ, EB, ASO; Funding acquisition: GS, TE, FHL, ASO.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study adheres to the tenets of the Declaration of Helsinki, as well as national legislation and ethical standards. This study was Approved by the Burkina Faso' National Health Research Ethics Committee (Approval Decision No.2017-5-057). All Participants, parents or guardians of children enrolled, provided informed consent.

Competing interests
The authors declare no competing interests.

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