World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(REVIEW ARTICLE)

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Characterization of *Campylobacter spp*. isolated from chicken and at-risk people (children) in Cameroon: A review

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World Journal of Advanced Research and Reviews, 2022, 15(01), 651–661

Publication history: Received on 16 June 2022; revised on 23 July 2022; accepted on 26 July 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.15.1.0728

Abstract

Campylobacteriosis is a disease caused by the bacteria called *Campylobacter spp*, it is considered a public health problem. *Campylobacter spp* has so far been identified as the pathogen most responsible for bacterial gastroenteritis. Emphasis was placed on controlling the foodborne route of exposure. The assessment of the actual burden of *Campylobacter* in the African context, particularly in Cameroon, is hampered by the lack of reporting of diarrheal incidents and the ineffectiveness of monitoring and surveillance programs for foodborne illnesses, as well as the lack of attention given to *Campylobacter* as a causative agent of diarrhea for the sole benefit of *Salmonella*. This article aims to report on the characterization of *Campylobacter spp* in infected chickens and children in Central Africa, more specifically in Cameroon. *Campylobacter* infection is more prevalent in the pediatric population and has been isolated from farm animals, particularly chickens and foods of animal origin. The prevalence of *Campylobacter* in children with diarrhea under five years of age ranges from 15% to 23% in Angola. In chickens, the prevalence varies from 90% in Cameroon to 41.2% in Congo. This review also highlights the increased resistance of *Campylobacter* to common important antimicrobials, such as ciprofloxacin, tetracycline and erythromycin, in food animals and humans in Central Africa. The solution to limit the incidence in humans is to control and prevent the spread of pathogens in animals constituting the main reservoir of infections.

Keywords: *Campylobacter spp*; Zoonoses; Antimicrobial resistance; Chicken; Children

1. Introduction

Campylobacter is one of the leading causes of foodborne bacterial gastroenteritis worldwide and is a public health problem [1]. It is responsible for around 500 million infections per year worldwide [2, 3]. *Campylobacter* are isolated from several pets, wildlife and the environment. *Campylobacter* outbreaks are caused by several sources, such as raw milk, water. However, the consumption of contaminated chicken meat is the primary cause of human Campylobacteriosis [4]. Reported cases of Campylobacteriosis is high in developed countries [5, 6,] while the disease is less reported in developing countries due to the lack of regular *surveillance* programs [7]. *Campylobacter* is increasingly becoming a major public health problem in sub-Saharan Africa, where the number of infections is likely to double by 2020 [7]. Deficiencies in food safety regulations and epidemiological data, which do not exist in Cameroon, limit the assessment and control of *Campylobacter* infections.

In young children, *Campylobacter* infection has been associated with diarrhea and malnutrition [8, 9, 10]. Environmental enteric dysfunction is a subclinical bowel disorder that is prevalent in low-resource settings and characterized by bowel

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inflammation and altered bowel structure and function [11, 12]. This is especially dangerous for young children who are more prone to dehydration and loss of nutrients, such as sodium and protein, due to diarrheal disease [13]. *Campylobacter* is one of the most isolated bacterial pathogens in the stools of infants with diarrhea in several developing countries [14, 7].

According to the World Health Organization [15], children under five account for 40% of the global burden of foodborne illness, with a predominantly African population. *C. jejuni* and *C. coli* are the main species responsible for Campylobacteriosis in humans [16]. Since antimicrobial therapy is generally not indicated in most cases of Campylobacteriosis, erythromycin and ciprofloxacin are considered the first and second choice of antimicrobials, respectively, for the treatment of severe human *Campylobacter* infections [15]. The spread of antibiotic resistant bacteria / genes to humans throughout the food chain could be facilitated by the massive and uncontrolled use of antibiotics for prophylaxis and treatment in primary animal production [17]. The state of knowledge of antimicrobial resistance in *Campylobacter* is not fully understood across Cameroon [18, 19,20]. This review brings together knowledge on the characterization of *Campylobacter* in chickens and children in Central Africa (Cameroon… etc.), Antimicrobial resistance patterns and proposals for management in Cameroon.

2. Campylobacter infection

Campylobacter infection is commonly caused by eating contaminated chicken, beef or pork (Figure 1). Consumption of poultry causes 30% of all cases of infections, of which 50-80% of *Campylobacter spp* strains are from chickens, 20-30% of cases from livestock pathogens and a low percentage of pathogenic strains from other sources, including game [21,22]. *Campylobacter* does not proliferate outside the digestive tract of warm-blooded animals but can survive for up to several weeks in food products, especially those stored at low temperatures [23].

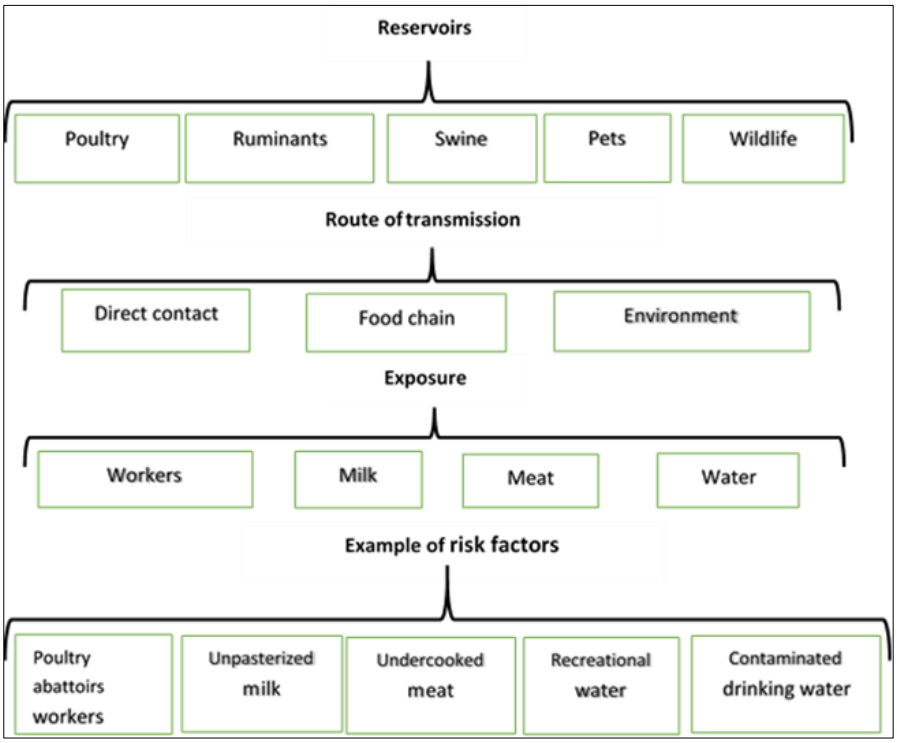

Figure 1 Reservoirs, transmission routs, and examples of source of infections caused by *Campylobacter* genus [23, 25]

In developing countries, Campylobacteriosis is endemic, the main sources of infections are environmental and food contamination [7]. In developed countries like the United States, food animals are considered the main source of *Campylobacter* infections in humans. There are many possibilities for transmission of *Campylobacter* infection through cross contamination through livestock farms, commercial food production. The main cause of foodborne illness (Campylobacteriosis) in humans is due to the consumption of chicken, beef and pork products. It is estimated that poultry (broilers, laying hens, turkeys, ducks and ostriches) are responsible for 50 to 70% of human infection with *Campylobacter* [24, 26,27].

Campylobacter spp colonizes the mucosa of the cecum and cloaca crypts of infected chickens, but can also be present in the spleen, blood and liver [28]. Each gram of chicken faeces can reach the bacterial level of 1010, causing no infection and leading to no changes in the cecal mucosa [29, 28]. Before the third week of life, *Campylobacter* is not detected in newborn chickens, [28, 30]. Beyond three weeks, if a single bird contracts the infection, it will be transmitted to the rest in a few days (about 3 days) by faeces containing pathogens, or by rodents, water, insects, or agricultural workers [28, 25]. The chicken meat available in retail markets containing *Campylobacter* is variable, in the EU it ranges from 60% to 80%, while in the US it can reach 98% [31].

2.1. Chicken

Campylobacter infection is widely regarded as a foodborne illness, with chickens considered the main vector of transmission. The consumption or handling of raw or undercooked chicken has been identified by studies as a major risk factor for Campylobacteriosis in humans [32, 33, 34,35]. The percentage of human Campylobacteriosis cases attributed to eating or handling raw chicken varies across countries and studies. Foodborne cases vary from 30% [36] to 58% -76% [35] and up to 80% can be attributed to the chicken tank as a whole [32]. Stafford et al in 2008, showed in their work that 75% of Campylobacteriosis were of food origin [37]. In 2009, Gillespie published are buttal of Stafford et al claiming to have overestimated the role of chicken consumption in Campylobacteriosis by a factor of 3.4 [38]. Often, the source of reported cases cannot be determined, which means that the actual number of foodborne cases is unknown [39].

2.2. *Campylobacter* infections in children

The isolation of *Campylobacter* in developing countries ranges from 5% to 20% [7, 50]. Data collected by the World Health Organization (WHO), combined with that of the Canadian Public Health Service, provided financial support to developing countries for epidemiological studies [7,51]. There is great heterogeneity in the incidence of Campylobacteriosis in developing countries compared to that in developed countries. A large number of children in developing countries are affected by *Campylobacter* infections. In developing countries in community areas, it has been estimated that 60,000 per 100,000 children under 5 suffer from Campylobacteriosis [7, 51, 52]. These data suggest that Campylobacteriosis is a pediatric disease in developing countries. In developed countries, over 90% of cases of human Campylobacteriosis occur during the summer due to undercooked meats from outdoor cooking facilities. People of all ages are affected, but especially children under 4 and young adults aged 15 to 44 [53, 54]. the unavailability of national Campylobacteriosis surveillance programs in developing countries does not allow for case incidence values in terms of population density. [7]. Most of the incidence estimates are made by laboratories where surveillance is based on the pathogens causing diarrhea. This is the case of Cameroon, which has a glaring lack of data on Campylobacteriosis in children and elderly people.

3. Prevalence of *Campylobacter*

In the Central African region, there are very few data on *Campylobacter* infection. An analysis of 194 stool samples from children with acute diarrhea and children without diarrhea under the age of five in the Angolan capital of Luanda found that *Campylobacter* was present in 15% of the samples overall, including 23 % in the stool of children with diarrhea compared to 6% in the stool of non-diarrheal children [55]. A multiplex real-time polymerase chain reaction (mPCR) was used to analyze the samples. Other pathogens, including *Escherichia coli, Salmonella, Cryptosporidium and Shigella,* were detected in all samples regardless of the state of the diarrhea.

Nzouankeu et al in Cameroun [42] showed that 90% of retail chickens obtained in eight markets in the capital Yaoundé contained *Campylobacter spp*. E. coli and Salmonella were also isolated from samples using culture-based methods. *Campylobacter* is commensal in chickens, its primary host, and the risk of cross-contamination of the carcass during slaughter and processing is high if not done with care and hygiene. Nzouankeu et al further suggested the need to monitor chickens for pathogens and to minimize cross contamination.

We are going to focus on some Central African countries. This in order to bring out a common reality in the country of Central Africa. Cameroon lacks a lot of well-detailed data on Campylobacteriosis. Thus, an overview of Central Africa will show an overview of reality in Cameroon.

Table 1 present the percentages of *Campylobacter* isolated in chicken in a few central african countries. We can see that the percentage of *Campylobacter spp* isolated in chicken in cameroon remains high. However, a statistical analysis predict that *Campylobacter* is increasingly becomming a major problem in sub-sahara africa where the number of infections is predicted to double by the year 2020. [7]

Table 1 Prevalence rates of *Campylobacter* in Children and chicken in some countries of central Africa

Country	Population/ Product	Sample size	Percentage %	Genus/species	Detection procedure	References
Cameroon/ yaounde	chicken	150	90	*Campylobacter*	Cultural	[42]
Congo/ DR/Lumbubashi	Goat meat	177	41,2	*Campylobacter*	PCR	[56]
	Goat stomach	86	37,2			
	Ready to eat goat skewer	139	23,7			
Angola Louanda	Diarrhoeal children under 5 year	194	15	*Campylobacter*	Multiplex PCR	[55]
		98	23			
	Non diarrhoeal children under 5year	96	6			

4. Methods

The main methods of identification are: traditional culture using selective agar, PCR or real-time PCR, and membrane filtration on blood agar [57, 58, 59]. The most widely used selective agar is cefaperazone deoxycholate charcoal agar containing 32 mg / L of cefoperazone. The plates are incubated at 37 ° C for two days in anaerobic jars. Selective culture is a rapid, inexpensive and efficient method for identifying *C. jejuni* and *C. coli* from faecal samples [58]. However, plaques are often invaded by faster growing microorganisms and this method does not identify less common species.

Real-time PCR allows identification of *Campylobacter spp* to the species level and results can be obtained within a day. However, it does not provide an isolate for further research, it is expensive and also very laborious [58]. Detection and enumeration of viable but nonculturable cells is performed using real-time PCR. The problem with this method is that the total counts can be overestimated due to the amplification of non-viable or killed cells. DNA from environmental samples can be very stable and can persist for long periods [60].

Filtration of samples through a cellulose triacetate membrane with 0.45 mm pores on blood agar separates *Campylobacter spp* from other larger bacteria which could invade the agar. This method can detect all *Campylobacter spp*. because there is no antibiotic used in the medium. Plates can also be incubated longer without being invaded, allowing the isolation of slower growing species [58]. The sensitivity of the membrane filtration technique is lower than that of the selective culture and these two techniques are less effective when applied to the isolation of *Campylobacter spp.* from water samples. [61]

4.1. Identification of the different species

The PCR method with specific primers, as described previously, can be used to identify colonies such as *C. jejuni* or *C. coli* [62, 63]. Bacterial DNA lysates are prepared from fresh *Campylobacter* cultures using the boiling method [64]. carried out in a volume of 25 mL containing 2.5 mL of 10 × PCR buffer (Thermo Fisher Scientific, Waltham, MA, US), 2.5 mL of 25 mM MgCl2 (Thermo Fisher Scientific, Waltham, MA, US), 1 , 0 mL of each PCR primer (10 mM - Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Warsaw, Poland), 1.0 mL of 10 mM dNTP mix (Thermo Fisher Scientific, Waltham, MA, States United States), 0.5 mL of Dream Taq DNA Polymerase (1U / mL – Thermo Fisher Scientific, Waltham, MA, US), 1.0 mL of matrix and 13.0 mL of purified water without DNA (Thermo Fisher Scientific, Waltham, MA, US). PCR is performed using the cycling conditions specified by the original authors [62, 63]. The amplified DNAs are analyzed by electrophoresis in a 1.5% agarose gel. The reference strains of *C. jejuni* (NCTC11322) and *C. coli* (NCTC11366) are used as a control strain.

Given the financial difficulty, in Cameroon the identification of *Campylobacter* is done using an API Campy tape. The API Campy band (Biomerieux), [42] consists of 20 microtubes containing dehydrated substrates. It consists of two parts. The first part of the strip (enzymatic and conventional tests) is seeded with a dense suspension which rehydrates the

substrates. During incubation (under aerobic conditions) the metabolism produces color changes which are either spontaneous or revealed by the addition of reagents. The second part of the strip (assimilation or inhibition tests) is inoculated with minimal medium and incubated under microaerophilic conditions. Bacteria thrive if they are able to use the corresponding substrate or if they are resistant to the antibiotic tested. The reactions are read according to the reading table. Identification is obtained by consulting the list of profiles in the notice or the identification table if necessary. Identification software can also be used.

4.2. *Campylobacter* virulence genes

The presence of the *rac*R, *sod*B, *csr*A, *vir*Bll, *cdt*B, *iam* and *wla*N genes is determined using the PCR method with the primers, as previously described by Linton et al., 2000 for the *wla*N gene; Bang, Scheutz and Ahrens, 2001 for the cdtB gene; Carvalho et al., 2001 for the *iam* gene; Datta, Niwa and Itoh, 2003 for the *rac*R gene, *vir*B11; Fields and Thompson, 2008 for the *csr*A gene and Biswas et al., 2011 for the *sod*B gene [65, 66, 67, 62, 68]. All PCRs were performed in reactions of 25 µL volume containing 2.5 µL of 10 × PCR buffer (Thermo Fisher Scientific, Waltham, Ma, US), 2.5 µL of MgCl2 (25 mM, Thermo Fisher Scientific, Waltham, MA, US) 1.0 µL of each PCR primer (10 µM, Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Warsaw, Poland), 0.5 µL of deoxynucleoside triphosphate mixture (10 mM, Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL of Dream Taq DNA Polymerase (0.5 U / µL, Thermo Fisher Scientific, Waltham, Ma, US), 2.0 µL of Matrix and 15,0 µL of purified water without DNA (Thermo Fisher Scientific, Waltham, MA, US). Visualization of DNA paths was achieved by adding Midori DNA Green Stain (Nippon Genetics, Duren, Germany) to 1% agar gel prior to electrophoresis. The size of the amplicon was compared using a 100 bp DNA size marker (Thermo Fisher Scientific, Waltham, MA, US).

5. Resistance to commonly used antibiotics

Virulence factors contribute to the pathogenicity of the microorganism in case of infection, as well as in persistent infection due to strains resistant to antibiotics [69]. Usually antimicrobials prescribed for the treatment of campylobacteriosis include antibiotics such as tetracycline, macrolides (erythromycin), and fluoroquinolones (ciprofloxacin) with aminoglycosides (gentamicin) prescribed for systemic infections [69]. For most of its antibiotics there are analogues used in veterinary practices especially for prophylaxis and treatment in food animals; for example, tylosin and kitasamysin (macrolides), enrofloxacin (quinolones) and doxycycline (tetracyclines) [70]. Resistance of *Campylobacter* to antibiotics has been reported for fluoroquinolones, tetracyclines, ß-lactams, aminoglycosides and macrolides. [71]. In establishing the infection, virulence factors play an important role. The virulence factors involved in *Campylobacter spp* are those responsible for adhesion, invasion, toxin production and thermo-tolerance [72]. Resistance of *Campylobacter* to erythromycin and fluoroquinolones has been identified at significant levels in many regions of the world according to the World Health Organization [73]. This appears to be associated with the use of these drugs in poultry and animal production. A relationship between antibiotic use and antimicrobial resistance has been established by some epidemiological studies in humans and animals [74, 75, 76]. The most commonly used antimicrobials for the treatment of *Campylobacter* infections in humans will receive our attention in this review. More specifically, we will focus on the resistance profiles of *Campylobacter spp* to fluoroquinolones, tetracycline and macrolides in a few studies.

Table 2 present the resistances encountered to quinolones/fluoroquinolones, macrolides, cyclins and phenolic of some work carried out. We can see that resistance in all families of antibiotics is higher in chicken than in humans. Nevertheless, the resistance observed in humains in these different families of antibiocs is increasing. This observation is also explained by the fact most of these antibiotic classes have analogues used in veterinary practices for growth promotion, prophylaxis, mataphylaxis and treatment in food animals. [70]**.**

Table 2 Antibiotics Resistance Trends Isolated of Humans and Chicken in few studies

Sources	Species	Antibiotic	Resistance %	Methods used	references
Humans diarrhoeal children	*Campylobacter*	Tetracycline	39.5	Kirby Bauer Disk Diffusion	[18]
		Chloramphenicol	31.6		
Backyard chicken	*C. jejuni*	Tetracycline	71	PCR	[77]
		Ciprofloxacin	71		
		Nalidixic acid	77.4		
		Chloramphenicol	25.8		
Diarrhoeal children	*Campylobacter*	Tetracyclin	22	Kirby Bauer Disk Diffusion	[78]
		Ciprofloxacin	11		
		Chloramphenicol	11		
		Nalidixic acid	11		
Chicken carcass and faeces	*Campylobacter*	Quinolones	41-86	Kirby Bauer Disk Diffusion	[79]
		Erythromycin	100		
		Tetracyclin	97-100		
Chicken	*C. jejuni*	Ciprofloxacin	38.5	Kirby Bauer Disk Diffusion	[80]
		Erythromycin	10.3		
		Nalidixic acid	79.5		
	C. coli	Ciprofloxacin	43.2		
		Erythromycin	8.1		
		Nalidixic acid	78.4		
Humans	*C. jejuni*	Erythromycin	31.5	Broth Microdilution	[81]
		Azithromycin	50		
	C. coli	Erythromycin	38.9		
		Azithromycin	77		
Retail meat product of chicken	*C. jejuni*	Clindamycin	75	PCR	[82]
		Ciprofloxacin	33		
		Erythromycin	79		
		Tetracyclin	16		
		Nalidixic acid	48		

6. *Campylobacter* risk management approach

The fight against *Campylobacter* is essential in the careful control of the production and management systems of animals. In Europe, it has been shown by some studies that a decrease in the load of *Campylobacter* in poultry poop would lead to a reduction of more than 90% of human infections attributed to the consumption of poultry meat [83].

In Cameroon, it would be important to minimize human-animal contact, practice personal and environmental hygiene and seek appropriate medical care for sick people in a home in order to minimize the risk of transmission. In addition to primary interventions at the farm level, it is necessary to apply interventions at the slaughter and processing levels

in order to reduce the contamination of poultry meat intended for human consumption. In Cameroon it would be important to implement chemical decontamination which is an effective intervention to reduce the load of *Campylobacter* on carcasses of animals intended for food, since this is not applied. On the other hand, there is a glaring lack of slaughterhouses in Cameroon there are more killings. Acidified sodium chlorite, chlorine dioxide, chlorine, trisodium phosphate and peroxyacid are generally used in the processing of poultry in several foreign countries, for example in the United States and Australia this is used in the form of sprays or washes for in-line reprocessing, or added to the cold-water reservoir [84,15]. The lack of continuous data surveillance in Cameroon hinders adequate assessment of the impact on public health and the burden of disease [84, 83].

Cameroon does not have a national *Campylobacter* surveillance program. But the permanent adoption of multisectoral collaborations with the different departments of the Ministry of Wildlife and Livestock would help strengthen the disease surveillance system, also strengthen laboratory capacities and support the implementation of prevention strategies and control. This would further improve public health and veterinary laboratories and create cross-sectoral links to control zoonoses [85].

7. Conclusion

This review aimed to present knowledge on the characterization of colonization by *Campylobacter spp* in chickens and children in Central Africa, in particular in Cameroon. A One Health intervention approach is needed to better understand, prevent and control *Campylobacter* in Cameroon. The management of foodborne transmission of *Campylobacter* can be addressed at the national, farm, processing and policy level. In chickens, the data collected indicates that the prevalence is 90% in Cameroon. On the other hand, 60,000 per 100,000 children under 5 suffer from Campylobacteriosis in developing countries. The collected data also highlight the alarming trend in several Central African countries of increasing resistance of *Campylobacter* to clinically important antimicrobials, such as ciprofloxacin, tetracycline and erythromycin, in humans and animals. intended for food.

The most appropriate approach to reduce the incidence of zoonoses in humans in Cameroon is to control and prevent the spread of pathogens in animals, which are the main reservoir for infections. However, these methods often prove ineffective in developing countries. For these reasons, it is essential that education in areas such as microbiology, sanitation, hygiene, food science, good agricultural and manufacturing practices, as well as the implementation of an assessment of risks by risk analysis and critical control points, is considered necessary.

Compliance with ethical standards

Acknowledgments

I would like to say thank you to the co-authors of this work for giving their time for writing and improving this review.

Disclosure of conflict of interest

The authors declare that they have no conflicts of interests.

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