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Bacteriological Profile of Campylobacter SPP Isolated from Pigs in Cameroon: A Review

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Authors' contributions

This work was carried out in collaboration among all authors. Author SDOA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EN and DN managed the analyses of the study. Author EN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Campylobacter is the most frequent bacteria implicated in acute gastroenteritis in the industrialized world and is considered as a major public health problem. The aim of this review is to improve our knowledge on the bacteriological profile of *Campylobacter* isolated from pigs. Porks, beef also represent sources of infection with these microorganisms. *Campylobacter* is a bacterium comprising seventeen species, including *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter fetus*. *C. coli* is the most common *Campylobacter* species recovered from pigs. The prevalence (46%, 52%, 75%) varies from one country to another, from the collection site. *C. jejuni* and *C. coli* grows best in a low oxygen or microaerophilic environment. The virulence markers varies among different sources of the isolates. The majority of genes were found at high levels in *Campylobacter* spp. isolated from pork meat (*csr*A, *sod*B, *cdt*B, and *rac*R). Moreover, this review revealed virulent properties of *Campylobacter* isolated from swine products and high resistance rates to Tetracycline (68%), Erythromycin (61%), which may represent difficulties in campylobacteriosis treatment.

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Fluoroquinolones resistance (2.2%, 7%, 11%,100%) varies according to the country and the source of the sample. It will be wise to insist on hygenic measures as a solution to limit the incidence of *Campylobacter* and prevent dissemination of pathogens in animals (chicken, pork and beef) within slaughter-houses/ killings Cameroon.

Keywords: Campylobacteriosis; pork; gastroenteritis; prevalence; resistance.

1. INTRODUCTION

Among food borne diseases, zoonoses takes a special place [1]. Studies indicates that a third of human infectious diseases are of zoonotic origin [2]. Indeed, these diseases are induced by pathogens of which the animal is the main carrier [3]. Zoonotic diseases are defined as diseases transmitted between animals and humans as a consequence of a direct contact, indirect environmental contact, or through food [4]. Among recognised pathogens causing human diseases, almost 60% are of animal origin [5]. *Campylobacter* spp is a typical zoonotic microo rganism/ zoonotic bacteria. *Campylobacter* spp are the most common cause of acute bacterial enteritis in humans [6,7]. They are typically considered foodborne pathogens and have been identified as the leading cause of food poisoning in Europe [8], the United States [9], Canada [10] and Australia [11].

Campylobacter is a gram-negative, non-spore forming, curved or spiral bacilli, which are oxygen sensitive and prefer to grow under micro-aerobic conditions [12,13]. Some *Campylobacter* species are thermotolerant; for instance, *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*), which are of critical importance to food safety, grow optimally at 42°C [14]. Meat obtained from poultry is the most common source of *Campylobacter* bacteria, but pork, beef, and unpasteurized milk also represent sources of infection with these microorganisms [15,16]. Pork and beef have long been preferred in many countries, and their level of consumption depends on the availability of the product in the market. The presence of *Campylobacter* in cattle and pig carcasses at slaughterhouses is well documented and significant [17,18]. However, the occurrence of *Campylobacter* in beef or pork is lower than that in poultry meat [19]. A lower rate of *Campylobacter* isolation from beef or pork meat can be associated with longer slaughter time, cooling of carcasses, and drying of the meat surface [20].

Resistance to antimicrobial substances among zoonotic bacteria is the current subject of research concerning the entire food chain, given the importance of this phenomenon in public health. Infections caused by drug-resistant strains requires a long term treatment, have a higher morbidity and mortality rates, and are associated with higher cost of treatment [21].

Several genes (i.e., *fla*A and *flh*A), are essential for the mobility/passage of *Campylobacter* through the stomach and gut environment [22]. In addition, several proteins (encoded by the *cad*F, *doc*A, *rac*R, *vir*B11, *cia*B, and iam genes) on the surface of Campylobacters have been shown to promote the adherence and invasion of epithelial cells of the intestine [23,24]. *Campylobacter* has also been found to excrete several cytotoxins (encoded by the *cdt*A, *cdt*B, *cdt*C, and *wla*N genes) that contribute to the development of human illness [25,26].

The aim of this study is to improve our knowledge of the bacteriological profile of *Campylobacter* isolated from pigs. This will help in understanding whether it is possible to eliminate *Campylobacter* from the pigs popu lation, to produce *Campylobacter*-free pigs (meat) and to prevent the spread of *Campylobacter* from pigs to the environment, particularly in Cameroon.

2. CAMPYLOBACTER SPP

Campylobacter are small, curved-to-spiral shaped, flagellated Gram-negative rods, ranging from 0.5 to 8 mm in length and from 0.2 to 0.5 mm wide [27]. *Campylobacter* is a bacterium comprising seventeen species, including *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter fetus*. Campylobacteriosis is an intestinal infection almost always caused by *Campylobacter jejuni* or *Campylobacter coli*, in the form of diarrhea. *Campylobacter fetus* can cause neonatal infection [27].

2.1 Survival in the Environment

Survival of *C. jejuni* and *C. coli* outside the gut is poor, and replication does not occur readily [28].

C. jejuni and *C. coli* grows best at 37°C to 42°C [29], the approximate body temperature of the pork (41°C to 42°C). such as an atmosphere of 5%O_2 10%CO_2, and 85%N. The organism is sensitive to freezing, drying, acidic conditions (pH 5.0), and salinity.

*Campylobacte*r survives in water thanks to the multilayer bag. The protozoan tetrahymena, is a unicellular organism living in fresh water. This feeds on bacteria. However, some bacteria, such as *Campylobacter*, which survive digestion, can become trapped there. Once evacuated, the multi-layered bag will offer them good protection against the vagaries of a decidedly hostile environment. Researchers have shown that the drinking water of farms and pigsties contained the bacteria, but also protozoa. Interestingly, some bacteria are able to survive in protozoa and therefore use them as a host [30]. This is the case with *Campylobacter.*

2.2 Pigs

Unlike poultry and cattle, *C. coli* is the more common *Campylobacter* species recovered from pigs [13]. In some studies, for instance, C. coli has been recovered from pigs fecal samples at greater than 99% [30,31]. Jensen et al. [32] studied the establishment of C. coli and C. jejuni in outdoor organically-reared pigs to monitor potential shifts from C. coli to C. jejuni in intestinal colonization. Their results demonstrated excessive fluctuations in numbers of swine colonized by C. jejuni, with recoveries ranging from 18.8 and 78.6% among the three trials, but C. jejuni was never more prevalent than C. coli [32]. Despite being recognized as the minor Campylobacter species in swine, high prevalence of C. jejuni has been observed in fecal or rectal contents of guilts, sows, and weaned piglets (76, 89, and 82%, respectively) [33]. In their report, C. coli (68%) was only more prevalent than C. jeuni (31.7%) in neonates when isolated within 24 h of birth [33].

3. GENUS OF CAMPYLOBACTER

The sequence of the genome of *Campylobacter jejuni* NCTC11168 was originally published in 2000. There is a considerable variation between strains and reannotation of the *C. jejuni* genome published in 2006 revealed that the complete sequence is 1,641,481 bp in length with 25 polymorphic regions [34]. Moreover, new information for 1,450 of the original 1,654 coding sequences revealed changes corresponding to over 300 product functions [34]. The infectious diseases caused by members of the bacterial genus *Campylobacter* are called campylobacteriosis [35]. Currently *Campylobacter jejuni* and *Campylobacter coli* are considered to be the most important enteropathogens among *Campylobacter* spp. The rate of *Campylobacter* infections are increasing worldwide, exceeding shigellosis [35,36]. Sequencing of the C. jejuni NCTC 11168 genome demonstrated the existence of genes that code some proteins with infectious potential. Despite numerous studies on the molecular genetics of *Campylobacter* spp. their mechanisms of pathogenicity and virulence remain poorly understood [37]. Although the bacteria are considered to be susceptible to stress associated with environmental conditions, in the course of evolution, they were able to develop some complex mechanisms of survival and virulence [38].

The occurrence of virulence markers various among different sources of the isolates. On the other hand depending on the species (*C.jejuni*, *C.Coli* …etc.) some virulence markers are different. Malgorzata et al. [39] in his study stated that the majority of genes were found at high levels in *Campylobacter* spp. isolated from pork meat (*csr*A, *sod*B, *cdt*B, and *rac*R). Low levels of the pathogenic markers virB11 and iam were noted in *Campylobacter* isolates from pork and beef meat. Also that significant differences in the occurrence of *iam*, *wla*N, and *vir*B11 genes were detected between *C. jejuni* and *C. coli* isolates.

4. PREVALENCE OF CAMPYLOBACTER IN PIGS

Pigs are well-recognized carriers of *Campylobacter* spp., particularly *C. coli* [40]. However, these agents are not commonly associated with enterocolitis in swine and a diagnosis of campylobacteriosis in pigs has historically been based upon the exclusion of other diseases [41]. As such, *Campylobacter* culture is not typically included in routine diagnostic testing for enteric disease in grow-finish pigs and thus the role of *Campylobacter* spp. infection is pigs with diarrhea is poorly characterized. A study detecting the prevalence of *Campylobacter* in swine through the different processing stations and comparing carcasses, colon and rectal samples throughout a slaughter operation was conducted by Pearce et al. [42]. When results from four different recovery methods were compared, *C. coli* was found in 151 of 202 isolates with recovery of *C. jejuni*

accounting for only 1% of the samples tested. Malakauskas et al. [43] reported that *C. coli* was prevalent in 92 of 120 isolates obtained from fecal, carcasses and slaughter line surfaces combined, while *C. jejun*i isolates accounted for 28 of 120 of positive samples recovered from carcasses and slaughter line surfaces.

All pigs excreted *Campylobacter* 10^3 - 10^7 CFU g^{-1} faeces from the age of 8 – 13weeks old. Pigs seem to be a natural reservoir of *Campylobacter* spp. with prevalence between 50% and 100% and excretion levels ranging from 10^2 to 10^7 CFU/g, but opposite to most animals, pigs show a dominance of *C.coli* [31,44]. Table 2 shows that the prevalence of *Campylobacter* spp varies from one country to another, from the site of collection. *C.coli* in pigs still remains the most common despite cohabitation with *C. jejuni*. In Africa few studies have had a work on pigs, particularly in Cameroon where there is none, the few studies are more interested in poultry. This observation led us to present the data already known in pigs and subsequently to carry out this study in Yaounde, Cameroon in order to describe the situation of *Campylobacter* in pigs in Cameroon.

5. IDENTIFICATION AND ANTIBIOTICS RESISTANCE PROFILE

Characterization methods make it possible to determine the genus and the bacterial species (biochemical identification and Polymerase Chain Reaction); other methods discriminate against strains within each species (serological methods or genotyic). Further to the limited technical platforms in Cameroon, identification is more focused on biochemical techniques.

5.1 Identification of Species by Biochemical Characterization

Biochemical tests performed on strains including morphological characters are suggestive of gender *Campylobacter* make it possible to identify the 4 major species of thermotolerant *Campylobacter*. These tests are carried out in conjunction with the assessment of sensitivity to two antibiotics, nalidixic acid and cephalotin. These tests are recommended in the ISO 10272 standard for the detection of *Campylobacter* in food. However, the emergence of resistance to nalidixic acid can pose problem for the use of this criterion for discrimination between *C. jejuni* and *C. upsialensis* on the one hand and *C. coli* and *C. lari* on the other. A *Campylobacter* identification kit is also available (API Campy; API Biomerieux). However, difficulties in identifying certain strains of *C. coli* and *C. lari* have been reported [45].

5.2 Antimicrobial Resistance

According to the World Health Organisation [46], surveillance of AMR in Campylobacter has identified important levels of resistance to erythromycin and fluoroquinolones in many studies of the world, which appears to be associated with the use of these drugs in pork production systems. [47,48]. Fluoroquinolones and macrolides such as ciprofloxacin and erythromycin, respectively, are recommended for the treatment of Campylobacter infections in humans [36,49]. However, given their abuse and misuse, resistance to these drugs has emerged [46].

Table 4 shows some studies carried out on the resistance of Campylobacter isolates from pigs in different countries. These different studies confirm the high resistance of Campylobacter to tetracycline the percentages of which are substantially the same. On the other hand fluoroquinoles (Ciprofloxacin) resistance varies according to the country and the source of sample. This reveals that despite the already known resistance to fluoroquinolones, it may happen that this resistance is not identical on all *Campylobacter* strains. The study that we will be carrying out in the coming days will enable us to obtain a percentage of resistance to ciprofloxacin in *Campylobacter* strains isolated from pigs in slaughterhouses in the city of Yaounde. Resistance to Erythromycin remains substantially the same in all studies. According to Jonker and Picard [50], in intensive poultry and pig rearing systems the use of oral antibiotics is essential to maintain health; hence there is a high risk for the *Campylobacter* in the intestinal tract of food animals to develop resistance to commonly used antibiotics. The increased resistance of bacteria to antibiotics has been associated with the continuous use of antibiotics either therapeutically, prophylactically, or as growth-promoting agents to maintain animal welfare in swine production systems. This practice creates a potential risk for human health care based on the present knowledge of gene transfer and co-resistance [51].

Table 1. Factors of *Campylobacter* genus bacteria allowing them to infect and survive in a host organism

The mechanism of survival/virulence	Description	References
Adherence to host's epithelial cells	Initial colonisation of intestinal epithelium *a*	[52-54]
	Mediation of the adhesins on the surface of bacterial cells, including: *Cad*F (an external membrane protein), PEB1 (periplasmatic binding protein), *Jlp*A (lipoproteins engaged in adhesion to Hep-2 cells), and *Cap*A (Camplyobacter A adhesion protein) *b*	
Invasion of host's cells	Avoiding immunological response *b*	[38,53]
	Significant role played by the external lipopolysaccharide bacterial core *b*	
Mobility	Moving against the persitalsis, reaching target sites in the intestine *a*	[52–55]
	adhesion to host's cells, formation of a biofilm, secretion of invasive proteins *a*	
	Required flagella and a chemosensory system (regulation of the flagellar movement depending on environmental conditions) *b*	
Production of Toxins-cytolethal distending toxin (CDT)	A protein composed of the subunits coded by genes *cdt*A, *cdt*B, and *cdt*C *b*	[56,52,57]
	Cdt B encodes the enzymatic part of the toxin b;	
	*cdt*A and *cdt*C encode subunits responsible for binding the toxin to the membrane of an eukaryotic cell b	
	Subunits CdtA, CdtB, and CdtC necessary for correct function of the toxin *b*	
	Halting the eukaryotic cell during the G2/M phase of the cellular cycle, stopping from transition into the phase of mitosis—cellular death *b*;	
	Not all strains produce CDT *b*	

[a] for C. jejuni; [b] for Campylobacter genus

Table2. Prevalence of *Campylobacter* isolates from Pigs in few country

Country	Samples types	Samples tested	Prevalence%	%of genes /species	Detection procedure	References
Poland	Pork chops	151	19 (12,6)	(47,4) *C. jejuni* 10(52,6) *C. Coli*	PCR	[39]
Ghana (Kumasi)	Pigs Carcass	102	(36,3)	*Campylobacter* spp	Cultural	[58]
Ivory Coast	Pigs faeca	270	3 (1.1)	1 (0.37) *C. jejuni* 2 (0.74) *C.coli*	Cultural	[59]
Brittany, France	Pigs caeca	582	86 (14,8)	*C. coli*	Multiplex PCR	[60]
Dannemark	Pigs rectal faecal	47	/	29 *C. jejuni* 0.3 – 46 *C.coli*	PCR	[61]
10 U.S states	Pigs faecal	838	472 (56,3)	*Campylobacter* spp	Cultural	[62]
USA	Pigs feces with diarrhea	155	128 (82.6)	75 *C. coli* 25 *C.jejuni*	PCR	[63]

Table 3. Biochemical tests used for identification of the 4 thermotolerant *Campylobacter* species

	C. jejuni	*C. coli*	*C. lari*	*C. upsialensis*
Culture à 42°C	+	+	+	+
Oxydase	+	+	+	+
Catalase	+	+	+	-or low
Hydrolysis of hippurate	+	-	-	-
nalidixic Acid (disc 32µg)	S	R	R	S
Cephalotine (disc 32µg)	R	R	R	S

Table 4. Antimicrobial resistances of *Campylobacter* isolated from pigs in few studies

Country	Sources	Species	Antibiotics	%Resistance	References
Poland	Pork (chops, meat)	*C. coli*	Ciprofloxacin	100	[39]
			Tetracycline	64.3	
			Gentamycin	10.7	
			Erythromycin	3.6	
			Azythromycin	7.2	
U.S Regions	Pork (pré, post evisceration)	*Campylobacter* spp	Ciprofloxacin	2.2	[62]
			Tetracycline	64.5	
			Erythromycin	47.9	
			Nalidixic acid	23.5	
			Chloramphenicol	11.5	
			Gentamycin	0.6	
Canada Quebec	Pork (feces,meat)	*C. coli*	Clindamycine	59	[64]
			Erythromycin	61	
			Streptomycin	67	
			Gentamycin	5	
			Tetracycline	68	
			Ciprofloxacin	11	

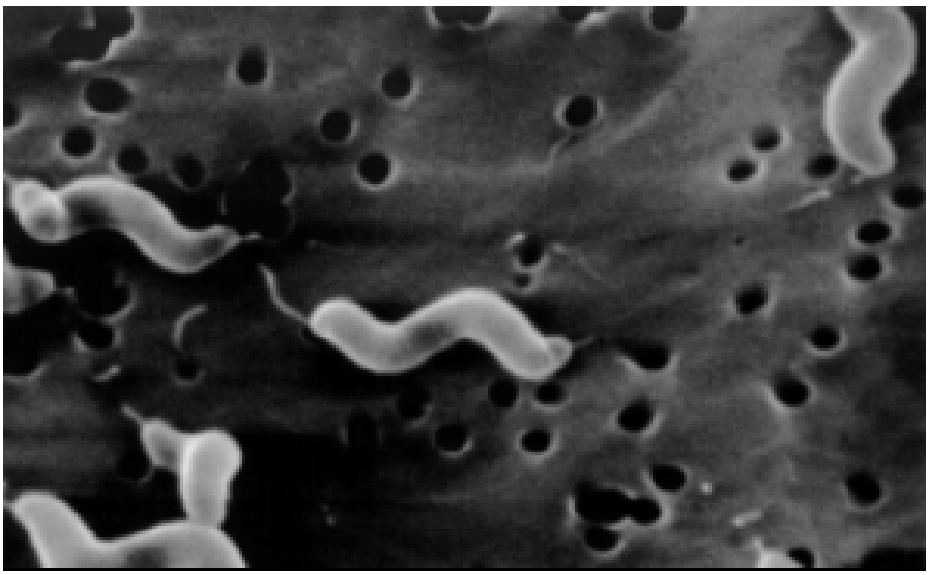

Fig. 1. Scanning electron microscope image of *Campylobacter jejuni*, illustrating its corkscrew appearance and bipolar flagella

Source: VirginiaMaryland Regional College of Veterinary Medicine, Blacksburg,Virginia

6. APPROACH TO PREVENT THE SPREAD OF CAMPYLOBACTER FROM PIGS TO THE ENVIRONMENT

Animal production and management systems plays an important part in Campylobacter control and must be carefully considered. Cameroon has no national surveillance programs on *Campylobacter*. Cameroon does not have slaughterhouses meeting international standards. According to SODEPA, Yaounde only has 02 slaughterhouses and several killings. To better control and limit the spread of *Campylobacter*, it would be wise to insist on hygiene measures. Butchers must wear personal protective equipment (PPE). Despite the lack of slaughterhouses, the killings should improve their work surfaces to limit the contamination of meat. Applying biosecurity interventions at swine production sites has resulted in different levels of success in different countries [65-67]. In Cameroon it would be important to minimise human-animal contact, practice personal and environmental hygiene, and seek proper medical care for sick persons in households in order to minimise risks of transmission. To reduce the risk of campylobacteriosis, careful management practices focus on innovative methods to avoid cross-contamination from raw meat products. Predominantly, the reduction of contamination of raw meats is handled at the processing plants through a post-harvest cleaning process. Pre-chilled carcasses that harbor *Campylobacter* may lead to contamination of retail consumer products [68,69].

7. CONCLUSION

To the best of our knowledge, this is the first review of bacteriological profile of *Campylobacter* spp isolated from pigs, in Yaounde Cameroon. *Campylobacter coli* is the more common *Campylobacter* species recovered from pigs. High resistance rates for tetracycline, fluoroquinolones, and emergence of MDR isolates from pork sample are reported. Moreover, a high level of resistance to ciprofloxacin and tetracycline among *C. jejuni* and *C. coli* species indicate the reduced clinical utility of these antibiotics for the treatment of patients. Pigs are also well-recognized as potential carriers of *Campylobacter* spp, particularly *Campylobacter* coli, yet enteric disease in swine associated with infection by these bacteria is considered uncommon and diagnosis has historically been based upon exclusion of other causes. It is crucial that education in areas such as microbiology, sanitation, hygiene, food science, good agricultural and good manufacturing practices should be considered as necessary in slaughterhouses/ killings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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