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Incidence of *Campylobacter* Species in Poultry Products and Beef Sold in Owerri, Imo State, Nigeria

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Abstract

The incidence of *Campylobacter* species in beef and poultry products sold in Owerri, Nigeria, was assessed. A total of 400 samples were analysed. Isolates were recovered on selective media and characterized using the API Campy Kits. Incidence data revealed that *Campylobacter jejuni ssp jejuni* 2 was predominant in all samples with an incidence of 53(13.25%), 51(12.75%), 51(12.75%) and 49(12.25%) respectively for the Ready-to-eat meat, Turkey meat, Chicken meat and Beef; *Campylobacter jejuni ssp jejuni* 1 had an incidence of 20% on the Ready-to-eat meat, 17% each on the others. On the other hand, *Campylobacter coli* had an incidence of 22%, 27%, 28% and 34% on the Ready-to-eat meat, Turkey meat, Chicken meat and Beef respectively while the least abundant isolate was the *Campylobacter jejuni bv fecalis* with an incidence of 5 (1.25%), 5 (1.25%), 4 (1%) and 0(0%) respectively.

Keywords: *Incidence; Campylobacter Species; API Campy Kit; Owerri*

Introduction

Campylobacter jejuni is one of the food borne pathogens with a world leading impact as a causative or aetiologic agent of diarrheal disease known as Campylobacteriosis, being a leading cause of zoonotic food borne illness in industrialized countries and the second or third cause in developing countries [1,2]. Transmission to human is usually by consumption of food substances of animal origin, handling of raw chicken and other poultry meat or offal and direct contact with animals or their exudates or faeces [1]. Most are adapted to the intestinal tract of homoeothermic animals. Although, certain *Campylobacter's* are commensals, but the majority of them are pathogens, infecting the human and animal gastrointestinal tracts [3,4].

Campylobacter jejuni/coli are also well established causative agents of diarrhoea in Nigeria. Gastroenteritis due to *Campylobacter jejuni* was first reported in the northern part of the country in 1981 [5] and the Southwestern part in Ile-Ife in 1983 [6]. In 1984, the most comprehensive report on *Campylobacter jejuni* as an agent of diarrhoea particularly in children was given by Coker and Dosunmu-Ogunbi [7] Coker [8]. Since then, the isolation rate of *Campylobacter* spp. from cases of diarrhoea in the country has increased and has led to a series of careful biological studies on the local strains [9]. Also, in the northern Nigeria, incidence of *Campylobacter jejuni* in Sokoto state was evaluated by Salihu., *et al* [10]. There appear to be paucity of information on *Campylobacter jejuni* in the eastern Nigeria.

The availability, safety and portability of food materials are of global concern [2]. The quality of food can be assessed by physical characteristics such as colour, flavour, taste, texture, etc. However, microbial quality of food to a large extent cannot be ascertained by mere physical means. Food substances that appear physically and organoleptically satisfactory may yet be highly contaminated with food borne pathogenic organisms. Routine check of food products for microbial quality is therefore highly required. This article reports on the incidence of *Campylobacter* species from raw and processed meat and poultry products from eateries and markets.

Materials and Method

Sample collection

Fresh beef, Chicken meat and turkey meat samples were collected from the five markets within Oweri zone which include Orji, Ekeonunwa, Ihiagwa, relief and Obinze markets. On the other hand, ready-to-eat (processed) chicken meat was purchased from five popular fast food eateries located in Owerri, Imo State, Nigeria. A total of 20 samples were collected of each type and from each location making a total of 400 samples. Each sample type was collected randomly and intermittently over a period of ten days. Samples were promptly transported immediately to Anthony van Leuwenhoek Research Center (AVLRC) for microbiological analysis.

Sample preparation

Each sample was blended in a sterile stomacher blender and emptied into a sterile 100ml beaker. One gram (1g) of representative blended sample was transferred into 9 mls of sterile distilled water to produce a stock solution from which a tenfold serial dilution was carried out with dilution up to the 5^{th} factor (10-5) [24].

Isolation of *Campylobacter* species from samples

Aliquot 1000µl of the 10-5 dilution was plated over the surface of a freshly prepared campy medium agar (eg Oxoid, England) using the spread plate technique. The inoculated plates were incubated at 370C for 24 hours. The colony forming units (CFU) were enumerated and recorded [24].

Characterization of *Campylobacter* isolates using API campy kit

API test strips consisting of microtubes (cupules) containing dehydrated substrates to detect the enzymatic activity or the assimilation/fermentation of sugars by the inoculated organisms was used. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. When the carbohydrates are fermented, the pH within the cupule changes and is shown by an indicator. An assimilation test was performed by inoculating pure cultures of test isolates into a minimal medium (API AUX medium), and the bacteria grow if they are able to utilize the corresponding substrate: a positive result is indicated by growth. Test results are entered into an online database to determine the bacterial identity. The procedure was carried out strictly according to the manufacturer's specification.

Catalase test

The catalase test is one of the recommended tests used as a basis of computing the unique 7 digits that can identify *Campylobacter* species according to the identification manual. Therefore, pure colonies of the isolates were tested for their catalase activity.

Characterization and identification of other bacterial isolates

Samples were analyzed for the presence of other microorganisms of public health importance. The Microorganisms isolated from the samples were characterized based on the colonial, morphological, microscopic and biochemical characteristics of the pure cultures [24]. The identities of the isolates were cross matched with features obtained in Standard microbiological manuals [25].

Results and Discussion

Incidence of *Campylobacter* Spp in the Meat Samples

The incidence of *Campylobacter* isolates from the ready-to-eat Chicken meat

Table 1 shows the incidence of *Campylobacter* isolates from the ready-to-eat Chicken meat obtained from fast food vendors within Owerri municipal. The results revealed that the incidence of *Campylobacter* spp from the samples from the five eateries Ren, Sun, Tren,

Crun and Twe had a count of 3.4 x 10^4 Cfu/mL (13%), 1.3 x 10^5 Cfu/mL (52%), 1.8 x 10^3 Cfu/mL (1%), 1.3 x 10^3 Cfu/mL (1%) and 8.4 x 10^4 Cfu/mL (33%) incidence out of a total of 100 samples comprising 20 samples from each eatery. The lowest incidence was observed in Crun and Tren with a 1% incidence while the highest incidence was observed in Sun foods with an incidence of 52%.

Fast foods samples	Total count on Campy medium (CFU/mL)
Sun	1.3 x 10^5
Crun	1.3 x 10^3
Tren	1.8 x 10^3
Twe	8.4 x 10^4
Ren	3.4 x 10^4

Table 1: *Incidence of Campylobacter sp in ready-to-eat chicken meat sold at selected fast foods in Owerri.*

The incidence of *Campylobacter* isolates from frozen Turkey meat purchased from markets within Owerri

Table 2 shows the incidence of *Campylobacter* isolates from Turkey meat purchased from markets within Owerri. The results revealed that the incidence of *Campylobacter* spp from the samples from Ihiagwa, Orji, Relief, Ekeonuwa and Obinze had a count of 1.23 x 10^5 Cfu/mL (9%), 3.4 x 10^5 Cfu/mL (25%), 1.1 x 10^5 Cfu/mL (8%), 3.4 x 10^5 Cfu/mL (25%) and 4.6 x 10^5 Cfu/mL (33%) incidence respectively out of a total of 100 samples comprising 20 samples from each Market. From the results, the least incidence was observed in Relief Market Turkey meat samples having a value of 8% while the highest incidence was observed in Obinze Turkey meat with a 33% value.

Turkey	Total count on Campy medium (CFU/mL)
Orji	3.4 x 10^5
Relief	1.1 x 10^5
Ekeonuwa	3.4 x 10^5
Obinze	4.6 x 10^5
Ihiagwa	1.23 x 10^5

Table 2: *The incidence of Campylobacter isolates from frozen Turkey meat purchased from markets within Owerri.*

The incidence of *Campylobacter* isolates from frozen Chicken meat purchased from markets within Owerri

Table 3 shows the incidence of *Campylobacter* isolates from Chicken meat purchased from markets within Owerri. The results revealed that the incidence of *Campylobacter* spp from Chicken samples purchased from Ihiagwa, Orji, Relief, Ekeonuwa and Obinze had a count of 1.8 x 10^4 Cfu/mL (9%), 8.4 x 10^4 Cfu/mL (41%), 2.3 x 10^4 Cfu/mL (11%), 1.54 x 10^4 Cfu/mL (7%) and 6.6 x 10^4 Cfu/mL (32%) incidence respectively out of a total of 100 samples comprising 20 samples from each Market. From the results, the least incidence was observed in Ihiagwa Market Chicken meat samples having a value of 9% while the highest incidence was observed in Orji Chicken meat with a 41% incidence.

Chicken	Total count on Campy medium (CFU/mL)
Orji	8.4 x 10^4
Relief	2.3 x 10^4
Ekeonuwa	1.54 x 10^4
Obinze	6.6 x 10^4
Ihiagwa	1.8 x 10^4

Table 3: *The incidence of Campylobacter isolates from frozen Chicken meat purchased from markets within Owerri.*

The incidence of *Campylobacter* isolates from beef purchased from markets within Owerri

Table 4 shows the incidence of *Campylobacter* isolates from beef purchased from markets within Owerri. The results revealed that the incidence of *Campylobacter* spp from the samples purchased from Ihiagwa, Orji, Relief, Ekeonuwa and Obinze had a count of 3.7 x 10^4 Cfu/mL (16%), 3.4 x 10^4 Cfu/mL (15%), 4.3 x 10^4 Cfu/mL (19%), 1.83 x 10^4 Cfu/mL (8%) and 9.8 x 10^4 Cfu/mL (42%) incidence respectively

out of a total of 100 samples comprising 20 samples from each Market. From the results, the least incidence was observed in Ekeonuwa market beef samples having a value of 8% while the highest incidence was observed in Obinze beef with a 42% incidence.

Beef	Total count on Campy medium (CFU/mL)
Orji	3.4×10^4
Relief	4.3×10^4
Ekeonuwa	1.83×10^4
Obinze	9.8×10^4
Ihiagwa	3.7×10^4

Table 4: *Incidence of Campylobacter isolates from beef purchased from markets within Owerri.*

Species Characterization and Abundance of *Campylobacter* Isolates in the Different Meat Samples

Campylobacter species characterization and abundance in Ready-to-eat Chicken meat

Figure 1 shows the *Campylobacter* Species characterization and abundance in Ready-to-eat meat. Among the *Campylobacter* spp isolated on the selective medium, it was observed that specific species of Campylobacter characterized using the API campy kit speciated *Campylobacter jejuni ssp jejuni* 2, *Campylobacter jejuni ssp jejuni* 1, *Campylobacter* coli and *Campylobacter jejuni bv fecalis*. The figure below presents the distribution of 20 selected isolates obtained from 20 samples of ready-to-eat meat. From the results, *Campylobacter jejuni ssp jejuni* 2 dominated in the samples compared to other selected species. The least isolate observed was the *Campylobacter jejuni bv fecalis* which was reportedly absent in Crun and Tren ready to eat Chicken meat.

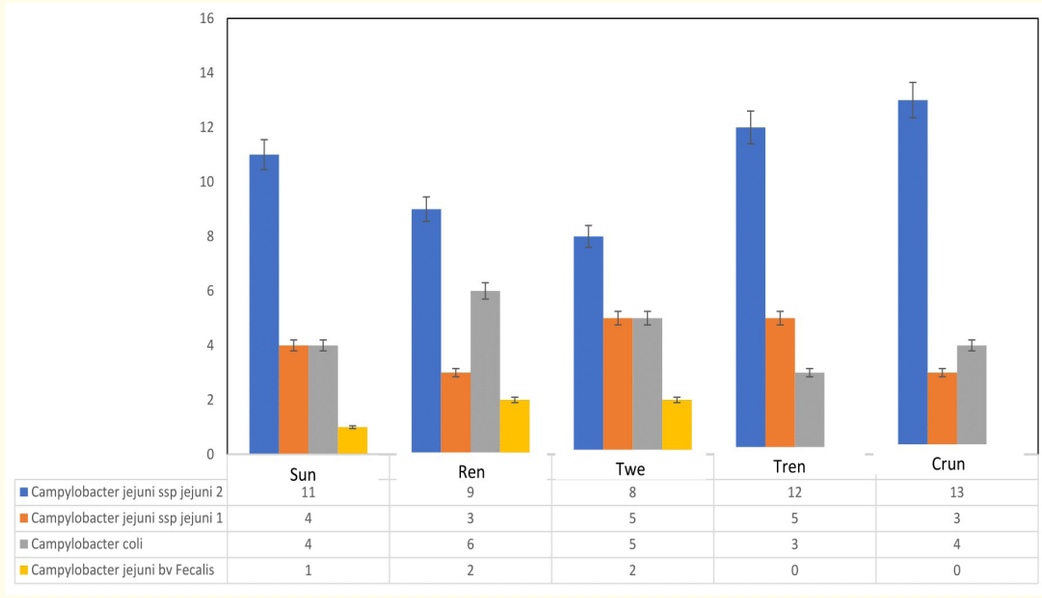
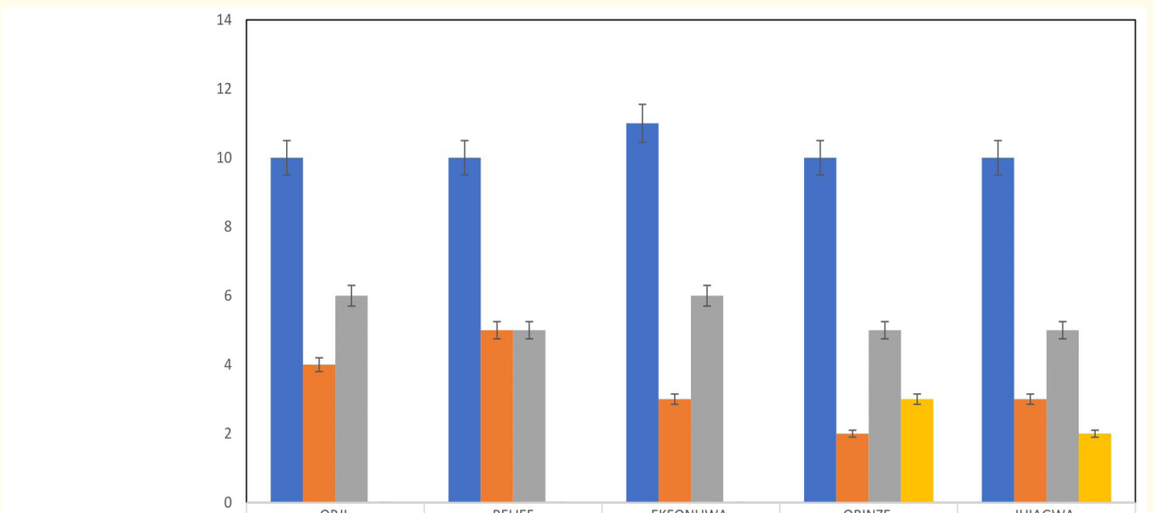


Figure 1: *Campylobacter Species characterization and abundance in Ready-to-eat meat.*

Campylobacter species characterization and abundance in Turkey meat

Figure 2 presents the distribution of 20 selected isolates obtained from 20 samples of Turkey meat from meat vendors in markets within Owerri. As observed earlier, in the isolates obtained from the ready to eat Chicken meat samples, *Campylobacter jejuni ssp jejuni* 2 was also the dominant species in the samples compared to other selected species. The least isolates observed was the *Campylobacter jejuni bv fecalis* which was reportedly absent in Turkey meat samples purchased from Orji, Relief and Ekeonuwa markets.

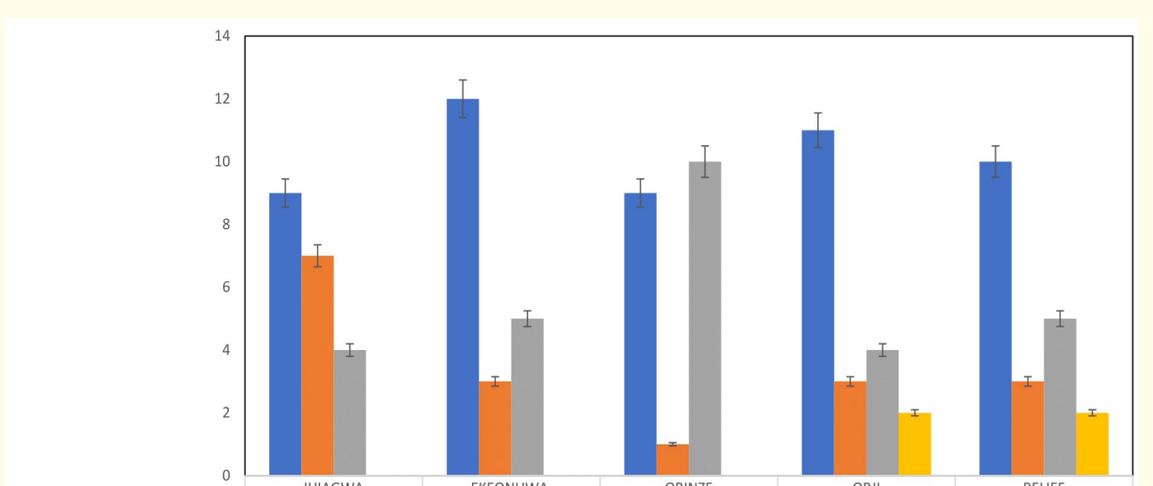


	ORJI	RELIEF	EKEONUWA	OBINZE	IHIAGWA
Campylobacter jejuni ssp jejuni 2	10	10	11	10	10
Campylobacter jejuni ssp jejuni 1	4	5	3	2	3
Campylobacter coli	6	5	6	5	5
Campylobacter jejuni bv Fecalis	0	0	0	3	2

Figure 2: *Campylobacter Species characterization and abundance in frozen Turkey meat.*

Campylobacter species characterization and abundance in Chicken meat

Figure 3 presents the distribution of 20 selected isolates obtained from 20 samples of Chicken meat from meat vendors in markets within Owerri. As observed in the isolates obtained from the ready to eat meat samples and the turkey meat samples vended at selected markets within Owerri. *Campylobacter jejuni ssp jejuni* 2 was the dominant isolates in the samples compared to other selected species with an exception of Obinze market Chicken meat samples that had *Campylobacter coli* in abundance. The least isolates observed was the *Campylobacter jejuni bv fecalis* which was also reportedly absent in Chicken meat samples purchased from Obinze, Ihiagwa and Ekeonuwa markets.

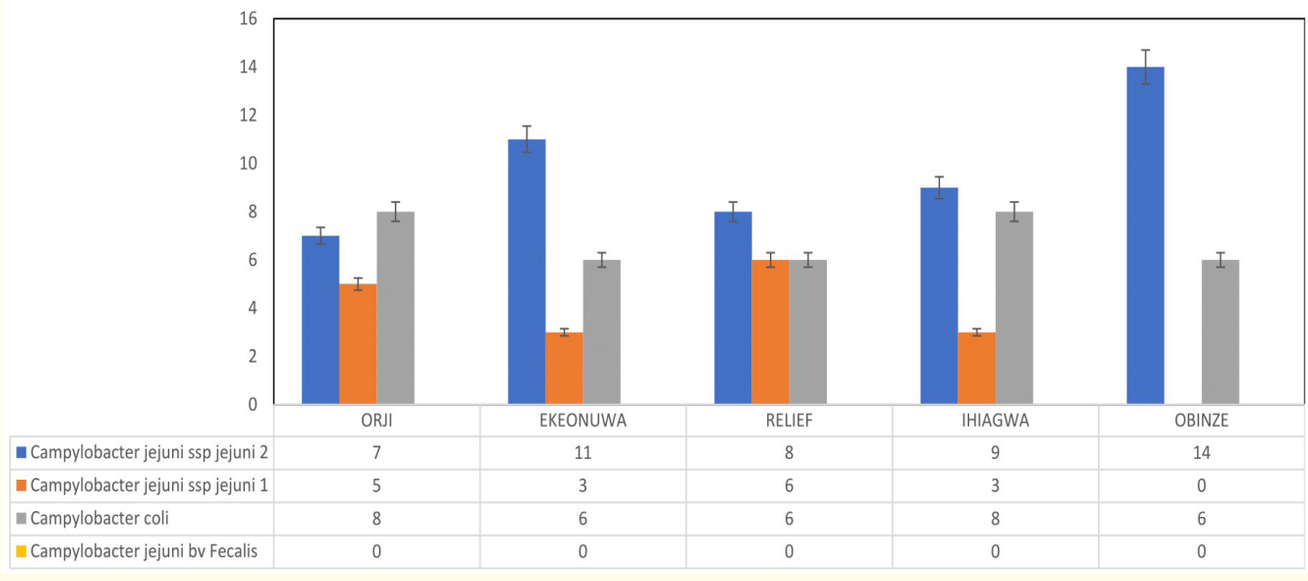


	IHIAGWA	EKEONUWA	OBINZE	ORJI	RELIEF
Campylobacter jejuni ssp jejuni 2	9	12	9	11	10
Campylobacter jejuni ssp jejuni 1	7	3	1	3	3
Campylobacter coli	4	5	10	4	5
Campylobacter jejuni bv Fecalis	0	0	0	2	2

Figure 3: *Campylobacter Species characterization and abundance in frozen Chicken meat.*

Campylobacter species characterization and abundance in Beef

Figure 4 presents the distribution of 20 selected isolates obtained from 20 samples of beef from meat vendors in selected markets within Owerri. As observed in the isolates obtained from the ready-to-eat meat samples and the turkey meat samples vended at selected markets within owerri, *Campylobacter jejuni ssp jejuni* 2 was the dominant species in the samples compared to other selected species with an exception of Orji market beef samples that had *Campylobacter coli* in abundance. The least isolate observed was *Campylobacter jejuni bv fecalis* which was absent in all samples. The incidence of isolates in the samples obtained from the meat samples



	ORJI	EKEONUWA	RELIEF	IHIAGWA	OBINZE
Campylobacter jejuni ssp jejuni 2	7	11	8	9	14
Campylobacter jejuni ssp jejuni 1	5	3	6	3	0
Campylobacter coli	8	6	6	8	6
Campylobacter jejuni bv Fecalis	0	0	0	0	0

Figure 4: *Campylobacter Species characterization and abundance in Beef.*

The incidence of isolates in the samples

The incidence of isolates in the samples obtained from the samples of the ready-to-eat chicken meat, turkey meat, chicken meat and beef is shown in table 5. The results revealed that *Campylobacter jejuni ssp jejuni* 2 was the most abundant isolate in all samples with an incidence of 53 (13.25%), 51 (12.75%), 51 (12.75%) and 49 (12.25%) respectively for the Ready-to-eat meat, Turkey meat, Chicken meat and Beef; *Campylobacter jejuni ssp jejuni* 1 had an incidence of 20% on the Ready-to-eat meat, 17% each on the Turkey meat, Chicken meat and Beef; on the other hand, *Campylobacter coli* had an incidence of 22%, 27%, 28% and 34% on the Ready-to-eat meat, Turkey meat, Chicken meat and Beef respectively while the least abundant isolate was the *Campylobacter jejuni bv fecalis* with an incidence of 5 (1.25%), 5 (1.25%), 4 (1%) and 0 (0%) respectively.

	Campylobacter jejuni ssp jejuni 2	***Campylobacter jejuni ssp jejuni 1***	***Campylobacter coli***	***Campylobacter jejuni bv fecalis***
Ready-to-eat meat	53 (13.25%)	20 (5%)	22 (5.5%)	5 (1.25%)
Frozen Turkey	51 (12.75%)	17 (4.25%)	27 (6.75%)	5 (1.25%)
Frozen Chicken	51 (12.75%)	17 (4.25%)	28 (7%)	4 (1%)
Beef	49 (12.25%)	17 (4.25%)	34 (8.5%)	0 (0%)

Table 5: *Incidence of the different species of Campylobacter in the meat samples.*

Discussion

Campylobacter jejuni is the leading cause of Campylobacteriosis which is largely considered as a food borne disease with poultry considered the principal vehicle of transmission. Studies have identified eating or handling raw or undercooked chicken as a major risk factor

for Campylobacteriosis in humans [11-13]. The percentage of human Campylobacteriosis cases that are attributed to eating or handling raw poultry varies between countries and studies. Estimates of cases that have a food borne origin range from 30% [14] to 58% - 76% [13] and up to 80% may be attributed to the chicken reservoir [11].

The result obtained from this study revealed different levels of contamination of meat samples sold in the different markets. For instance, an incidence of 78%, 73%, 72% and 66% *Campylobacter jejuni* was recorded on the Ready-to-eat meat, frozen Turkey meat, Chicken meat and Beef respectively. The results revealed that *Campylobacter jejuni ssp jejuni* 2 was the most dominant species in all samples with an incidence of 53%, 51%, 51% and 49% respectively for the Ready-to-eat meat, frozen Turkey meat, Chicken meat and Beef. *Campylobacter jejuni ssp jejuni* 1 had an incidence of 20% on the Ready-to-eat meat, 17% each on the frozen Turkey meat, Chicken meat and Beef; on the other hand, *Campylobacter coli* had an incidence of 22%, 27%, 28% and 34% on the Ready-to-eat meat, frozen Turkey meat, Chicken meat and Beef respectively while the least isolate was the *Campylobacter jejuni bv fecalis* with an incidence of 5%, 5%, 4% and 0% respectively isolated from the samples.

The results recorded in tables 1-3 confirm the presence of *Campylobacter* sp in the meat samples. Muller., *et al.* [13] reported that the primary source of contamination is from the meat handlers in slaughter houses as well as the rigorous processes that are involved in meat packaging and preservation. The high incidence of *Campylobacter* sp in the ready to eat meat sample may suggest that the meat samples were purchased from same poultry farm and the differences in handling, preparation and preservation attributes for the variation in microbial load [12].

It is evident that meat handling plays an important role in the transmission of *Campylobacter* spp. Obinze had a high count in most of the samples analysed owing to handling and environment. It recorded a 33% incidence in frozen Turkey meat and a 42% incidence in beef samples. However, Orji frozen Chicken meat had a higher incidence with a 41% incidence. It have been reported that up to 90% of chickens are colonized with *Campylobacter* spp but the colonization rate varies from place to place. There are a range of environmental sources poultry are exposed to both on the farm and at the processing plant that can result in contamination with *Campylobacter* spp. Studies have shown that three days contact with a single *Campylobacter* spp. infected bird is sufficient for the majority of the flock to be colonized [15]. Horizontal transmission can occur via contaminated water, litter, faecal contact and other vectors such as insects [16,17], rodents and farm personnel (Stern et al., 2001). When these contaminated poultry enter the processing plant having *Campylobacter* spp. populations ranging from 105 to 10^8 CFU/g of faeces, these high levels allow the bacteria to be easily spread throughout the plant [18]. The scalding and defeathering procedures have the potential for cross-contamination. *Campylobacter* spp. has periodically been removed from scald water and it has been postulated that the opening and closing of follicles may allow the retention of *Campylobacter* spp. within the carcass [18]. The use of recycled water throughout processing plant is another procedure that results in cross contamination. *Campylobacter* spp. may also be transported throughout a processing plant by personnel moving from one area of the plant to another [19].

Raw beef has also been identified as sources of foodborne Campylobacteriosis in humans [20-22]. *Campylobacter* spp. may be present in milk from faecal contamination during the milking process or an udder infection [21]. A recent study from the United States found that 5% (12/262) of Campylobacteriosis outbreaks from 1997 - 2008 were due to consumption of contaminated pork, beef or game [23]. Therefore, the presence of *Campylobacter jejuni* in beef as seen in this study is not coincidental.

Conclusion

It is important to justify that *Campylobacter* jejuni and other species of *Campylobacter* are present in meat samples including some poorly cooked meat samples. Since the organism is heat labile, proper cooking is necessary to kill the pathogen in foods. On the other hand, contamination of meat products in the market could be as a result of cross contamination and therefore, standards should be set and enforced on meat handlers to limit the spread across tables. More so, the presence of the isolates in some ready to eat meat samples is alarming and therefore calls for proper monitoring of eateries and ensure that such microbes are kept within the WHO standard. As reported, ≥500 organisms can cause campilobacteriosis [26].

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