Occurrence and Antibacterial Response Patterns of *Campylobacter jejuni* in Beef and Vegetables

[1*] Shamsuddeen, U., [1]Haladu, A. and [2]Panda, T. W.
[1]Department of Microbiology Faculty of Life Science Bayero University Kano PMB: 3011
[2]Department of Microbiology Aminu Kano Teaching Hospital Kano-Nigeria
*Correspondence author: ushamsudeen.bio@buk.edu.ng

Abstract: Food borne Campylobacteriosis is distributed all over the world. Raw meats become contaminated during processing when intestinal contents come in contact with meat surfaces. The aim of the research is to study the occurrence and antimicrobial response pattern of *Campylobacter jejuni* isolated from beef and vegetables. Fifty (50) processed and unprocessed food samples (20 beef and 30 vegetables) were collected from different sites. The samples were subjected to aerobic bacterial counting and higher counts were obtained in unprocessed vegetables (3.80×10^6 cfu) and raw beef (2.22×10^6 cfu) samples. All the samples were inoculated on mCCDA, selective media for isolation of *Campylobacter* species. The isolates were confirmed to be *Campylobacter jejuni* using standard procedures. Extracts were subjected to phytochemical analyses for the detection of secondary metabolites. Alkaloids and flavonoids were generally present in all the extracts tested; while anthraquinone was conspicuously absent. Ethanolic and aqueous extracts of *Syzigium aromaticum, Allium sativum, Zingiber officinale* and *Piper nigrum* as well as commercially prepared antibiotics were tested against the bacterial isolates via disc diffusion techniques. Out of the 50 samples examined, 26% samples yielded *Campylobacter* spp*.,* from which 8% were identified as *Campylobacter jejuni*. Statistical analysis revealed that there were significant differences in the bacterial count between raw and processed samples of beef (P = 0.004), cabbage (P = 0.019) and cucumber (P = 0.048), while there is no significant difference in bacterial count between unprocessed and processed lettuce samples (P = 0.058). *S. aromaticum, Allium sativum,* and *Zingiber officinale* extracts were active against *Campylobacter* jejuni. Among the antibiotics tested against *C. jejuni,* Ciproflaxacine (100%) and Erythromycin (100%) were active.

INTRODUCTION

Campylobacter jejuni infection is one of the most common gastroenteritis worldwide. It occurs more frequently than do infections caused by S*almonella* species, *Shigella* species and *Escherichia coli* (Sharfadi *et al*., 2015). *Campylobacter jejuni* is Gram negative, slender, curved and motile rod like bacterium. It is microaerophilic organism, relatively fragile and sensitive to environmental stress (Bukar and Ajagbe, 2016)

Campylobacter species are widely believed to be among the most common causes of acute bacterial enteritis in human worldwide. Most of infections have been linked to handling and consumption of contaminated water and food, which includes unpasteurized milk, meat, poultry, shellfish, fruits and vegetables. Clinical features of infection through contaminated foods or drinks are usually similar across the different species with an incubation period of 2-10days (Nwankwo *et al.,* 2016). Food poisoning caused by *Campylobacter* species can be severely debilitating, but is rarely life threatening. It has been linked with subsequent development of Guillain- Barre Syndrom (GBS) which develops two to three weeks after initial illness (Ajagbe *et al.*, 2016). Although most patients with *Campylobacter* infections do not require antibiotic treatment, antimicrobial therapy is necessary for patients with severe or prolonged systemic diseases (Luangtongkum *et al.*, 2007).

The aim of the research is to study the occurrence and antimicrobial response pattern of *Campylobacter jejuni* isolated from beef and vegetables.

MATERIALS AND METHODS

Study area

Kano is located in north western Nigeria; Kano state has been a commercial and agricultural state, producing variety of vegetables. It has a total area of $18684km^2$, and 44 local governments, with the coordinates $11^0$30'N $8^0$30'E.

Samples collection

The samples were collected from vegetable markets and beef retailers from various sites in Kano metropolis. *Syzygium aromaticum*, *Piper nigrum, Allium sativum* and *Zingiber*

officinale were purchased from Kurmi market; while antibiotics were purchased from pharmacies.

Sample size

Fifty (50) processed and unprocessed food samples (15 each of unprocessed and processed vegetables, and 10 each of raw and processed beef) were collected for the analysis.

Sample Preparation and Serial Dilution for Bacterial Enumeration

According to method described by Nwachukwu and Chukwu (2013), twenty-five grams (25 g) of each sample was aseptically collected and placed in a sterile blender to which 225 ml of buffered peptone water was added and homogenized for 2 min at normal speed. A millilitre (1ml) of the homogenate was then 10 fold serially diluted. Serial dilution and pour plating procedures were carried out according to procedure described by Madigan *et al.,* (2012).

Isolation of *Campylobacter jejuni* from beef and vegetables

Each individual sample was inoculated into modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) medium supplemented with cefoperazone and amphotericin B for selective isolation of *Campylobacter* spp. at 42^0C for 48 hours to 96 hours in an anaerobic jar containing microaerophilic generating pack. Presumptive *Campylobacter* colonies were then suspended in protease peptone glycerol (10%) and stored at -7^0C for subsequent species identification (Mohammed *et al.,* 2009).

Confirmatory tests *Campylobacter jejuni*

Gram's staining, cell morphology and motility test, catalase test and oxidase test were carried out (Hadush and Pal, 2013).

The hippurate hydrolysis test: The hippurate hydrolysis test was used to identify *C. jejuni* among the confirmed isolates. A small quantity of 24h growth culture was suspended in 0.4ml of 0.1%(W/V) sodium hippurate (Sigma) solution and incubated at 37^0 C for 2h, 0.2ml of 2% Ninhydrin solution (Sigma)was added and incubated for further 15min. The development of a purple-violet color identified the isolate as *C. jejuni* (Salihu *et al.,* 2009).

Identification and preparation of Spices Materials

Spices were *Piper nigrum*, *Syzygium aromaticum*, *Allium sativum* and *Zingiber officinale* that were purchased from Kurmi market in Kano city were identified and authenticated by a botanist from Plant Biology Department of Bayero University Kano.

Preparation of extracts

Fresh *P. nigrum* (seeds), *Syzigium aromaticum* (seeds), *Allium sativum* (bulbs) and *Zingiber officinale* (roots) were thoroughly washed using tap water and rinsed with distilled water. They were dried for 5 min in an oven at 60^oC to stop enzyme activity. They were then air dried to a constant weight and milled to a fine powder. Two solvents were used for the preparation of the extracts, namely distilled deionized water and ethanol 60% conc. The aqueous extract was prepared by weighing (250 g) of the milled powdered plant materials and 200 ml of distilled deionized water was added in a 500 ml beaker and stirred vigorously with a glass rod. The combination was allowed to settle for 3 hrs using the infusion method. The extracts were then filtered using Whatman no.1 filter paper. The ethanol extracts were obtained by weighing same fraction 250 g of the different plants and wrapping it in Whatman no.1 filter paper and placed in the holding chamber of the soxhlet extractor. About 500 mL of the60% ethanol was used as solvent for the extraction of the plant materials using the reflux method for a period of 48 hr. This was carried out exhaustively. The extracts were then concentrated by evaporating to dryness using rotary evaporator at a temperature 40^oC (Nwinyi *et al.,* 2009).

Phytochemical Screening of the Extracts

Extracts were subjected to phytochemical analysis for the detection of secondary metabolites such as alkaloids, phenolics, flavonoids, tannins, saponins, steroids and

anthraquinones as described by Ogbebe *et al.,* 2017.

Standardization of bacterial inoculum

Campylobacter isolates were sub-cultured twice on Columbia agar supplemented with 5% sheep blood and incubated at 41.5 ℃ for 44 ± 4 h in microaerophilic conditions. After incubation, a suspension equivalent to 0.5 McFarland standard was prepared and transferred to Mueller-Hinton broth supplemented with 5% of sheep blood and 100 µl was used to inoculate antibiotic plates. The plates were incubated in microaerophilic conditions for 48 h at 37 °C and the minimal inhibitory concentration (MIC) was recorded and then read as described by wieczorek *et al.*, (2012).

Determination of antibacterial activity of the extracts against the isolates by disc diffusion method

Two grams (2g) of each of the extracts were dissolved in 2ml of appropriate diluent (water for water extract and dimethyl sulphoxide (DMSO) for ethanolic extract), to yield 1.0g/ml (1,000,000µg/mL) solution. This was labeled as stock solution. From the stock solution 0.1ml was transferred in to a bijou bottle containing 0.9ml diluents, to effect 10 times dilution this will give a concentration of 100000µg/ml. Then, 0.1ml was transferred in to another bottle containing 0.9ml diluents which gave a concentration of 10000µg/ml and this was further diluted to yield 1000µg/ml, 100µg/ml, 10µg/ml, 1.0µg/ml, 0.1µg/ml and 0.01µg/ml on pro- rata basis. One hundred (100) discs of 6.0mm in diameter were impregnated with the extracts to arrive at 100, 10, 1.0 and 0.01µg/disc. Greater disc potencies of 2000 and 3000µg/disc were prepared and stored in refrigerator before use (Shamsuddeen, 2015). The organism was grown overnight in thioglycollate broth. Discs containing different extracts were placed on Mueller-hinton agar which were lawn cultured with *Campylobacter jejuni* colonies (Chetana *et al.,* 2007).

Determination of Minimum Inhibitory Concentration (MIC)

The determination of minimum inhibitory concentration MIC was carried out to obtain an idea of the antibacterial activities of basic metabolites of the plant extracts. The MIC of active extracts was evaluated by tube dilution method. The MICs of all the extracts were determined by dilution of the extract to various concentrations (1500µg/ml, 1400µg/ml, 1300µg/ml, 1200µg/ml, 1100µg/ml, 1000µg/ml, 900µg/ml, and 800µg/ml). Decreasing concentrations of ethanolic and water extracts dilutions were prepared using Muellar Hinton Broth (MHM). Controls were included. After an overnight incubation at 37°C, the tubes were examined for turbidity indicating the growth of the microorganisms. The lowest solution of the extract that inhibited the growth of the microorganism as detected by the lack of visual turbidity (matching the negative growth control) was designated the minimum inhibitory concentration (Ramalivhana *et al.,* 2014).

Minimum Bactericidal Concentration (MBC)

Sterile Mueller – Hinton agar plates were inoculated with sample from the MIC tubes that showed no visible turbidity (bacterial growth) and incubated the plates at 37^0C for 24hrs, the lowest concentration in which no growth occurred on the medium was taken as the MBC (Aliyu *et al.,* 2009).

RESULTS

Occurrence of *Campylobacter spp.* in raw and processed beef and vegetable samples

Table 1 shows the occurrence of *Campylobacter* spp. in raw processed beef and vegetable samples. The occurrence of *Campylobacter* spp. was 4/10 (40%)in raw beef sample, 3/5 (60%)in unprocessed lettuce and 2/5 (40%) cabbage samples. From the processed beef samples the occurrence was 2/10 (20%), and 1/5 (20%), 1/5 (20%) for lettuce and cabbage samples respectively.

Table 1: Occurrence of *Campylobacter* spp. in raw and processed Beef and Vegetable samples

S/N	Food type	No. of samples examined	No. of *Campylobacter* species isolated raw samples (%)	No. of *Campylobacter* species isolated processed samples (%)
1	Beef	20	4 (40)	2 (20)
2	Lettuce	10	3 (60)	1(20)
3	Cabbage	10	2 (40)	1 (20)
4	Cucumber	10	0 (0)	0 (0)

Confirmatory tests for *Campylobacter jejuni*.

Table 3 shows that thirteen isolates were confirmed to be *Campylobacter spp.,* four (4) *Campylobacter* spp. isolates were hippurate hydrolysis positive which confirmed *Campylobacter jejuni*.

Table 3: Result for confirmatory tests

Sample	Catalase	Oxidase	Gram staining	Motility (cockscrew)	Hippurate hydrolysis
BR1	+	+	–	+	+
BR4	+	+	–	+	+
BR5	+	+	–	+	–
BR6	+	+	–	+	+
BP3	+	+	–	+	–
BP6	+	+	–	+	+
LU1	+	+	–	+	–
LU3	+	+	–	+	–
LU4	+	+	–	+	–
CAU2	+	+	–	+	–
CAU3	+	+	–	+	–
LP1	+	+	–	+	–
CAP2	+	+	–	+	–

Key: BR = Raw beef, BP = Processed beef, LU = Unprocessed lettuce, LP = Processed lettuce, CAU = Unprocessed cabbage, CAP = Processed cabbage, Gram staining (–) = Gram negative, – = absence, + = Presence

Phytochemical analyses of the plants extracts

Table 4 shows the phytochemical analyses of the plants extracts (aqueous and ethanolic) and revealed that alkaloids and flavonoids were present in all the extracts while anthraquinone was absent in all the extracts of the plant. Phenols, steroids, saponin and tannins were found in some extracts and absent in others.

Table 4: Phytochemical composition of *Syzigium aromaticum, Piper nigrum, Allium sativum* and *Zingiber officinale*

Extract	Alkaloids	Anthraquinone	Phenols	Steroids	Flavonoids	Saponins	Tannins
Syzigium aromaticum (ethanolic)	+	–	+	–	+	–	+
Syzigium aromaticum (aqueous)	+	–	–	–	+	+	–
P. nigrum (ethanolic)	+	–	+	+	+	+	+
P. nigrum (aqueous)	+	–	–	–	+	+	–
Allium sativum (ethanolic)	+	–	–	+	+	+	–
Allium sativum (aqueous)	+	–	–	–	+	+	+
Z. officinale (ethanolic)	+	–	–	+	+	+	–
Z. officinale	+	–	+	+	+	+	+

Key: EE = Ethanolic extract; AE = Aqueous extract; + = detected; – = not detected

Aerobic Mesophilic Bacterial Counts of raw and processed beef and vegetables samples

The aerobic mesophilic bacterial counts of raw and processed beef were presented in Table 5, while the aerobic bacterial counts of unprocessed and processed vegetables (lettuce, cabbage and cucumber) were presented in Table 6. The highest and lowest aerobic mesophilic bacterial counts of raw beef were 2.56×10^5 and2.22×10^6cfu/g respectively while that of processed beef samples were 4.70×10^3 and 4.60×10^4cfu/g. respectively. The highest aerobic mesophilic bacterial count among unprocessed lettuce samples was observed on LU4 (3.20×10^6) while that of processed lettuce samples was observed on LP1 (5.10×10^5). The high aerobic bacterial count among unprocessed cabbage samples were observed on CAU (2.22×10^6) while among processed sample, the highest count were observed in CAP3(2.55×10^5). The high aerobic bacterial count among unprocessed cucumber samples were observed on CBU5 (3.80×10^6) while among processed samples highest count was observed in CBP5 (5.40×10^4).

Table 5: Aerobic mesophilic Bacterial counts of raw and processed beef.

Raw beef sample	AMBC (cfu/g)	Processed beef sample	AMBC (cfu/g)
BR1	3.80×10^5	BP1	4.70×10^3
BR2	3.90×10^5	BP2	1.40×10^4
BR3	2.22×10^6	BP3	3.40×10^4
BR4	2.56×10^5	BP4	4.60×10^4
BR5	3.10×10^5	BP5	2.64×10^4
BR6	3.80×10^5	BP6	3.40×10^4
BR7	2.95×10^5	BP7	5.80×10^3
BR8	4.50×10^5	BP8	2.30×10^4
BR9	1.63×10^6	BP9	3.30×10^4
BR10	2.95×10^5	BP10	5.50×10^3

P = 0.004

Key: BR = Raw beef sample; BP = processed beef sample; AMBC = aerobic mesophilic bacterial count

Table 6: Aerobic mesophilic bacterial count of unprocessed and processed vegetables samples.

Unprocessed vegetables samples	AMBC (cfu/g)	Processed vegetables samples	AMBC (cfu/g)
LU1	1.79×10^5	LP1	5.10×10^5
LU2	3.50×10^5	LP2	6.10×10^4
LU3	3.00×10^5	LP3	6.40×10^4
LU4	3.20×10^6	LP4	1.51×10^5
LU5	2.35×10^6	LP5	2.63×10^4
CAU1	2.00×10^6	CAP1	4.10×10^4
CAU2	2.22×10^6	CAP2	1.49×10^4
CAU3	4.70×10^5	CAP3	2.55×10^5
CAU4	3.90×10^6	CAP4	3.70×10^4
CAU5	1.83×10^5	CAP5	2.85×10^4
CBU1	2.55×10^6	CBP1	2.01×10^4
CBU2	2.41×10^5	CBP2	3.10×10^4
CBU3	4.80×10^5	CBP3	1.67×10^4
CBU4	2.11×10^5	CBP4	2.67×10^5
CBU5	3.80×10^6	CBP5	5.40×10^4

Key: LU = Unprocessed lettuce sample; LP = Processed lettuce sample; AMBC = aerobic mesophilic bacterial count; CAU = Unprocessed cabbage sample; CAP = Processed cabbage sample; AMBC = aerobic mesophilic bacterial count; CBU = Unprocessed cucumber sample; CBP = Processed cucumber sample; AMBC = aerobic mesophilic bacterial count.

Antibacterial activities of plants (*Syzigium aromaticum, Piper nigrum, Allium sativum* and *Zingiber officinale*) extracts on *Campylobacter jejuni.*

Table 7 shows the antibacterial activity of *Syzigium aromaticum* extracts that were tested against *Campylobacter jejuni.* The highest bioactivity of *Syzigium aromaticum* against *Campylobacter jejuni* was observed in both 3000µg/disc ethanolic (19 – 29mm zones of inhibition) and aqueous (18 -25mm zones of inhibition) concentrations of the extracts. The antibacterial activity of both ethanolic and aqueous extracts of the plants were initially observed in 1000µg/disc concentration.

Table 8 indicates the antibacterial activity of *Allium sativum* extracts that were tested against *Campylobacter jejuni.* The highest antibacterial activity of *Allium sativum* against *Campylobacter jejuni* was observed in both 3000µg/disc ethanolic (21 – 29mm zones of inhibition) and aqueous (19 -27mm zones of inhibition) concentrations of the extracts. The antibacterial activity of both ethanolic and aqueous extracts of the plants were initially observed in 1000µg/disc concentration.

The antibacterial activity of *Zingiber officinale* extracts was tested against *Campylobacter jejuni*. The highest antibacterial activity of *Zingiber officinale* against *Campylobacter jejuni* was observed in both 3000µg/disc ethanolic (20 – 29mm zones of inhibition) and aqueous (19 -28mm zones of inhibition) concentrations of the extracts. The antibacterial actvity of both ethanolic and aqueous extracts of the plants was initially observed in 1000µg/disc concentration as shown in Table 9.

The antibacterial activity of ethanolic and aqueous extracts of *Piper nigrum* was tested against *Campylobacter jejuni.* There was no antibacterial activity of ethanolic and aqueous extracts of *Piper nigrum* on *Campylobacter jejuni* isolates as shown in table 10.

Table 11 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. aromaticum, Allium sativum* and *Zingiber officinale* on *Campylobacter jejuni*.

Table 7: Antibacterial activity of S*yzigium aromaticum* extracts on *Campylobacter jejuni* isolates

Isolate ID	Ethanolic extract (mm)					Aqueous extract (mm)					
	10 (µg)	100 (µg)	1000 (µg)	2000 (µg)	3000 (µg)	10 (µg)	100 (µg)	1000 (µg)	2000 (µg)	3000 (µg)	Control CIP
BR1	06	06	10	15	21	06	06	09	18	20	33
BR4	06	06	09	17	20	06	06	11	16	22	29
BR6	06	06	11	20	26	06	06	08	14	20	29
BP6	06	06	09	14	20	06	06	09	15	19	22

Key: BR = Raw beef; BP = processed beef; 6mm = size of disc

Table 8: Antibacterial activity of *Allium sativum* extracts on *Campylobacter jejuni* isolates

Isolate ID	Ethanolic extract (mm)					Aqueous extract (mm)					
	10 (µg)	100 (µg)	1000 (µg)	2000 (µg)	3000 (µg)	10 (µg)	100 (µg)	1000 (µg)	2000 (µg)	3000 (µg)	Control CIP
BR1	06	06	13	19	24	06	06	11	18	20	30
BR4	06	06	10	17	20	06	06	10	16	22	25
BR6	06	06	12	20	28	06	06	12	18	27	34
BP6	06	06	10	15	21	06	06	09	14	20	26

Key: BR = Raw beef; BP = processed beef; 6mm = size of disc

Table 9: Antibacterial activity of *Zingiber officinale* extracts on *Campylobacter jejuni* isolates

Isolate ID	Ethanolic extract (mm)					Aqueous extract (mm)					
	10 (µg)	100 (µg)	1000 (µg)	2000 (µg)	3000 (µg)	10 (µg)	100 (µg)	1000 (µg)	2000 (µg)	3000 (µg)	Control CIP
BR1	06	06	10	19	23	06	06	12	20	26	21
BR4	06	06	10	16	21	06	06	09	18	23	22
BR6	06	06	09	17	29	06	06	10	15	19	25
BP6	06	06	12	18	20	06	06	13	19	28	24

Key: BR = Raw beef; BP = processed beef; 6mm = size of disc

Table 10: Antibacterial activity of *Piper nigrum* extracts on *Campylobacter jejuni* isolates

Isolate ID	Ethanolic extract (mm)					Aqueous extract (mm)					
	10 (µg)	100 (µg)	1000 (µg)	2000 (µg)	3000 (µg)	10 (µg)	100 (µg)	1000 (µg)	2000 (µg)	3000 (µg)	Control CIP
BR1	06	06	06	06	06	06	06	06	06	06	22
BR4	06	06	06	06	06	06	06	06	06	06	31
BR6	06	06	06	06	06	06	06	06	06	06	21
BP6	06	06	06	06	06	06	06	06	06	06	29

Key: BR = Raw beef; BP = processed beef

Table 11: MIC and MBC of *S. aromaticum, Allium sativum* and *Zingiber officinale* on *Campylobacter jejuni*

Extract	Ethanolic extract(µg/ml)		Aqueous extract(µg/ml)	
	MIC	MBC	MIC	MBC
S. aromaticum	900	1300	1000	1300
A. sativum	1000	1500	1000	1500
Z. officinale	900	1400	1000	1400

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

Antibiotic susceptibility pattern of *Campylobacter jejuni* isolates

All *Campylobacter jejuni* isolates tested were sensitive to Ciprofloxacin. Tetracycline, Cotrimoxazole and Erythromycin have activity against some *Campylobacter jejuni* isolates, while Augmentin and Clindamycin have no activity against all the isolates tested as shown in Table 12.

Table 12: Antibiotic susceptibility pattern of *Campylobacter jejuni* isolates from beef and chicken samples

S/N	Isolate ID (mm)	AMC (mm)	TE (mm)	DA (mm)	SXT (mm)	E (mm)	CIP (mm)
1	BR1	06	30	06	18	16	29
2	BR4	06	08	06	06	14	22
3	BR6	10	24	06	25	15	23
4	BP6	06	06	06	06	14	23

Key: BU = unprocessed beef; BP = processed beef; AMC = Augmentin; TE = Tetracycline; DA = Clindamycin; SXT = Cotrimoxazole; E = Erythromycin; CIP = Ciprofloxacin; ID = Identity;

DISCUSSION

From the results obtained, *Campylobacter jejuni* was isolated in 4/50 (8.00%) food samples examined in this study. *Campylobacter jejuni* was isolated from raw beef samples and processed beef samples. *C. jejuni* was not isolated from both fresh and processed vegetables. Raw meat and poultry, raw milk, and field-grown vegetables are potential sources of *Campylobacter* infection (Wong *et al.,* 2007). The occurrence of *Campylobacter jejuni* in raw beef (30%) in this study is contrary to the findings of Wong *et al.,* (2007) who reported 3.5% incidence of *C. jejuni* in New Zealand; Wieczorek *et al.,* (2012) who reported 0.00% prevalence of *C. jejuni* in beef samples in Poland; 2% from the report of Osano and Arimi (1999) in Kenya; and also 0.5% as reported by Zhao *et al.,* (2001) in Turkey.

The high occurrence (20%) of *C. jejuni* from beef samples may not be unconnected with the fact that chicken and beef are mostly sold together at the point of sampling and the possibility of cross contamination may occur.

Zero (0%) percentage occurrence of *Campylobacter jejuni* observed in cucumber is not similar to the findings of (Khalid *et al.,*2015) who reported high prevalence in farm samples (vegetables). The absent of *Campylobacter jejuni* isolate from vegetables might be due to minimum of contact between vegetables and chicken (natural reservoirs of *Campylobacter jejuni*).

From the results of aerobic mesophilic bacterial count, the bacterial count of processed beef samples ranged between 4.70 $\times 10^3$to4.60 $\times$ 10^4cfu/g. the highest bacterial count of unprocessed vegetable samples was recorded on cucumber 3.80 x 10^6while highest bacterial count was recorded in lettuce as 5.10 x 10^5among processed vegetable samples.

The International Commission for Microbiological Specification for Foods (ICMSF, 1996) states that ready-to-eat foods with plate counts between 0 – 10^3 is acceptable, between 10^4 - ≤ 10^5 is tolerable and10^6 and above is unacceptable. Therefore, all the processed food samples examined in this study have aerobic plate counts ranging from 4.70 x 10^3 – 6.80 10^5cfu/g which is within acceptable or tolerable limits.

Higher aerobic bacterial counts were recorded in unprocessed and raw food samples. These higher counts might be due to the fact that there was absence of any form of treatment on the food samples. The high bacterial count recorded in unprocessed vegetables is not unconnected with the fact that many vegetables become contaminated because they grow low to the ground where they are likely to come in contact with the soil which might be treated with improperly treated animal manure as fertilizer or irrigated with contaminated waters. Besides this, other sources of contamination are improper handling and improper storage and transportation conditions. (Chaturvedi *et al.,* 2013).

In this study, *C. jejuni* are to some extent resistant to all the antibiotics tested (except ciprofloxacin) with clindamycin having total resistance. On the other hand, all the isolates were sensitive to ciprofloxacin (100%). Highest resistance was recorded on Clindamycin (100%) and Augmentin (100%).

The 100% susceptibility of *Campylobacter jejuni* to Ciproflaxacin was similar with the findings of Khalid *et al.*, (2015).

The zero (0%) resistance of ciprofloxacin found in this study was contrary to the work of Manyl-Loh *et al.,* (2018) who reported 31.1%, 18.8%, 87.5%, 12.5% and 37.5% resistance of *Campylobacter jejuni* to ciprofloxacin, Erythromycin, Cotrimoxazole, Tetracyclin and Augmentin respectively. It is also contrary to the work of Salihu *et al.,* (2012) who reported resistance of *Campylobacter jejuni* to Erythromycin 12.9%, Ciproflaxacin 21.4% and Tetracyclin 18.6%. The resistance by *C. jejuni* to some antibiotics observed in this study was also reported by Manyl-Loh *et al.,* (2018) who reported that increased resistance causes rise in costs due to morbidity and mortality of infected individuals, human therapies associated with severe and persistent infections and long hospital stays, laboratory workloads, the discovery and production of new antibacterial agents against drug resistant bacteria as well as increase in resources for suitable infection control programs.

The resistance observed particularly in Clindamycin and Augmentin may likely be due to the transfer of resistance genes via lateral gene transfer to human pathogens.

From the result of the present study, *Allium sativum* and *Zingiber officinale* was found to contain some phytochemical substances that possess antibacterial potentials which includes atleast one of tannins, saponins, alkaloids and flavonoids. According to previous reports of Huzaifa *et al.,* (2014) these classes of compounds were known to have curative activity against several pathogens (Gazuwa *et al.,*2013) also reported the presence of these compounds as reason behind antibacterial activity of some plant extracts. Antimicrobial activity observed in garlic and ginger is due to the presence of sulfide/ thiols (not tested in this study) and phenolics respectively (Jarriyawattaachaikul *et al.,*2016). The antibacterial activity found in Garlic (*A. sativum* and *Z. officinale*) is in agreement with the work of Babu *et al.,* (2002) who reported that garlic and clove essential oils were found to inhibit growth of *C. jejuni* .Jarriyawattaachaikul *et al.,* (2016) also reported the antimicrobial activity of ginger and garlic against *C. jejuni.* Sunilson *et al.,* (2009) also reported moderate antibacterial activity of ginger extract against *C. jejuni.*

CONCLUSION

Based on the findings of this research work, occurrence of *Campylobacter jejuni* is (20%) from the beef samples (raw and processed) tested, occurrence of this

bacterium particularly in processed beef sample is of serious public health importance.

High bacteria count was observed which indicates poor hygienic practices from slaughter of the animal to the retailers.

The phytochemical screening the crude extracts of *S. aromaticum, Piper nigrum, Allium sativum,* and *Zingiber officinale* confirmed the presence of active chemical components responsible for their antimicrobial activities.

Ciprofloxacin and Erythromycin are strongly active against the isolates. Tetracycline and Cotrimoxazole are also active but showed some level resistance. Augmentin and Clindamycin have no antibacterial activity against *Campylobacter jejuni* isolates.

S. aromaticum, Allium sativum, and *Zingiber officinale* were active against *Campylobacter jejuni*

RECOMMENDATIONS

- Adequate treatment of food especially minimally processed foods should be given top priority.
- Food handlers should be trained on hygienic food handling and processing.

REFERENCES

Adeleye S. A., Braide W., Chinakwe E.C., Esonu C. E. and Uzoh C. V. (2018). Chicken meat, Beef and Vegetables: Potential Sources of *Campylobacter jejuni* Contamination in Imo State, Nigeria. *Sustainable Food Production*. Vol 3: 63-71

Ajagbe J. M., Bukar A. and Kawo A. H. (2016). Antibiotic Susceptibility Profile of *Campylobacter jejuni* Isolated From Chicken Meat and Faeces in Kano Metropolis, Kano State, Nigeria.

Aliyu M. S., Hanwa U. A., Tijjani M. B. and Ya'u B. (2009). Pytochemical and antibacterial properties of leaf extract of *Sterospermum kunthianum* (Bignoniaceae). *Nigeria Journal of Basic and Applied Science,* 17(2); pp 235-239.

Babu B., Jisha V. K., Salitha C. V., Mohan S. and Valsa A. K. (2002). Antimicrobial Activity of Different Plant Extracts. *India J. Microbiol.* Vol. 42: 361-363

Bukar A. and Ajagbe J. M. (2016). Incidence of *Campylobacter jejuni* in chicken's meat and faeces in Kano Metropolis, Kano State, Nigeria. *British Microbiology Research Journal.* 14(6). 1-7

Chaturvedi M., Kumar V., Singh D. and Kumar S. (2013). Assessment of Microbial Load of Some Common Vegetables Among Two Different Socio-economic Groups. *International Food Research Journal.* 20 (5): 2927-2931

Chetana V., Sukhminderjit K. and Manpreet K. (2007). Bactericidal activity of chicken spices and condiments on enteropathogens. *Natural Products Radiance;* 6(1). Pp 40-45

Ejele A. E., Duru I. A., Ogukwe C. E. and Iwu I. C. (2012). Phytochemistry and Antimicrobial Potential of Basic Metabolites of *Piper umbellatum, Piper guineense, Ocimum gratissimum* and *Newbouldi alaevis* Extracts. *Journal of Emerging Trends in Engineering and Applied Sciences.* 3(2): 309-314

Gazuwa S. Y., Makanjuola E. R., Jaryum K. H., Kutshika J. R. and Mafulul S. G. (2013). The Phytochemical Composition of *Allium cepa/Allium sativum*and the Effects of Their Aqueous Extracts (Cooked and Raw Forms) on the Lipid Profile and Other Hepatic Biochemical Parameters in Female Albino Wistar Rats. *Asian J. Exp. Biol. Sci.* 4(3): 406-410

Hadush A. and Pal M. (2013). Detection of *Campylobacter jejuni* from food and its epidemiology. *Journal of Public*

Health and Epidemiology. 5(9). P 357 – 361

Hammad A. M. (2016). Antimicrobial Effect of Cinnamon and Clove on *Staphylococcus aureus. Journal of Veterinary Sciences* .Vol. 48(1): 1-6

Huzaifa U., Labaran I., Bello A. B. and Olatunde A. (2014). Phytochemical Screening of Aqueous Extract of Garlic (*A. sativum*) bulbs. *Report and Opinion.* 6(8): 1-4

International Commission on Microbiological Specification for Foods (ICMSF). (1996). Microorganisms in Foods 5: Microbiological Specifications of Pathogens.

Jarriyawattanachaikul W., Chareerach P. and Chokesajjawatee N. (2016). Antimicrobial Activity of Thai-Herbal Plants against Foodborne Pathogens *Escherichia coli, Staphylococcus aureus* and *Campylobacter jejuni. Agriculture and Agricultural Sciences.* Vol. 11: 20-24

Khalid M. I., Tang J.W.H., Baharuddin N. H., Rahman N. S., RahimiN. F. and Radu S.(2015). Prevalence, Antibiogram and cdt Genes of Toxigenic *Campylobacter jejuni* in Salad Style Vegetables (Ulam) at Farm and Retail Outlets in Terengganu. *Journal of Food Protection.* 78(1) 65-71

Luangtongkum T., Morishita T. Y., El-Tayeb A. B., Ison A. J. and Zhang Q. (2007). Comparison of Antimicrobial Susceptibility Testing of *Campylobacter* spp. by the Agar Dilution and the Agar Disk Diffusion Methods. *Journal of Clinical Microbiology*. Vol.45 (2):590-594

Madigan M. T., John M. M., Stahl D. A. and Clark D. P. (2012). *Biology of Microorganisms.* Thirteenth Edition; Pearson Press Inc., publishing. Pp 129-130

Many-Loh C., Mamphweli S., Meyer E. and Okoh A. (2018). Characterization and Antibiotic Resistance of Selected Bacterial Pathogens Recovered from Diary Cattle Manure During Anaerobic Mono-Digestion in a Ballon-Type Digester. *Applied Sciences.* Vol. 8: 1-12

Mehdi R., Mohammad B. B., Mohammad M. and Mostafa R. (2016). Assessment of disk diffusion and E-test methods to determine the antimicrobial activity of cefalotin and vancomycin on clinical isolates of *Staphylococcus aureus. International Journal of Medical Research & Health Sciences.* 5(11). Pp 122-126

Mohammed D. S., Junaidu U. A., Steve I. O., Godwin O. E., Abdullahi A. M., Mohammed L., and Yahaya H. (2009). Isolation and Prevalence of *Campylobacter* Species in Cattle from Sokoto State, Nigeria. *Veterinari aItaliana.* 45 (4). 501 – 505

Nwachukwu E. and Chukwu C. M. (2013). Effect of chemical treatments on the microbial load of fruits and vegetables. *Applied Microbiology and Biotechnology Research,* 1(2); 16-22

Nwankwo I. O., Faleke O.O., Salihu M. D., Magaji A. A., Musa U., Garba J. and Ibitoye E. B.(2016). Detection and viability of *Campylobacter species* isolates from different species of poultry and humans in Sokoto State, Nigeria. *International Journal of One Health.*Vol. 2(4):19-23

Nwinyi O. C., Chinedu N. S., Ajani O. O., Ikpo C. O. and Ogunniran K. O. (2009). Antibacterial Effects of Extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus. African Journal of Food Science.* 3(1): 022-025

Ogbebe J., Iruolaje F. O. and Dogo B. A. (2017). Antimicrobial efficacy of *Guiera senegalensis* and *Prosopis africana* leave extract on some

bacterial pathogens. *European Journal of Biology and Medical Science Research,* 5(2); pp 27-36

Osano O. and Arimi S. M. (1999). Retail Poultry and Beef as Sources of *Campylobacter jejuni. East African Medical Journal.* 76(3): 141-144

Salihu M. D., Junaidu A. U., Abubakar M. B., Magaji A. A. and Muhammed L. G. (2009). Isolation and Characterization of *Campylobacter* species from Camel (*Camelus dramedarius)* in Sokoto State, Northern Nigeria. *International Journal of Animal and Veterinary Advances.* 1(1): 25 -27.

Salihu M. D., Junaidu A. U., Magaji A. A. and Yakubu Y. (2012). Prevalence and Antimicrobial Resistance of Thermophilic *Campylobacter* Isolates from Commercial Broiler Flocks in Sokoto, Nigeria. *Research Journal of Veterinary Sciences.* 5(2): 51-58

Shamsuddeen U. (2015). Microbiological hazard and critical control point analysis of dried and minced meat snacks produced in Kano Nigeria. *Lambert Academic Publishing*. Pp 20-25

Sharfadi R. S., Whong C. M. Z., Kabir J., Yahaya H., Ibrahim A. and Muhammad A. (2015). Incidence of *Campylobacter jejuni* in Poultry Handlers. *Scholars Journal of Agriculture and Veterinary Sciences.* 2(3A): 169-173

Sunilson J. A. J., Suraj R., Rejitha G., Anandarajagopal K., Kumar A. B. A. and Promwichit P. (2009). In vitro Antimicrobial Evaluation of *Zingiber officinale, Curcuma longa* and *Alpinia galanga* Extracts as Natural Food Preservatives. *American Journal of Food Technology.* Vol. 1: 1-8

Wieczorek K., Szewczyk R. and Osek R. (2012). Prevalence, Antimicrobial Resistance, and Molecular Characterization of *Campylobacter jejuni* and *Campylobacter coli* Isolated from Retail raw meat in Poland. *Veterinary Medicina*. 57(6): 293-299.

Wong T. L., Hollis L., Cornelius A., Nicol C., Cook R. and Hudson J. A. (2007). Prevalence, Numbers, and Subtypes of *Campylobacter jejuni* and *Campylobacter coli* in Uncooked Retail Meat Samples. *Journal of Food Protection.* 70(3): 566-573.

Zhao C., Ge B., Villena J. D., Sudlre R., Yeh E., Zhao S., White D. G., Wagner D. and MengJ. (2001). Prevalence of *Campylobacter spp., Escherichia coli,* and *Salmonella Serovars* in Retail Chicken, Turkey, Pork, and Beef from the Greater Washington, D. C., Area. *Applied and Environmental Microbiology:*5431-5436