Occurrence Of *Campylobacter Jejuni* In Chicken And Its Response To Some Antibiotics And Extracts Of Some Plant Materials

[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 poultry become contaminated during processing when intestinal contents contact the meat surfaces. Chicken meat is considered the primary source of infection with *Campylobacter* spp. in humans. Twenty (20) chicken samples were collected from different sites in Kano State which is located in North western Nigeria. All the samples were processed and analyzed in the laboratory using modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) selective media for isolation of *Campylobacter species*. Presumptive tests were carried out against the isolates Gram's stain, catalase, oxidase and mortility tests; and confirmed to be *Campylobacter jejuni* using Hippurate hydrolysis test. Ethanolic and aqueous extracts of *Syzigium aromaticum, Allium sativum, Zingiber officinale* and *Piper nigrum*were tested against *Campylobacter jejuni* via disc diffusion techniques. Commercially prepared antibiotics (Erythromycin, Tetracycline, Clindamycin, Ciprofloxacine and Cotrimoxazole) were also tested against *Campylobacter jejuni.*From the 20 chicken samples examined, 10 isolates were identified as *Campylobacter jejuni*. Based on the findings of this research work, occurrence of *Campylobacter jejuni* is (50%) from the samples tested, occurrence of this bacterium particularly in processed food samples is of serious public health importance. Statistical analysis revealed that there are significant differences ($P< 0.05$) in the bacterial count between (raw and processed) samples of chicken. *S. aromaticum, Allium sativum,* and *Zingiber officinale* extracts were active against *Campylobacter* jejuni. Among the antibiotics tested against *C. jejuni,* Ciproflaxacine (100%) and Erythromycin (79%) were active. All the plant extracts except *Piper nigrum,* showed antibacterial activity on *C. jejuni.* Both the ethanolic and aqueous plant extracts were not toxic ($LC_{50} >$ 1000).

INTRODUCTION

Campylobacter specie, particularly *C. jejuni* and *C. coli*, are a major cause of enteritis in humans. Additional species cause reproductive disease in sheep and cattle. Many animals carry *Campylobacter* specie asymptomatically and shed the organism in their faeces Poultry, particularly broiler chickens, are an especially important source of the bacterium, though they usually do not become ill (Wieczorek *et al.*, 2012). The birds usually do not show any signs of disease, but bacteria from the intestines can contaminate carcass surfaces during evisceration in the slaughterhouse and subsequently may be transmitted to humans (Granic *et al.*, 2009).

Food borne Campylobacteriosis is distributed all over the world. It habituates intestinal tract of a wide range of warm blooded animals. The principal route by which *C. jejuni* contaminates the food is through fecal contamination by *C. jejuni* infected carriers. Raw meats and poultry become contaminated during processing when intestinal contents contact the meat surfaces (Hadush and Pal, 2013). Chicken meat is considered the primary source of infection with *Campylobacter* spp. in humans (Awadallah *et al.,* 2014).

Campylobacter species are Gram-negative microaerophilic bacilli, having a somewhat curved, rod-like appearance, with two cells forming a short chain resembling seagull wings. Campylobacter is characterized by flagella-mediated corkscrew motility. It is a typical microaerophilic microorganism with respiratory type metabolism, requiring 3-15% O_2 and 3-5% CO_2. These microorganisms do not produce acids from carbohydrates and use amino acids as a source of energy (Granic *et al.*, 2009).

The aim of the research is to study the occurrence and antimicrobial response patterns of *Campylobacter jejuni*in chicken.

MATERIALS AND METHODS

Samples Collection Identification and preparation of Spices Materials

The samples (chicken whole parts) were collected from chicken retailers from various sites in Kano metropolis. *Syzygium aromaticum*, *P. nigrum, Allium sativum* and *Zingiber officinale* were purchased from Kurmi market in Kano city. The spices were identified and authenticated by the vendors and a botanist from Plant Biology Department of Bayero University Kano; while commercially prepared antibiotics were purchased from renowned pharmaceutical shops that were certified and licensed to sell antibiotics.

Sample size

Twenty (20) samples of chicken (raw and processed) were collected for this analysis.

Isolation of *Campylobacter jejuni* from chicken.

The chicken 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. The presumptive *Campylobacter* colonies were then suspended in protease peptone glycerol (10%) and stored at -7^0C for subsequent species identification (Mohammed *et al.,* 2009).

Presumptive Tests for Isolation of *Campylobacter jejuni*

Gram's staining: A drop of distilled water was placed on the slide and an inoculating wire loop was flamed to a red hot and allow to cool before a loopful of culture was collected, smeared on the slide and air dried; the smear was heat-fixed by passing the slide over a flame three times. The slide was flooded with crystal violet and allowing to stand for a minute before rinsing with water; Gram's iodine was added to the slide and left for 1 minute before being washed off with alcohol and rinsed with water. A few drops of Safranin was also added and left to react for 15 seconds before rinsing off with water. The slide was air dried and viewed under microscope. Gram positive bacteria picked up the primary dye (purple) while gram negative organisms picked up the secondary stain and were stained red (Adeleye*et al.,*2018).

Cell morphology and motility tests: Cell morphology and motility tests were carried out by preparing a wet preparation and using phase contrast microscope. Presence of highly motile, slender rods with curved morphology and a characteristic darting or corkscrew like movement indicated the presence of *Campylobacter spp*. (Hadush and Pal, 2013).

Catalase test: A quantity of 2-3ml of hydrogen peroxide was poured in a test tube. A colonies suspected to be *Campylobacter jejuni* were picked using a sterilized wire loop into the hydrogen peroxide solution. Presence of bubbles indicate positive and absence of bubbles shows negative result (Shamsuddeen, 2015).

Oxidase test: A swab was immersed in freshly prepared oxidase reagent and touched lightly the surface of the colony to be tested, the immediate appearance of a dark purple color at the point of contact denoted a positive reaction which confirmed *C. jejuni* (Hadush and Pal, 2013).

Confirmatory Test for *Campylobacter jejuni*

The hippurate hydrolysis test: 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 presenceof*C. Jejuni*(Salihu*et al.,* 2009).

Sample Preparation and Serial Dilution for Bacterial Enumeration

According to method described by Nwachukwu and Chukwu (2013), twenty-five grams (25g) 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 mililitre (1ml) of the homogenate was then 10 fold serially diluted. Serial dilution was carried out according to procedure described by (Madigan *et al.,* 2012).

Using sterile pipette, 1ml of sample was withdrawn and mixed with 9ml of diluent (buffered peptone water) contained in a test tube to make 10-fold (10^{-1}) dilution. The dilution was well shaked. From the 10-fold (10^{-1}) dilution, 1ml of the dilution was withdrawn and transferred to 9ml of the diluent to obtain 100-fold (10^{-2}) dilution. Successive dilutions of the sample (10^{-3}, 10^{-4} and 10^{-5}) were made following the above procedure.

Plating of Sample

The serially diluted samples were plated using pour-plate method and then incubated at 37^0C for 24 hours. Colonies on a plate (between 30 and 300) were counted and recorded (Madigan *et al.,* 2012)

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 out (250 g) of the milled powdered plant materials and adding in 200 ml of distilled deionized water in a 500 ml beaker and stirring 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 out 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 the 60% 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).

Test for Alkaloids

Two drops of the Mayer's Reagent were added to 2 ml of the extract in a test tube, formation of white (cream) precipitate indicated the presence of alkaloids.

Test for flavonoids (*Shinoda test)*

A quantity of 2 ml of the test solution of the extracts, a few magnesium turnings and a few drops of concentrated Hydrochloric acid were added and boiled for 5 minutes. Appearance of red or orange colour indicated the presence of flavonoids

Test for Saponins (Frothing test)

This was carried out by adding 5 ml of distilled water to the test solution of the extract and then shaken vigorously for 30 seconds and allowed to stand for 30 minutes. A honey comb-like froth formed for more than 30 minutes indicated the presence of saponin in the extract.

Test for Steroids

Two (2 ml) of acetic anhydride was added to 2 ml of each extract in a test tube. One (1ml) of concentrated Sulphuric acid was added down the side of the tube. Appearance of purple colour which changed to blue or green colour confirmed steroids.

Test for Phenolics

To 2ml of the test solution of the extract, a few drops of ferric chloride solution was added, a blue-black or green precipitate confirmed the presence of phenolics.

Test for anthraquinones (Borntrager's test)

A few drops of magnesium acetate solution was added to the test solution of the extract. The production of a pink or violet color indicated the presence of anthraquinones.

Test for Tannins

The test solution of the extracts was mixed with drops of basic lead -acetate solution. Formation of a white precipitate indicated the presence of tannins.

Preparation of 0.5 McFarland standards and Standardization of bacterial inoculum

0.5 McFarland standard was prepared (Mehdi *et al.,* 2016), and the innocolum was standardized to 0.5 McFarland standards (wieczorek *et al.*, 2012).

Determination of antibacterial activity of the extracts against the isolates by disc diffusion method (using commercially prepared discs)

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) 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. A one hundred (100) discs, 6.0mm in diameter of Whatman No. 1 filter paper was 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. Two grams (2g) and three grams (3g) of each of the extracts were dissolved in 1ml of the appropriate diluents to yield 2g/ml and 3g/ml solutions. Subsequently, the solutions were serially diluted to 2000 and 3000 µg/disc. (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. Zone diameter breakpoints of augmentin, tetracycline, clindamycin, cotrimoxazole,erythromycin, and ciprofloxacin for *Campylobacter jejuni* isolated from chicken were recommended by the CLSI. (Chetana *et al.,* 2007).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The determination of MIC was carried out by agar method to obtain an idea of the antibacterial activities of basic metabolites of the plant extracts. Standard solutions of the extracts were prepared: 1.0mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml and 0.0625mg/ml, and distributed into sterile test tubes. One milliliter (1ml) of each metabolite dilution was separately added into the agar plate and poured into Petri-plates. The test microorganism was spotted onto the surface of the solidified extract-agar mixture and the plates were inoculated, starting from the lowest concentration to the highest. After inoculation the plates were allowed to dry for 30 min and incubated at 37oC for 18h, after which the samples were examined for microbial growth. The lowest concentration of metabolite which showed no growth of microorganisms was taken as the MIC of the extract (Ejele*et al.,* 2012).

Sterile Mueller – Hinton agar plates were inoculated with sample from the MIC plates that showed no visible 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

Table 1 presented the result for isolation of *Campylobacter* spp in raw and processed chicken samples by cultural method using mCCDA and biochemical tests. Fourteen (14) out of 20 raw and processed chicken samples generated colonies on mCCDA. The fourteen (14) isolates were Gram negative bacilli slightly curved, catalase and oxidase positive and exhibits corkscrew motility when viewed under microscope which confirmed *Campylobacter* spp.; while 10 isolates were Hippurate hydrolysis positive thereby confirming *Campylobacter jejuni.*

Table 2 shows the aerobic mesophilic bacterial counts in which the highest aerobic bacterial count among raw chicken samples was observed on CR9 (1.98×10^6 cfu/g) while in processed samples the highest count was observed in CP6 (6.80×10^5cfu/g). In contrast, the lowest counts of raw and processed chicken samples were 1.77×10^5cfu/g (CR10) and 1.24×10^4cfu/g (CP5) respectively.

The ethanolic and aqueous extracts of *S. aromaticum, Piper nigrum, Allium sativum* and *Zingiber officinale* were physically observed (table 3). Some are red, brown or dark brown in colour, while some are oily, gummy or powdery in texture, all are spicy in smell.

Table 4 shows the phytochemical analyses of the plants (*Syzigium aromaticum, Piper nigrum, Allium sativum* and *Zingiber officinale)* extracts (aqueous and ethanolic) which revealed that alkaloids and flavonoids were present in all the extracts while anthraquinone was absent in all the extracts of the plants examined. Phenols, steroids, saponin and tannins were found in some extracts and absent in others.

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 as shown on table 5. The bioactivity of both ethanolic and aqueous extracts of the plants was initially observed in 1000µg/disc concentration. The lowest antibacterial activity was recorded in 1000µg/disc of ethanol and aqueous concentrations (12-8 zones of inhibition).

Table 6 show the highest antibacterial activity of *Allium sativum* against *Campylobacter jejuni* as observed in both 3000µg/disc ethanolic (21 – 29mm zones of inhibition) and aqueous (19 -27mm zones of inhibition) concentrations of the extracts. The bioactivity of both ethanolic and aqueous extracts of the plants was initially observed in 1000µg/disc concentration. The lowest antibacterial activity of ethanolic and aqueous extracts of the plant was 9mm zones of inhibition for the former and 8mm zones of inhibition for the latter, both at 1000µg/disc.

Table 7 indicated the highest bioactivity of *Zingiber officinale* against *Campylobacter jejuni* as observed in both 3000µg/disc ethanolic (20 – 29mm zones of inhibition) and aqueous (19 -28mm zones of inhibition) concentrations of the extracts. The bioactivity of both ethanolic and aqueous extracts of the plants was initially observed in 1000µg/disc concentration.

The antibacterial activity of ethanolic and aqueous extracts of *Piper nigrum* was tested against *Campylobacter jejuni* and no activity on ethanolic and aqueous extracts of *Piper nigrum* on *Campylobacter jejuni* isolates was observed (table 8).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. aromaticum, Allium sativum* and *Zingiber officinale* on *Campylobacter jejuni* were presented on table 9. The MICs of the ethanolic extracts of *S. aromaticum, A. sativum* and *Z. officinale* were 900, 1000 and 900 µg/ml respectively, while the MICs of their aqueous extracts were each 1000 µg/ml. The MBCs of ethanolic extracts of *S. aromaticum, A. sativum* and *Z. officinale* were 1300, 1500 and 1400 µg/m respectively, while the MBCs of their aqueous extracts were 1300, 1500 and 1400 µg/ml respectively.

Antibiotic susceptibility pattern of *Campylobacter jejuni* isolates chicken samples that was tested by commercially prepared antibiotic discs of Augmentin, Tetracycline, Clindamycin, Cotrimoxazole, Erythromycin and Ciprofloxacin as shown on table 10 shows that *Campylobacter jejuni* isolates 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.

Zone diameter breakpoints of augmentin, tetracycline, clindamycin, cotrimoxazole, erythromycin, and ciprofloxacin for *Campylobacter jejuni* isolated from beef and chicken were recommended by the CLSI. S, susceptible; I, intermediate; R, resistant.

CLSI zone diameter breakpoints (S, susceptible; I, intermediate; R, resistant) are given below:

	S	I	R
AMC	≥17	14 - 16	≤13
TE	≥19	15 -18	≤14
DA	≥21	15 -20	≤14
SXT	≥16	11 -15	≤10
E	≥23	14 -22	≤13
CIP	≥21	16 -20	≤15

Table 1: Isolation and occurrence of *Campylobacter jejuni*

Sample	Colony presence on mCCDA	Catalase	Oxidase	Gram staining	Motility (cockscrew)	Hippurate hydrolysis	*Campylobacter jejuni*
CR1	+	+	+	–	+	+	+
CR2	+	+	+	–	+	+	+
CR3	+	+	+	–	+	–	–
CR4	+	+	+	–	+	+	+
CR5	+	+	+	–	+	–	–
CR6	+	+	+	–	+	+	+
CR7	+	+	+	–	+	–	–
CR8	+	+	+	–	+	+	+
CR9	+	+	+	–	+	+	+
CR10	+	+	+	–	+	–	–
CP1	+	+	+	–	+	+	+
CP2	–	NT	NT	NT	NT	NT	–
CP3	+	+	+	–	+	–	–
CP4	+	+	+	–	+	+	+
CP5	+	+	+	–	+	–	–
CP6	–	NT	NT	NT	NT	NT	–
CP7	–	NT	NT	NT	NT	NT	–
CP8	+	+	+	–	+	+	+
CP9	+	+	+	–	+	+	+
CP10	–	NT	NT	NT	NT	NT	–

Key CR = Raw chicken, CP = Processed chicken, Gram staining (–) = Gram negative, Gram staining (+) = Gram positive, – = absence, + = Presence

Table 2: Aerobic mesophilic bacterial count of raw and processed chicken samples

Raw chicken Sample	AMBC (cfu/g)	Processed chicken sample	AMBC (cfu/g)
CR1	1.58×10^6	CP1	2.20×10^4
CR2	1.49×10^6	CP2	1.45×10^5
CR3	4.90×10^5	CP3	3.60×10^5
CR4	6.70×10^5	CP4	1.64×10^5
CR5	1.98×10^6	CP5	1.24×10^4
CR6	5.50×10^5	CP6	6.80×10^5
CR7	1.61×10^6	CP7	4.40×10^5
CR8	1.44×10^6	CP8	7.20×10^4
CR9	6.00×10^5	CP9	2.70×10^4
CR10	1.77×10^5	CP10	1.16×10^5
P = 0.001	mean = 10.587		mean= 2.9294

Key: CR =Raw chicken sample; CP = Processed chicken sample; AMBC = aerobic mesophilic bacterial count

Table 3: Physical characteristics of *S. aromaticum, Piper nigrum, Allium sativum* and *Zingiber officinale*

Extracts	Color	Texture	Solubility	Smell
SAEE	Red	Oily	DMSO	Spicy
SAAE	Dark brown	Powdery	Water	Spicy
PNEE	Brown	Oily	DMSO	Spicy
PNAE	Dark brown	Gummy	Water	Spicy
ASEE	Brown	Oily	DMSO	Spicy
ASAE	Dark brown	Gummy	Water	Spicy
ZOEE	Brown	Gummy	DMSO	Spicy
ZOAE	Dark brown	Gummy	Water	Spicy

Key: SAEE= *S. aromaticum* ethanolic extract, SAAE=*S. aromaticum* aqeous extract, PNEE= *Piper nigrum* ethanolic extract, PNAE= *Piper nigrum* aqeous extract, ASEE=*Allium sativum*ethanolic extract, ASAE= *Allium sativum* aqeous extract, ZOEE= *Zingiber officinale* ethanolic extract, ZOAE=*Zingiber officinale* aqeous extract.

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)	+	–	–	–	+	+	–
Piper nigrum (ethanolic)	+	–	+	+	+	+	+
Piper nigrum (aqueous)	+	–	–	–	+	+	–
Allium sativum (ethanolic)	+	–	–	+	+	+	–
Allium sativum (aqueous)	+	–	–	–	+	+	+
Zingiber officinale (ethanolic)	+	–	–	+	+	+	–
Zingiber officinale (aqueous)	+	–	+	+	+	+	+

Key: + = detected; – = not detected

Table 5: Antibacterial activity of S*yzigium aromaticum* extracts on *Campylobacter jejuni* isolates

Isolate ID	Ethanolic extract					Aqueous extract					
	10µg	100µg	1000µg	2000µg	3000µg	10µg	100µg	1000µg	2000µg	3000µg	Control CIP
CR1	06	06	10	16	26	06	06	12	18	23	32
CR2	06	06	08	15	19	06	06	08	14	18	23
CR3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CR4	06	06	10	14	20	06	06	09	14	19	24
CR5	06	06	10	16	23	06	06	09	16	21	22
CR6	06	06	09	14	21	06	06	09	13	21	21
CR7	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CR8	06	06	11	16	24	06	06	11	15	22	25
CR9	06	06	10	17	27	06	06	09	14	24	31
CR10	06	06	10	15	26	06	06	10	15	24	23
CP1	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP2	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP4	06	06	12	18	29	06	06	08	15	25	29
CP5	06	06	10	14	23	06	06	10	14	22	24
CP6	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP7	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP8	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP9	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP10	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Key: CR = Raw chicken; CP = processed chicken; NT = not tested

Table 6: Antibacterial activity of *Allium sativum* extracts on *Campylobacter jejuni* isolates

Isolate ID	Ethanolic extract					Aqueous extract					
	10µg	100µg	1000µg	2000µg	3000µg	10µg	100µg	1000µg	2000µg	3000µg	Control CIP
CR1	06	06	13	20	27	06	06	13	18	25	21
CR2	06	06	09	14	21	06	06	09	13	19	27
CR3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CR4	06	06	12	18	26	06	06	12	17	26	31
CR5	06	06	11	16	24	06	06	10	16	23	29
CR6	06	06	12	19	28	06	06	12	18	30	24
CR7	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CR8	06	06	11	18	26	06	06	11	18	24	33
CR9	06	06	13	21	27	06	06	12	19	25	27
CR10	06	06	11	16	25	06	06	10	15	25	20
CP1	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP2	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP4	06	06	12	18	29	06	06	08	15	25	29
CP5	06	06	10	16	27	06	06	10	14	24	22
CP6	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP7	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP8	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP9	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP10	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Key: CR = Raw chicken; CP = processed chicken; NT = not tested

Table 7: Bioactivity of *Zingiber officinale* extracts on *Campylobacter jejuni* isolates

Isolate ID	Ethanolic extract					Aqueous extract					
	10µg	100µg	1000µg	2000µg	3000µg	10µg	100µg	1000µg	2000µg	3000µg	Control CIP
CR1	06	06	11	20	26	06	06	12	15	25	31
CR2	06	06	10	16	26	06	06	09	16	24	23
CR3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CR4	06	06	09	15	27	06	06	11	14	20	23
CR5	06	06	09	21	20	06	06	12	18	25	32
CR6	06	06	10	20	24	06	06	12	15	27	29
CR7	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CR8	06	06	10	18	26	06	06	10	14	25	22
CR9	06	06	08	14	21	06	06	08	13	22	24
CR10	06	06	09	19	20	06	06	10	16	25	29
CP1	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP2	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP4	06	06	10	18	23	06	06	11	14	20	30
CP5	06	06	11	16	20	06	06	10	17	24	33
CP6	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP7	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP8	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP9	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP10	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Key: CR = Raw chicken; CP = processed chicken; NT = not tested

Table 8: Bioactivity of *Piper nigrum* extracts on *Campylobacter jejuni* isolates

Isolate ID	Ethanolic extract					Aqueous extract					
	10µg	100µg	1000µg	2000µg	3000µg	10µg	100µg	1000µg	2000µg	3000µg	Control CIP
CR1	06	06	06	06	06	06	06	06	06	06	33
CR2	06	06	06	06	06	06	06	06	06	06	32
CP3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CR4	06	06	06	06	06	06	06	06	06	06	23
CR5	06	06	06	06	06	06	06	06	06	06	25
CR6	06	06	06	06	06	06	06	06	06	06	26
CR7	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CR8	06	06	06	06	06	06	06	06	06	06	26
CR9	06	06	06	06	06	06	06	06	06	06	22
CR10	06	06	06	06	06	06	06	06	06	06	22
CP1	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP2	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP4	06	06	06	06	06	06	06	06	06	06	23
CP5	06	06	06	06	06	06	06	06	06	06	32
CP6	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP7	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP8	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP9	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CP10	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Key: CR = Raw chicken; CP = processed chicken; NT = not tested

Table 9: 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
P. nigrum	NT	NT	NT	NT

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; NT = not tested

Table 10: Antibiotic susceptibility pattern of *Campylobacter jejuni* isolates from beef and chicken samples

S/N	Isolate ID	AMC (mm)	TE (mm)	DA (mm)	SXT (mm)	E (mm)	CIP (mm)
1	CR1	06	06	06	16	10	22
2	CR2	06	25	06	21	15	25
3	CR3	NT	NT	NT	NT	NT	NT
4	CR4	06	06	06	06	13	23
5	CR5	06	06	06	06	06	29
6	CR6	06	32	06	20	21	31
7	CR7	NT	NT	NT	NT	NT	NT
8	CR8	06	06	06	06	14	21
9	CR9	06	21	06	06	14	24
10	CR10	06	34	06	20	20	32
11	CP1	NT	NT	NT	NT	NT	NT
12	CP2	NT	NT	NT	NT	NT	NT
13	CP3	NT	NT	NT	NT	NT	NT
14	CP4	06	06	06	15	20	29
15	CP5	06	20	06	22	14	33
16	CP6	NT	NT	NT	NT	NT	NT
17	CP7	NT	NT	NT	NT	NT	NT
18	CP8	NT	NT	NT	NT	NT	NT
19	CP9	NT	NT	NT	NT	NT	NT
20	CP10	NT	NT	NT	NT	NT	NT

Key: CR = raw chicken; CP = processed Chicken; AMC = Augmentin; TE = Tetracycline; DA = Clindamycin; SXT = Cotrimoxazole; E = Erythromycin; CIP = Ciprofloxacin; ID = Identity; NT = not tested

DISCUSSION

From the 20 chicken samples examined for *Campylobacter jejuni.,* 10 (50%) were positive for the organism. Occurrence of *Campylobacter jejuni* in raw chicken samples was 80%, while in processed chicken samples the occurrence was 20%. From the results obtained, *C. jejuni* occurrence in this study was (50%). This might be due to the fact that chickens are primary reservoirs of *Campylobacter* as reported by Adeleye *et al.,*(2018). Evidence of high occurrence of *C. jejuni* in chicken indicates that the meat has been contaminated by faecal materials during processing, because the organism is found in the intestine of poultry. The possible reason for high occurrence of *C. jejuni* in raw chicken was that raw chicken samples underwent no treatment, contrasting the low occurrence of the organism in the processed samples (Bukar and Ajagbe, 2016).

Ilida and Faridah (2012) indicated that the high level of contamination of raw chicken with *C.jejuni* has been shown to contaminate food preparation surfaces and subsequent re-infection of the cooked chicken or other foods, through unhygienic practices. The overall result of this study has shown that the occurrence of *Campylobacter jejuni* was higher in raw chicken samples than processed samples.

This above finding agrees with the report of Adeleye *et al.,* (2018) who reported that the highest incidence of the isolates was from chicken meat followed by beef meat, and also beef meat higher than the vegetables. Consumption or handling of raw or poorly processed chicken is a cogent risk factor for campylobacteriosis in humans (Adeleye *et al.,* 2018).

The occurrence of *C. jejuni* (50%) in this study is higher than the incidence rate of 20% reported by El-Zamkan and Abdel-Hamid (2016), the findings of Bukar and Ajagbe, (2016) , 37% reported by Ilida and Faridah (2012),7.2% by Abdulazeez, (2006) , 9.7% reported by Medeiros *et al.,* (2008) and 17.5% prevalence from the work of Uaboi-Egbenni *et al.,* (2011).

However, the occurrence found in this study 10/20 (50%) is nearly similar with the findings of Quinones-Ramirez *et al.,* (2000) who reported 54%.

The findings of this study is lower than that of Sharfadi *et al.,*(2015) who reported 74% incidence and the 62.2% reported by Salihu *et al* (2012).

From the results of aerobic mesophilic bacterial count , the bacterial count of Processed chicken samples had bacterial count ranging from 1.24 x 10^4 to 6.80 x 10^5 cfu/g.

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 - $\leq 10^5$ is tolerable and10^6 and above is unacceptable. Therefore, all the processed chicken samples examined in this study have aerobic plate counts ranging from 1.24 x 10^4 to 6.80 x 10^5cfu/g which is within acceptable or tolerable limits.

Higher aerobic bacterial counts were recorded in raw chicken samples. These higher counts might be due to the fact that there was absence of any form of treatment on the chicken samples. Antibiotic treatment may be regarded only in severe cases of *C. jejuni.* Antibiotic resistant *Campylobacter* spp. can infect and colonize human through occupational exposure or food chain (Salihu *et al.,* 2012).

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

Zero (0) resistance of Ciproflaxacin 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 ,*A. sativum, Allium sativum* and *Zingiber officinale* were 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 *C. jejuni.* Jarriyawattaachaikul *et al.,* (2016) also reported the antimicrobial activity of ginger and garlic against *C. jejuni.* Clove extract were reported to contained phenolic acid (Hammad, 2016) which is in agreement with the current study. Sunilson *et al.,* (2009) also reported moderate antibacterial activity of ginger extract against *C. jejuni.*

Our finding on the antibacterial activity of Ginger (*Zingiber officinale*) extract opposed the previous finding of Sa-Nguanpuag *et al.,* (2011) who reported absence of antibacterial activity of the plant.

CONCLUSION

Based on the findings of this research work, occurrence of *Campylobacter jejuni* in raw and processed chicken samples is (50%) from the samples tested, occurrence of this bacterium particularly in processed chicken samples 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 of 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 of resistance. Augmentin and Clindamycin have no antibacterial activity against *Campylobacter jejuni* isolates.

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

It is therefore concluded that use of *S. aromaticum, Allium sativum,* and *Zingiber officinale* holds a great prominence source of easily available and effective antibacterial against *Campylobacter jejuni*

RECOMMENDATIONS

1. Adequate treatment of food should be given top priority.
2. Food handlers should be trained on hygienic food handling and processing.
3. Application of spices (*S. aromaticum, A. sativum* and *Z. officinale*) should be encouraged to inhibit *Campylobacter jejuni* in chicken

REFERENCES

Abdulazeez A. A. (2006). Prevalence of *Campylobacter jejuni* infections among Japanese quails and exotic chickens in Vom. *Nigerian journal of Medical Laboratory Science.* 15(2): 6 - 24

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.* 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.

Awadallah, M. A.I., Ahmad, H.A., El-Gedawy A.A., and saad, A.M. (2014). Molecular identification of *Campylobacter jejuni* and *C. coli* in chickens and humans at Zagzzig Egypt, with reference to the survival of *C. jejuni* in chicken's meat at refrigeration on freezing temperature. *International food research journal* 21(5): 1801-1812

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

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 *Newbouldia laevis* 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 Journal of Experimental Biological Sciences.* 4(3): 406-410

Granic K., Krcar D., Uhitil S. and Jaksic S. (2009). Determination of *Campylobacter spp.* in Poultry Slaughterhouses and Poultry Meats. *Veterinarski Arhiv.* 79 (5):491-497

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* . 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

Ilida M. N. and Faridah M. S. (2012). Prevalence of *Campylobacter jejuni* in Chicken meat and Chicken-based products. *Journal of Tropical Agriculture and Food. Sciences.* 40(1): 63-69

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

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

Medeiros D. T., Sattar S. A., Farber J. M. and Carrillo C. D. (2008). Occurrence of *Campylobacter spp.* in raw and Ready-to- Eat Foods and in a Canadian Food Service Operation. *Journal of Food Protection.* 71(10): 2087-2093

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. *Veterinaria Italiana.* 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

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

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

Sa-Nguanpuag, K., Kanlayanarat S., Srilaong V., Tanprasert K. and Techavuthiporn C. (2011). Ginger (*Zingiber officinale*) oil as an antimicrobial agent for minimally processed produce: a case study in shredded green papaya. *International Journal of Agriculture and Biology*, 13: 895–901

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*. 1: 1-8

Uaboi-Egbenni P. O., Bessong P. O.,Samie A. and Obi C. L.(2011). Prevalence and Antimicrobial Susceptibility Profiles of *Campylobacter jejuni* and *Campylobacter coli* isolated From Diarrheic and Non-diarrheic Goat Faeces in Vendra Region, South Africa. *African Journal of Biotechnology*. 10(64):14116-14124

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