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Incidence of *Campylobacter jejuni* in Chicken's Meat and Faeces in Kano Metropolis, Kano State, Nigeria

A. Bukar[1*] and J. M. Ajagbe[1]

[1]Department of Microbiology, Bayero University, P.M.B. 3011, Kano State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author JMA performed the research, statistical analysis and wrote the first draft of the manuscript. Author AB designed the study, wrote the protocol, supervised the research, read, corrected and approved the final manuscript.

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ABSTRACT

This study was conducted to determine the incidence of *Campylobacter jejuni* in Chicken meat and faeces in Kano metropolis. A total of 300 samples comprising of 180 faecal samples and 120 chicken's meat were collected from Poultry markets located within 6 local government areas of Kano metropolis, over a period of 6 months, from July, 2014 to January, 2015. All the samples were analyzed using Cultural and Biochemical methods. The confirmed isolates of *C. jejuni* were randomly selected and subjected to further identification by Molecular techniques (polymerase chain reaction and Nucleotide sequencing). Out of the 300 samples examined, 162 (54%) were *Campylobacter* positive, while 142 (47.3%) were identified as *C. jejuni*. Statistical analysis revealed that there is no significant difference ($P < 0.05$) in the isolation rates of *C. jejuni* at the different locations sampled. Based on this study, the recovery of *C. jejuni* at high rate in processed chicken's meat is of a serious public health importance as consumption of improperly processed or contaminated chickens has been implicated as the root cause of sporadic infection and outbreaks of Campylobacteriosis worldwide.

**Corresponding author: E-mail: al_amsak2004@yahoo.com, aminubukar80@gmail.com;*

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1. INTRODUCTION

Thermophilic *Campylobacter* species mainly *Campylobacter jejuni* and *Campylobacter coli* are recognized worldwide as an important cause of food borne illness and are major concern to the Poultry industry [1]. *Campylobacter jejuni* is a Gram – negative, slender, curved and motile rod – like bacterium. It is a microaerophilic organism, which means it has requirement for a reduced level of oxygen. It is relatively fragile and sensitive to environmental stress (e.g. 21% oxygen, drying, heating, disinfectants, and acidic conditions). Because of its microaerophilic characteristics, the organism requires 3–5% oxygen and 2-10% carbon dioxide for optimal growth condition). The bacterium is now recognized as an important enteric pathogen [2].

Campylobacter jejuni is one of the most common causes of human gastroenteritis in the world. 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 syndrome (GBS) which develops two to three weeks after initial illness [3]. The bacterium is commonly associated with poultry, and it naturally colonises the digestive tract of many bird species [3]. One study found that 30% of European starlings in farm settings, Oxford shire, United Kingdom, were carriers of *C. jejuni* [4]. *C. jejuni* is also common in Cattle, although it is normally harmless commensal of their gastro intestinal tract.

A study carried out in 2008 at Makurdi, North-central Nigeria on prevalence of *Campylobacter jejuni* in Duck faeces around drinking Water sources (Ponds and Wells) revealed that, out of 192 faecal samples cultured for *Campylobacter jejuni,* the overall incidence rate was 63% [5].

Surveys have shown that *C. jejuni* is the leading cause of diarrhoea illness in the United States and causes more disease than *Shigella* spps and *Salmonella* spps combined [2]. However, in developing countries, for example, Nigeria, bacteria diarrheal illnesses are rather assumed for enteric pathogens such as *Salmonella*, *Shigella, Escherichia coli* etc., but this may not be the fact in all cases of the disease. A thorough investigation could reveal other uncommon food-borne pathogens, particularly *Campylobacter jejuni*. The research on Incidence of *Campylobacter jejuni* in Chicken meat sold in Kano metropolis is important because bacterial food poisoning, diarrhea diseases, and gastroenteritis are still very serious health challenges confronting Nigeria, particularly, Kano state. Evidences from past and current literature available has shown that, no research work has been done on isolation of *Campylobacter jejuni* in Chicken meat and bird's faeces within Kano metropolis. Therefore, there is an urgent need for thorough investigation and detailed research as a means of generating a baseline information on the incidence of *Campylobacter jejuni*.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Kano metropolis and the surrounding six local governments. The local governments are Tarauni, Fagge, Gwale, Nassarawa, Dala and Ungogo Local government areas. Kano state is located in North West geo-political Zone of Nigeria. It lies on latitude 12^0N and longitude 9℄E. The area is the commercial nerve centre of northern Nigeria.

2.2 Collection of Sample

Samples for this research work are roasted Chicken meat, raw Chicken meat and Chicken faeces. A total of 300 samples were collected and analysed during the research. The Sample collection and analysis was carried out for a period of 7 months, between July, 2014 and January, 2015. The samples comprised of 180 Broilers Chicken faeces, 60 Roasted Chicken meat and 60 raw Chicken meats collected from Poultry markets across the six (6) local government areas located within Kano metropolis. Thirty (30) Faecal samples, 10 Raw Chicken meat and 10 Roasted Chicken meat were collected from each local government during sampling period. A pea size amount of Chicken's faeces was collected in different spots from the Chickens cage and placed into sterile Universal bottles and then taken to the Laboratory for Culture. Roasted and raw Chicken parts were purchased from the same location

and placed separately in sterilized sample bottles. The samples collected were then packed in ice box and transported to the Laboratory for analysis [6]. Transport to the Laboratory and subsequent processing were carried out as rapid as possible, within at most 24 hours. The samples were protected from light and elevated temperature [7]. The chicken meat was homogenized using a sterile food blender.

2.3 Isolation and Identification of *Campylobacter jejuni* from Samples

A loopful of the Chicken meat homogenate and faecal samples were cultured directly into Butzler Selective supplement media (Oxoid SR0085E) which contain Bacitracin, Colistin sulphate, Cyclohexamide, Cephazolin sodium and Novobiocin to inhibit growth of other bacteria [8]. The inoculated selective media plates were incubated microaerobically at 42℃ for 72 hours. This was achieved by placing the plates in anaerobic incubation jar without Catalyst. The gas was produced with a commercially available CO_2 gas generating pack (Lobal Chemie C10067). Incubation at 42℃ is to prevent growth of most of the other bacteria present in the foods. The colonies of *C. jejuni* appeared grey in colour, which was watery and spreading or round and convex [9]. The suspected isolates were sub-cultured in freshly prepared Butzler selective media for *Campylobacter* and stored at 4℃ for subsequent species identification.

2.4 Phenotypic and Biochemical Characterization of *C. jejuni*

The isolates were identified to species level using Standard Phenotypic identification tests as recommended by Attabay et al. [10]. Suspected colonies were first examined macroscopically for color, shape, texture and haemolysis. After which they were Gram stained and examined microscopically for morphology and Gram reaction. Motility testing by microscopic examination of wet preparation was also carried out on the isolates [11]. Catalase test [8], Oxidase test [12] and hippurate hydrolysis were also conducted [13].

2.5 Quality Control

Some confirmed Isolates of *C. jejuni* isolated from the Chicken meat and chicken faeces were randomly selected and subjected to molecular identification, which involved DNA extraction, Polymerase Chain Reaction and sequencing [14]. The primer sequence for *C. jejuni* types I and II is presented in Table 1.

2.6 Statistical Analysis

Data generated from the research was statistically analyzed using Chi square (X^2) at 5% Probability level and 10 degree of freedom using the software Excel Package developed by Microsoft Co-operation. The computed value was compared with the table value of X^2 and appropriate level of significance was determined [15].

Table 1. Primer sequence for *C. jejuni* types I and II

Target gene	Sequence (5' – 3')	Gene location	PCR product base pairs
Dideoxynucleotide Sub Unit (*C. jejuni*)Type I	GAG GAG GGC GTG GGT GTG AG CGT GGC CCA AAA GTT GCA TAG T TTG TGT GAA GCG GAC GCA GCT A GTT GTT CGA CAG CGG CGT GGT C GTG GTG ACG TGG GTG CAT GCA C GCT GCG TTG GAG AGA ACT GAG ATA GCA GTG TCG TGT GGG AGA GAG AT	780 - 1000	800 bp
Dideoxynucleotide Sub Unit (*C. jejuni*)Type II	TCA TAT CCG GTG TGT GTA TGA TTT TTT AGT T AA T TT GTT TTT TTT ATA TGC TCG GGT TTT TTT TAT TCT CTT AA A GTT TC A TTA ATA CAC GTT CCT CG CGT TGC TTT TTA TCT ATG TTC TTA ATT ATT TAC TTC ATC TCA TAA TTC GGC TGG ATA TT	780 - 1000	800 bp

3. RESULTS

All *Campylobacter jejuni* isolates appeared grey in colour, non haemolytic, round or convex colonies with spreading characteristics. The Organism is a gram negative rod, motile, Catalase positive, Oxidase positive and Hippurate hydrolysis positive. The results of Chicken meat and faeces examined for *Campylobacter jejuni* are shown in Table 2. Out of the 300 samples analyzed, a total of 162 (54%) samples were *Campylobacter* positive. One hundred and forty two 142 (47.3%) of the isolated species were identified to be *Campylobacter jejuni.* The phenotypic characterization of the isolates is shown in Table 4. Of the 224 total number of the isolates obtained, 204 were Gram-negative and 20 Gram positive bacteria, 188 isolates were found to be motile and 18 non-motile; 206 were Catalase Positive and 18 Catalase negative; 162 were Oxidase positive and 62 Oxidase Negative; 142 isolates were Hippurate hydrolysis positive and 82 were Hippurate hydrolysis negative. Therefore 142 isolates were finally confirmed to be *Campylobacter jejuni* by phenotypic identification.

Results of the PCR are shown in Plate 1. The PCR assay generates products with a length of 800 base pairs.

The 3 *C. jejuni* positive isolates by phenotypic identification were confirmed as C. *jejuni* (Type 1) and *C. jejuni* Type 2 (Table 4) and also 2 non *C. jejuni* isolates initially identified phenotypically were confirmed accordingly by molecular identification.

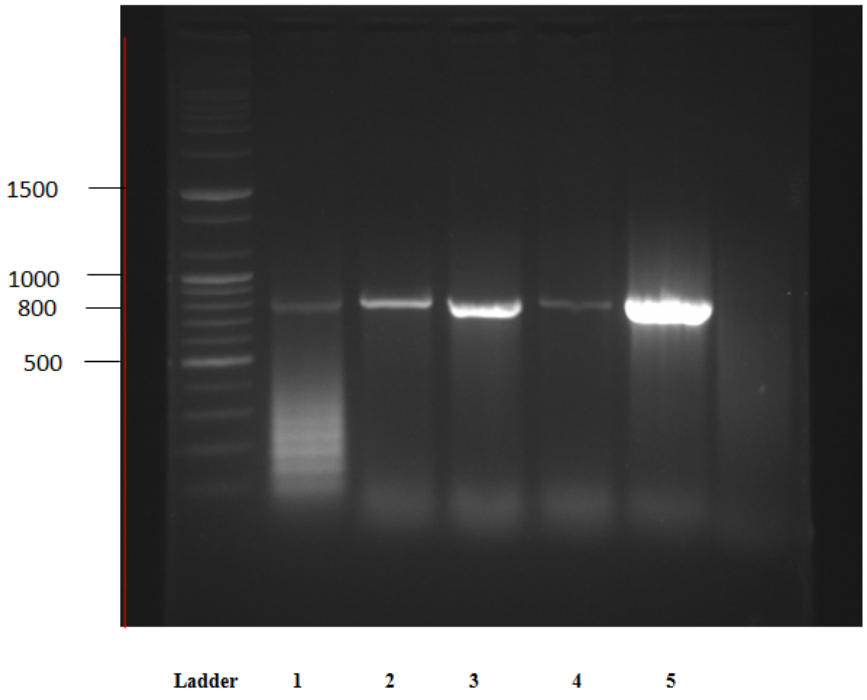

Plate 1. Agarose gel electrophoresis showing various bands of amplicons with 1500 bp ladder

Table 2. Percentage incidence of *C. jejuni* in chicken meat and faecal samples in local government areas within Kano metropolis

Sample	Total number of sample examined	Total number positive (%)
Chicken faeces	180	113(37.66)
Roasted chicken meat	60	5(1.66)
Raw chicken meat	60	24(8.00)
Total	300	142(47.3)

Key: Values in parenthesis ( ) are percentages

Table 3. Distribution of *Campylobacter jejuni* in chicken's meat and faecal samples within the local government areas of Kano metropolis

Sampling area	Faecal sample n = 30	Roasted chickens meat n = 10	Raw chicken's meat n = 10	Total n = 50
Fagge	20 (66.6)	1 (10)	5 (50)	26 (52)
Nassarawa	19 (63.3)	1 (10)	4 (40)	24 (48)
Tarauni	21 (70)	1 (10)	3 (30)	25 (50)
Dala	20 (66.6)	0 (0)	3 (30)	23 (46)
Gwale	15 (50)	1 (10)	5 (50)	21 (42)
Ungogo	18 (60)	1 (10)	4 (40)	23 (46)
	113 (62.7)	5 (8.3)	24 (40)	142 (47.3)

Key: Values in parenthesis ( ) are percentages

Table 4. DNA nucleotide sequence of *C. jejuni*

Target gene	Sequence (5'-3')	PCR product
Dideoxynu deoxynucleotide subunit (*C. jejuni*) Biotype 1	06E CTCCTCGCCCGCACACCACACTCG CACCGGGTTTTCAACGTATCAAAC ACACTTCGCCTGCGTCGATCAACA AGCTGTCGCCGCACCAGCACCACT GCACCCACGTACGTGCGACGCAA CCTCTCTGACTCTATCGTCACAGC ACACCCTCTCTCTATTTGCCGCCC ACGTCACGCTCGCAGCCTCGCAC GCACGCACAGACCTC	800 bp
Dideoxynu deoxynucleotide (*C. jejuni*) Biotype 2	10D AGTATATGGGCACACATACTAAAAA AAAAATTAAACCAAAAAATATACGA GCCCAAAAAAAAAGAATTTCAAAGT AATTATGTGCAAGGAAGGGACACG AAAATAGATACAAAGAATTAATAAA TGAAGTAGAGTATTAAGCCGACCT ATAATTGATGGCCACATAAATTCAA CCATCGATATAATGAACAGCACCC AAAG	800 bp

4. DISCUSSION

The presence of *Campylobacter jejuni* in chicken's meat and faeces has been established in this research work. The overall prevalence of *Campylobacter spp.* from this study was found to be 54%. *Campylobacter jejuni* was the most commonly isolated, with prevalence of 47.3%, while other *Campylobacters* prevalence was 6.7%. The prevalence rate in this study is lower (67.2%) than that obtained from indigenous Chickens in Sokoto State, Nigeria [6] but higher than the prevalence rate (7.2%) obtained from chicken broilers and layers in Vom, Plateau State, Nigeria [16]. It is most likely that the high level of hygienic standard maintained in the environment accounts for the low prevalence rate recorded from the Chicken broilers sampled in Vom. The free-range nature of rearing the Chickens exposes them to both human and animal wastes and other sources of enteric pathogens. The high isolation rate in this study is in agreement with reports from other studies obtained from broilers chicken's, 46% by Atanassova and Ring [17] in Germany, 50% by Salihu et al. [6] in Sokoto State. The obtained prevalence (47.3%) could be due to poor sanitary standard observed in feeding, housing and the environment where the Chickens are kept.

From this research, it was observed that, the occurrence of *Campylobacter jejuni* in roasted Chicken meat was relatively lower (8.3%) as compared to that of raw Chicken meat which was found to be 40%. The reason for this disparity was because, the roasted Chicken meat had

been subjected to various treatment including moist heat at a very high temperature during cooking. Hence, the organism is expected not to survive such treatment and must have been killed. *C. jejuni* occurrence in roasted meat was likely due to improper cooking and post processing contamination as a result of unsafe handling and sanitation practices. Consumption of contaminated foods is of significant public health implications, as it could lead to outbreak of food-borne diseases in a community. *C. jejuni* infection is mainly transmitted through consuming raw or undercooked meat, poultry or shell fish. Unsafe food handling and sanitation practices in Kitchens, food processing plants, farms and retail establishments can also transmit the infection [18]. On the other hand, the raw chicken meat, having a higher occurrence of *C. jejuni* justifies the fact that the meat has been faecally contaminated during washing as the organism is found in intestinal tracts of Poultry birds [2].

*Campylobacter jejun*i frequently contaminates raw chicken meat. Surveys show that 20 to 100% of retail chickens are contaminated. This is not overly surprising since many healthy chickens habour the bacteria in their intestinal tracts [2]. In Nigeria, the indigenous Chickens are always in close contact with other Animals and Humans and considering the zoonotic nature of *C. jejuni*, it can be contracted through close contact with infected animals [6].

Therefore, proper sanitary check on food selling spots and poultry markets must be executed regularly in view of its public health significance as well as strict observance of personal hygiene by the handlers. This would prevent or minimize transmission of infection caused by the food-borne pathogen.

The quality control of phenotypic identification was carried out through random selection of few positive *C. jejuni* isolates for molecular analysis. From the result, 3 identified as *C. jejuni* were confirmed positive by PCR, while 2 isolates initially identified as negative for *C. jejuni* by phenotypic method were also confirmed negative by molecular identification.

Chi-square result indicated the calculated value (3.41) was lower than the table value (18.307) at 10 degree of freedom (df). Therefore, there is no significant difference in the isolation rates of *C. jejuni* at the different locations sampled.

5. CONCLUSION

Based on this research work, the incidence of *Campylobacter jejuni* in Chickens meat and faeces is high (47.3%). The isolation of *C. jejuni* from roasted chicken's meat is of a serious public health importance, because consumption this type of food has been implicated as the cause of sporadic infections and outbreaks of Campylobacteriosis worldwide.

6. RECOMMENDATIONS

Based on findings of this study and the public health implication, the following are suggested:

1. Further research work should be carried out about incidence of *Campylobacter jejuni* in humans.
2. Clinicians should include among other relevant Laboratory investigations, the test for *Campylobacter jejuni* in their routine Laboratory request.
3. Government and individuals should intensify public enlightenment on proper and aseptic methods of handling processed chickens in order to avoid contamination.
4. Poultry farmers and markets should be enlightened about proper disposal of Chicken's faeces and other waste in order to avoid contamination of our environment and other foods by the organisms.
5. Infected individuals and animals should be properly diagnosed and treated properly to avoid transmission to others.

There should be regular inspection of processed chickens and other foods marketing spots by National Agency for Food, Drug Administration and Control (NAFDAC) in order to examine the sanitary quality of their product and disciplinary measures should be allotted to those found wanting.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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