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Occurrence of *Campylobacter* species in chickens reared in Maiduguri, Nigeria, and their antibiotic susceptibility patterns

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Abstract

Campylobacter species are one of the foremost causes of foodborne gastroenteritis globally, often linked to poultry products. The present study determined the occurrence and antibiotic susceptibility patterns of *Campylobacter* species isolated from layers and broilers sampled at local market abattoirs in Maiduguri, Nigeria. Four hundred intestinal tracts eviscerated from layers (n = 200) and broilers (n = 200) were collected and processed using standard microbiological methods for isolation of *Campylobacter*. Presumptive *Campylobacter* isolates were confirmed using biochemical tests, and species identification was performed using polymerase chain reaction (PCR) technique. Antibiotic susceptibility testing was performed using Kirby-Bauer disc diffusion method. The overall occurrence of *Campylobacter* species in the sampled chickens was 33.1% (125/400), with *Campylobacter coli* (17.5% = 70/400) being more frequently isolated than *Campylobacter jejuni* (13.8% = 55/400). The occurrence of *Campylobacter* species was higher in layers (33.5% = 67/200) than broilers (29.0% = 58/200), though the difference was not statistically significant (p = 0.3315). PCR results showed amplicon sizes of 735 base pairs (bp) and 500 bp for *hippurase* genes and *aspartokinase* genes of *Campylobacter jejuni* and *Campylobacter coli,* respectively. The *Campylobacter* isolates from both the layer and broiler chickens showed remarkable resistance to ciprofloxacin (71.6% vs. 79.3%), tetracycline (98.5% vs. 94.8%), ampicillin (97.0% vs. 93.1%), nalidixic acid (65.7% vs.72.4) and erythromycin (64.2% vs. 48.3%). However, the isolates, exhibited susceptibility to chloramphenicol (88.1% vs. 89.7%) and gentamicin (77.6% vs. 89.7%). Intermediate resistance rate, in a range of 4.5% to 13.4% was observed among the isolates. The study revealed a fairly high occurrence of *Campylobacter* spp. in the layers and broilers sampled in the study area, and a high level of resistance of the isolates to some antibiotics. These findings emphasize the need for improved biosecurity measures in poultry production and marketing systems, as well as judicious antibiotic use in poultry farms in Maiduguri, Nigeria. Further researches are recommended, to address foodborne illnesses in Maiduguri, Nigeria.

Keywords: *Campylobacter* species; Occurrence; Chickens; Antibiotic susceptibility patterns; Maiduguri, Nigeria.

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Introduction

Campylobacter species are microaerophilic Gram negative short curved or spiral rods that are commensals in the intestinal tracts of mammals and birds (Osimani *et al*., 2017; Varga *et al*., 2019). They are one of the leading causes of bacterial foodborne illness worldwide (EFSA, 2013). Poultry, particularly chickens, has been documented as reservoirs of the pathogen and important source of human infection (Ma *et al*., 2017).

Campylobacter species are transmitted to poultry by wild birds, insects, pets, farm workers and contaminated equipment (EFSA, 2013; Ma *et al*., 2017). They are adapted to living in poultry, because the high body temperature of birds creates conducive environment for their growth (Ma *et al*., 2017). *Campylobacter* resides in intestinal tracts of birds, in symbiotic association, without harm (Ma *et al*., 2017). *Campylobacter jejuni* and *Campylobacter coli* are the major species frequently associated with chicken contamination and human infection (Varga *et al*, 2014). Other species of *Campylobacter* including, *C. lari, C. upsaliensis,* and *C. hyointestinalis* have also been reported in birds, but are less frequently associated with infection (Akosua *et al*., 2017).

Inadequate sanitation, suboptimal hygiene, poor biosecurity in poultry industry and marketing systems had been documented as significant risk factors for *Campylobacter* contamination of poultry products (Mageto *et al*., 2014). A rupture of the intestinal tracts during evisceration have been associated with substantial contamination of carcass surfaces and increased risk of illness in humans (Ma *et al*., 2017). Studies indicate that about 50% to 70% of *Campylobacter* infections are acquired through the consumption and mishandling of raw poultry products, especially chickens (Nwankwo *et al*., 2016; Igwaran *et al*., 2019).

Campylobacter species cause mild to severe diarrhoeal illness, often accompanied with high fever and abdominal cramps (Akosua *et al*., 2017). The enteric infection sometime can give rise to long-term complications, such as reactive arthritis, irritable bowel syndrome and Guillain-Barre Syndrome (Igwaran *et al*., 2019).

The increasing demand for poultry meat in developing countries, such as Nigeria, has led to the widespread use of antimicrobial agents in poultry production to boost growth, leading to higher antibiotic consumption and development of antibiotic resistance by foodborne pathogens, like *Campylobacter* (Akosua *et al*., 2017; Ma *et al*., 2017). Consumption of chicken products with antibiotic-resistant *Campylobacter* strains can lead to infections that are harder to treat.

Studying the occurrence of *Campylobacter* in chicken helps to assess possible risk of human infections. In Nigeria, data on the epidemiology and antibiogram of chicken *Campylobacter* strains is scanty. This study investigated the occurrence and antibiotic susceptibility patterns of *Campylobacter* species in layers and broilers sampled in Maiduguri, Nigeria.

Materials and Methods

Study Design/Study Area: The design of the study was cross-sectional survey, conducted between February 2017 and July 2019 to determine the prevalence of *Campylobacter* species in slaughtered layers and broilers in poultry abattoirs at Kasuwa Shanu, Bulukutum Kasuwa, Custom Market and Abbaganaram Market in Maiduguri Metropolis, Borno State, Nigeria. Maiduguri is the capital of Borno State in northeastern Nigeria. The town is situated in the semi-arid zone with an area of approximately 69,436 km^2. The city is located on latitude 11^o.48' N to 11^o52'N and longitude 13^o.15 E to 12^o12E. Maiduguri city experiences both hot and dry climate most period of the year, with a rainy season ranging from late June to early October. The mean

annual rainfall is around 600 – 650 mm, with an average annual temperature of about 32°C. The relative humidity ranges from 13% in the driest months (February and March) to 70 – 80% during the peak of wet season (July and August). The population of the city is about 1.2 million people according to the 2019 population estimate (NPC, 2019). The primary occupation of the people is agriculture, trading and livestock herding, making it a hub for livestock and poultry production in the northeast zone of Nigeria. Poultry farming and trading in Maiduguri is majorly informal, creating greater opportunities for the spread of zoonotic pathogens, like *Campylobacter*.

Ethical clearance for the study was obtained from the University of Maiduguri, Maiduguri Institutional Review Board, with Ethical Certificate Reference No. UM/RCS/01/2715/2017.

Sample size estimation: Sample size was determined using the Kish (1965) formula. A previous *Campylobacter* prevalence rate of 30% in chicken (Nwankwo *et al*., 2016) was used. The estimated sample size was 323, but was rounded off to 400.

Sample collection and transportation: A total of 400 intact intestinal tract samples were collected from eviscerated layers (n = 200) and broilers (n = 200) from various poultry abattoirs sampled. Samples were placed in isothermal cool boxes with ice packs and transported within 2 – 4 hours of collection to the Department of Veterinary Microbiology Laboratory, University of Maiduguri, for analysis.

Isolation and Identification of *Campylobacter* species: Each intestinal tract was open with a sterilized lancet. One gram of the intestinal contents was collected using a spatula into a *Campylobacter* enrichment broth with cefoperazone, vancomycin, trimethoprim and natamycin (IDG Ltd, UK). The enrichment culture was incubated at 42°C for 24 hours under microaerophilic conditions (12% CO_2, 3% H_2, 11% O_6, 74% N_2) provided using GENbag microaer (BioMérieux- France). *Campylobacter* selective agar (LAB M Ltd, UK) containing cefoperazone and vancomycin was inoculated with 100 µl of the enrichment culture. The inoculated plates were incubated at 42°C for 48 hours in microaerophilic conditions generated as described above. After the incubation, colonies that grew on *Campylobacter* selective agar were examined on the basis of colony morphology, typical cell morphology and motility (by handing drop method). Subsequent to sub-culture of the isolates on Columbia blood agar plates (Oxoid code CM331), oxidase and catalase tests with hippurate hydrolysis tests were carried out on the isolates for further identification, and polymerase chain reaction was performed for confirmation and speciation of the isolates (Gharst *et al*., 2013).

DNA Extraction: Genomic DNA extraction from isolated *Campylobacter* strains was performed using the phenol-chloroform methods (Sambrook, *et al*., 1996). *Campylobacter* colonies on blood agar plates were scooped into 1.5 ml Eppendorf tube containing 400 µl lysis buffer and 10 µl proteinase, incubated at 56°C for 1 hour and vortexed at 20-minute interval. The supernatant was discarded and the deposits mixed with 400 µl of phenol-chloroform. The mixture was vortexed and centrifuged at 14,000 revolutions per minute (rpm) for 10 minutes. The supernatant was again removed and mixed with 400 µl of chloroform, vortexed and spun at high speed as above. The lysate was mixed with equal volumes of 3 M sodium acetate and 100% cold ethanol and incubated at 20°C for 24 hours. Following incubation, the mixture was spun at 14,000 rpm for 10 minutes in a refrigerated centrifuge. The upper layer was removed, and 200 µl of 70% alcohol was added to the residues and spun at 14,000 rpm for 4 minutes at 4°C in a refrigerated centrifuge. Finally, the upper layer was removed, and the DNA pellets were dried

at room temperature and re-suspended in 30 µl of sterile water.

Molecular Identification of *Campylobacter* spp.: The multiplex PCR was used for the confirmation of C. *jejuni* and *C. coli* colonies recovered using phenotypic methods. Genotyping was performed by a slight modification of techniques described by Wang *et al*., (2002). Two genes for identification of *Campylobacter jejuni* and *Campylobacter coli* were used for amplification: hipO (encoding *C. jejuni* hippurase) and AspK (encoding *C. coli* aspartokinase). The primers used for gene amplification were HIP 400 F'5 GGA GAG GGT TTG GGT GGT G-3' and HIP 1134 R 5' AGC CGC ATA ATA ACT TAG CTTTG-3' and asp CC18 (F 5' GGT ATG ATT TCT ACA AAG CGA G-3' and CC519 R 5' ATA TAT CGT CGG GTG AAAGAC-3'. PCR was performed in a final volume of 20 µl containing 2x Tag premix PCR mastermix (Bioneers, Korea), 1 µl of forward primer, 1 µl reverse primer, 2 µl template DNA and 14µl deionized water min. Amplification reactions were performed in thermal cycler (TC-100 Peltier; MJ Research, California, USA). The amplification conditions were initial denaturation at 96 °C for 3 minutes followed by 35 cycles of denaturation at 96 °C for 30s, annealing at 55 °C for 30s, and polymerization at 72 °C for 30s. Final extension was performed at 72 °C for 5 minutes. The amplification generated 735 bp and 500 bp DNA fragment, conforming to *Campylobacter jejuni* and *Campylobacter coli,* respectively. The amplicons were stained with 0.6% solution of ethidium bromide and visualized under UV light after gel electrophoresis on 1.5% agarose. A 100 bp DNA marker (Bioneers, Korea) was used as marker for the PCR amplicons.

Antibiotic Susceptibility Test: Antibiotic susceptibility of *C. jejuni and C. coli* isolates was determined by the Kirby-Bauer disk diffusion method on Muller-Hinton agar supplemented with 5% defibrinated sheep-blood, according to Clinical and Laboratory Standards Institute (CLSI) guidelines for *Campylobacter* spp. (CLSI, 2016). Pure colonies from 48h microaerophilic cultures were suspended in sterile normal saline and turbidity adjusted to 0.5 McFarland standard. The suspension was swabbed onto Muller-Hinton places using a sterile cotton swab. Antibiotic discs, including ampicillin (25 µg), tetracycline (25µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), erythromycin (10 µg), chloramphenicol (30µg) and gentamicin (10 µg) were aseptically placed onto the inoculated plates using sterile forceps. Plates were incubated at 42^{o}C for 24 – 48 hours under microaerophilic conditions (5% O_2, 10% CO_2, 85%N_2) generated using Campygen Sachets (bioMérieux, France). Zones of inhibition were measured to the nearest mm using a ruler and interpreted as Susceptible, Intermediate, or Resistant, based on CLSI M45 breakpoints for *Campylobacter* species (CLSI, 2016). *C. jejuni* ATCC 111684 and *C. coli* ATCC 3246 were used as quality control strains.

Statistical Analysis: Data obtained in the present study were subjected to descriptive statistics using SPSS 20.0 software version. Chi-square test was used to determine associations, at 95% confidence interval.

Results

The occurrence of *Campylobacter* species in the intestinal contents of the chickens sampled is shown in Table 1. Out of 400 faecal contents analyzed, 125/400 (31.3%) was positive for *Campylobacter* spp. The occurrence rate of the organism was higher in layers (67/200; 33.5%) than broilers (58/200; 29.0%). However, there was no significant difference ($p < 0.05$) in occurrence rate between the two chicken types. C*ampylobacter coli* occurrence (70/400 = 17.5%) was higher compared to that of *C. jejuni* (13.8% = 55/400), though this difference was not statistically ($p > 0.05$) significant (Table 2).

The antibiotic susceptibility testing results of the 125 *Campylobacter* isolates are presented in Table 3. *Campylobacter* isolates from layer chickens showed high rate of resistance to ampicillin (97.0%), tetracycline (98.5%), ciprofloxacin (71.6%), nalidixic acid (65.7%) and erythromycin (64. 2%), while, isolates from broiler chickens exhibited high resistance levels to tetracycline (94.8%) ampicillin (93.1%), ciprofloxacin (79.3%), nalidixic acid (72.4%) and erythromycin (48. 3%). Majority of the isolates from both the layers and broilers were generally susceptible to chloramphenicol (88.1% for layers and 89.7% for broilers) and gentamicin (77.6% for layers and 89.7% for broilers) [Table 3]. Intermediate susceptibility rates among the isolates to different antibiotic classes ranged between 4.5 to 13.4% in layers and 3.4 to 6.9% in broilers (Table 3).

Table 1. Occurrence of *Campylobacter* spp. in intestinal contents of layers and broiler chickens sampled at poultry abattoirs in Maiduguri, Nigeria.

Poultry category	No of samples	No. of isolates identified	Prevalence (%)	P-value
Layers	200	67	33.5%	0.3315
Broiler	200	58	29.0%	
Total	400	125	31.3%	

Table 2. Distribution of the occurrence of species of *Campylobacter* among the intestinal contents of layers and broiler chickens sampled at poultry abattoirs in Maiduguri, Nigeria.

Poultry category sampled	No. of isolates Identified	*Campylobacter* species identified, with percentage out of the number of chickens sampled, in brackets.	
		C. jejuni	*C. coli*
Layer	67/200	30 (15.0%)	37 (18.5%)
Broiler	58/200	25 (12.5%)	33 (16.5%)
Total	125/400	55 (13.8%)	70 (17.5%)

Table 3. Antibiotic resistance and susceptibility patterns of *Campylobacter* species isolated from the intestinal contents of layers and broiler chickens sampled at poultry abattoirs in Maiduguri, Nigeria.

Antibiotic	Layers (n = 67)			Broilers (n = 58)		
	Percentage Sensitive	Percentage Intermediate	Percentage Resistant	Percentage Sensitive	Percentage Intermediate	Percentage Resistant
Ampicillin	3.0%	0%	97.0%	6.9%	0%	93.1%
Tetracycline	1.5%	0%	98.5%	5.2%	0%	94.8%
Ciprofloxacin	14.9%	13.4%	71.6%	15.5%	5.2%	79.3%
Nalidixic Acid	34.3%	0%	65.7%	20.7%	6.9%	72.4%
Erythromycin	22.4%	13.4%	64.2%	46.3%	5.2%	48.3%
Chloramphenicol	88.1%	4.8%	7.5%	89.7%	3.4%	6.9%
Gentamicin	77.6%	4.5%	17.9%	89.7%	1.7%	8.6%

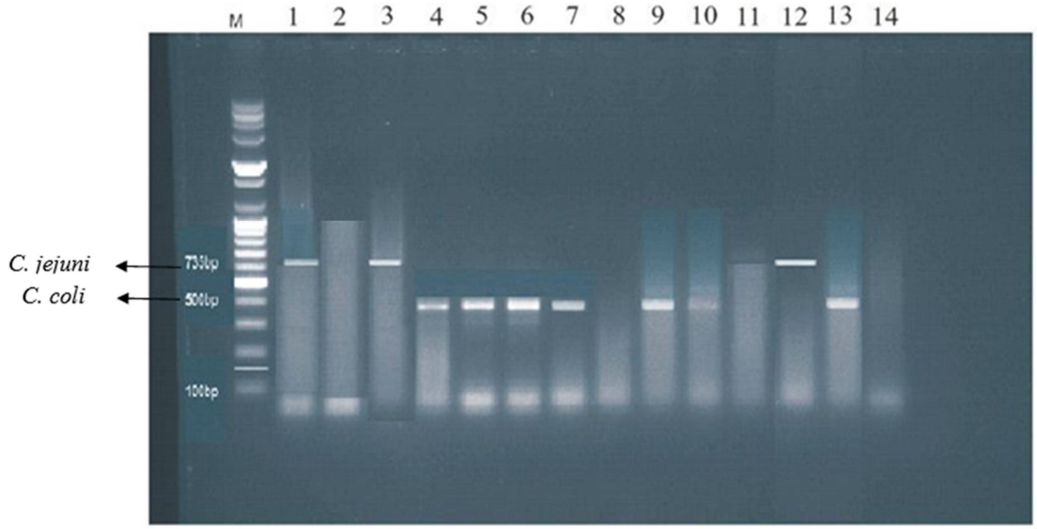

Figure 1. Gel picture of multiplex PCR detection of *C. jejuni* and *C. coli* from intestinal contents of layers and broiler chickens sampled at poultry abattoirs in Maiduguri, Nigeria. Lane M 100 bp ladder as DNA marker: Lanes 1 and 13 are positive controls for *C. jejuni* and *C. coli* respectively, while Lane 14 is negative control for both species. Lanes 4, 5, 6, 7, 9 and 10 are positive for *C. coli,* showing specific bands at 500 bp. Lanes 3 and 12 are positive for C*. jejuni* showing specific bands at 735 bp. Lane 2, 8 and 11 are negative for both *C. jejuni* and *C. coli*.

Molecular analysis of 65 representative isolates selected from the total culture-positive isolates (52% of 125) showed distinct amplicons at 735 bp and 500 bp (Figure 1), corresponding to the expected sizes of *Hip*O and *Asp*K genes in the reference strains *C. jejuni* ATCC 111684 and *C. coli* ATCC 3246, respectively.

Discussion

Overall, findings of this study highlight the significant burden of *Campylobacter* species in layer and broiler chickens sampled in Maiduguri, Nigeria. The overall occurrence of 31.3% recorded in this study concurs with earlier reports by Nwankwo *et al*. (2016), who reported 30% occurrence in broiler chickens in Sokoto, northwest Nigeria. Occurrences lower than the one obtained in this study has been reported elsewhere: 13.7% in China (Ma *et al*., 2017), and 21.6% in Egypt (Awadallah *et al*., 2014). In contrast, higher occurrence of 63.8% has been reported in Cote d'Ivoire (Goualié *et al*., 2012), 64.1% in Ecuador (Vinueza-Burgos *et al*., 2017) and 87.2% in Poland (Wieczorek and Osek, 2013).

The relatively higher occurrence recorded in this study among layers when compared to the broilers, may be attributed to the longer rearing period of layers with the consequent exposure risk to infection, while broilers with shorter lifespans has less time to acquire and spread the bacteria. The higher occurrence in layers than broilers, suggests that eggs may play a role in the spread of *Campylobacter* to humans.

Our finding of high occurrence of *C. jejuni* and *C. coli*, in the present study, aligned with the global trend that linked these two *Campylobacter* species with chicken contamination (EFSA 2013). The relative dominance of *Campylobacter coli* over *C. jejuni* observed in this study concurs with reports by

Vinueza-Burgos *et al*. (2017), but differed from the reports of *C. jejuni* as foremost chicken contaminant (Messad *et al*. 2014; Akosua *et al*., 2017). *Campylobacter coli* has been more recognised as the second most isolated species in chicken (Akosua *et al*., 2017; Bouhamed *et al*., 2018). The predominance of *C. coli* in the present study, suggest that it might be more adapted to semi-arid climate than *C. jejuni* strains.

The high occurrence of resistance to tetracycline and ampicillin, among *Campylobacter* from layers and broilers in this study corroborates reports in Algeria (Mouffok, *et al*. 2013), which found high resistance of *Campylobacter* isolates to commonly used antibiotics in treatment of poultry infections, but contradicts reports from Côte d'Ivoire (Goualié *et al*. 2012), which showed high resistance of chicken *Campylobacter* isolates to amoxicillin and tetracycline Our observation of high occurrence of resistance to nalidixic acid and ciprofloxacin contrasted the lower rate of quinolone-resistance in a similar study in Algeria (Messad *et al*. 2014). The higher susceptibility of the isolates to chloramphenicol and gentamicin may be explained by restricted use of these drugs in poultry farming (Goualié *et al*., 2012). The resistance rate to macrolides recorded in the present study, did not agree with the reports by Bouhamed *et al*. (2018), which showed low levels of erythromycin and azithromycin resistance in chicken. The higher occurrence of resistance to ampicillin, tetracycline and erythromycin, recorded for the *Campylobacter* isolates from layers compared to broiler strains concurs with report by Adiguzel *et al*. (2018), while the higher frequency recorded for *Campylobacter* isolates obtained from broilers when compared to layers against ciprofloxacin and nalidixic acid, agreed with the resistance profile to fluoroquinolones reported in chicken elsewhere by Olufemi and Oluseye (2016). The intermediate resistance to ciprofloxacin, tetracycline and ampicillin recorded from the layer and broiler isolates in the present study, suggest a potential for emergence of resistant *Campylobacter* strains. A similar study by Akosua *et al*. (2017) in Ghana also found intermediate antibiotic resistance rates in chicken isolates. The high resistance rates to certain antibiotics by isolates from the chicken sampled in this study may be attributed the sale of antibiotics over-the-counter and lax enforcement of antibiotic regulation. This observation highlights the need to strengthen antibiotic use regulation in both veterinary and human medical practice in Nigeria. The pattern of antibiotic resistance exhibited by *Campylobacter* isolates in this study underscores the importance of professional veterinary prescription to guide antibiotic use in animal husbandry.

The identical band sizes to reference strains *C. jejuni* and *C. coli* in this study implied that phenotypic identification was reliable. The 735 bp and 500 bp amplicons obtained in this study concurs with report by Frasao *et al.,* (2017) which showed amplicons sizes at 735 bp for *C. jejuni* and 500 bp for *C. coli*, and also aligns with molecular data reported by Banowary *et al*., (2015) regarding species-specific band sizes for *C. jejuni* and *C. coli*. The high level of agreement observed here validates reliability of culture and biochemical tests for preliminary identification of *Campylobacter* species in poultry.

Conclusion: This study showed a high (33.1%) overall occurrence of *Campylobacter* species in layers and broilers sampled at poultry abattoirs in Maiduguri Nigeria, with *Campylobacter coli* (17.5%) being more frequently isolated than *Campylobacter jejuni* (13.8%). The *Campylobacter* isolates exhibited remarkable resistance majorly to ciprofloxacin, tetracycline and ampicillin, but were highly susceptible to chloramphenicol and gentamicin. These results emphasize the need for enhanced biosecurity measures in poultry production and marketing systems as well as

enactment of regulations that will ban use of medically important antibiotics in animal husbandry. Further researches are necessary to address food safety concerns in Maiduguri, Nigeria.

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Conflict of interest

The authors declare that they have no conflicts of interest

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