



## Antibiotic Susceptibility Profile of *Campylobacter jejuni* Isolated from Chicken Meat and Faeces in Kano Metropolis, Kano State, Nigeria.

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### Abstract

This study was conducted to determine the susceptibility of *Campylobacter jejuni* isolated in chicken's meat and faeces to commonly available of antibiotics in Kano metropolis, Kano State, Nigeria. Isolation of *C. jejuni* was carried out by culture method followed by biochemical and molecular tests for identification of the isolates. The antibiotic susceptibility profile of the isolates was done using the Disc Diffusion Technique. Out of 142 (47.3%) *C. jejuni* isolates, all (100%) isolates were resistant to Augmentin, Cefixime, Ceftazidime and Cefuroxime, however, 140 (98.5%), 138(97.2%) and 27(19%) of the isolates were sensitive to Ciprofloxacin, Ofloxacin and Nitrofurantoin respectively. The result confirmed Ciprofloxacin and Ofloxacin as the drugs of choice in the treatment of infections caused by *C. Jejuni*.

**Key words:** *Campylobacter jejuni*, Chicken meat, Chicken faeces, Kano metropolis, Antibiotics.

### Introduction

*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. The bacterium is commonly associated with poultry, and it naturally colonises the digestive tract of many bird species.<sup>[1]</sup> One study found that 30% of European starlings in farm settings were carriers of *C. jejuni*.<sup>[2]</sup> *C. jejuni* is also common in Cattle, although it is normally harmless commensal of their gastro intestinal tract.<sup>[1]</sup> The incidence of *C. jejuni* in 6 local government areas of Kano metropolis has been reported to be 47.3% in a previously related publication by same authors.<sup>[3]</sup>

Campylobacteriosis is an infection by *Campylobacter*. The common routes of transmission are faecal oral, ingestion of contaminated food or water and eating of raw meat. Infection can also be acquired by contact

with infected animal or animal products.<sup>[4]</sup> *C. jejuni* is susceptible to gastric acid, and ingestion of about 10<sup>5</sup> organisms is usually necessary to produce infection. This inoculum is similar to that require for *Salmonella* and *Shigella* infection but less than that for *Vibrio* infection.<sup>[4]</sup>

The organisms multiply in the small intestine, invade epithelium, and produce inflammation that results in the appearance of red and white blood cells in the stools. Occasionally, the blood stream is invaded and a clinical picture of enteric fever develops.<sup>[4]</sup>

The sites of tissue injury include the jejunum, the ileum, and the colon. Most strains of *C. jejuni* produce a toxin (Cytolethal distending toxin) that hinders the cells from dividing and activating the immune system. This helps the bacteria to evade the immune system and survive for a limited time in the cells.<sup>[5]</sup>

Cholera like enterotoxin was once thought to be also made, but this appears not to be the case. The organism produces diffuse, bloody, edematous, and

exudative enteritis. Although, rarely has the infection been considered a cause of Haemolytic Uraemia Syndrome and thrombotic thrombocytopenic purpura, no unequivocal case report exist. In some cases, a *Campylobacter* infection can be underlying cause of Guillian–Barre Syndrome. Gastrointestinal perforation is a rare complication of ileal infection.<sup>[5]</sup>

Few years ago, the antimicrobial therapy indicated for *Campylobacter* infection were fluoroquinolones. However, in the past few years, a rapidly increasing proportion of *Campylobacter* strains all over the world have been found to be fluoroquinolone resistant, including resistant to many other choice antibiotics.<sup>[6]</sup>

Ciprofloxacin is a broad-spectrum antibiotic of the fluoroquinolone class. It is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase and a type II topoisomerase and topoisomerase IV, necessary to separate bacterial DNA, thereby inhibiting cell Division.<sup>[7]</sup> Ciprofloxacin for systemic administration is available as immediate release tablets, extended release tablets, oral suspension and as a solution for intravenous infusion.<sup>[8]</sup> The drug rapidly distributes into tissues, with levels in tissues exceeding those in serum. Penetration into the central nervous system is relatively modest, with cerebrospinal fluid levels normally less than 10% of peak serum concentrations. The serum half life of Ciprofloxacin is about 4-6 hours, with 50-70% of administered dose being excretion in urine as metabolites. Urinary excretion is virtually complete 24 hours after administration. Dose adjustment is required in elderly and those with renal impairment.<sup>[8]</sup>

The fluoroquinolone class of drug is about 70% when administered orally, so a slightly higher dose is needed to achieve the same exposure when switching from I.V to oral administration. A 750mg immediate- release oral tablet given every 12 hours produces about the same area under serum concentration curve (AUC) and peak plasma concentration ( $C_{max}$ ) as a 400mg dose given every 8 hours I.V (US FDA, 2009). Maximum serum concentrations are achieved between 1 and 4 hours after administration. The 500mg and 1000mg tablets provides higher  $C_{max}$  compared to 250mg but 24 hours AUCs are equivalent.<sup>[9]</sup>

The aim of this research is to test for the susceptibility of *Campylobacter jejuni* to profile of antibiotics with a view to determining the most active amongst them.

## Materials and Methods

### Study Area

The study area for this research work was within and around Kano metropolitan City comprising of six local governments. The local governments are Tarauni, Fagge, Gwale Nassarawa Dala and Ungogo Local Government Areas. Kano is located in North West geo-political Zone of Nigeria. It lies on latitude 12°N and longitude 9°E at a distance of 442Km from Abuja, the FCT, 1150 Km from Lagos, the biggest Industrial and commercial nerve centre of the Country, which is 615Km from Lake Chad. The area is the commercial nerve centre of northern Nigeria.<sup>[10]</sup>

### Collection of Sample

Samples for this work are roasted chicken's meat, raw chicken's meat and Chicken faeces. A total of 300 samples was collected and screened during this study. The sample collection and analysis were carried out for a period of 7 months, between July, 2014 and January, 2015. Three hundred (300) samples, comprising 180 broiler chicken faeces, 60 roasted chicken meat and 60 raw chicken meat were collected from poultry markets across the six (6) local government areas located within Kano metropolis. Thirty (30) faecal samples, 10 raw chicken's meat and 10 roasted chicken meat were collected from each local government during sampling period. A pea size amount of chicken 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 sterilised sample bottles. The samples collected were then protected from light, packed in ice box and immediately transported to the laboratory for analysis.<sup>[11]</sup>

### Isolation and Identification of *Campylobacter jejuni* from Samples

The procedure for isolation of *C. jejuni* and subsequent identification by biochemical (catalase, oxidase, hippurate hydrolysis and motility tests) and molecular (DNA extraction, Polymerase Chain Reaction, electrophoresis and sequencing) techniques is comprehensively reported in another published work by the same authors.<sup>[3]</sup>

### Standardization of Inoculum

Inoculum for the test was prepared and standardized by McFarland Turbidity Standard Method. The turbidity standard was prepared by mixing 99.5ml of 1% V/V Sulphuric acid and 0.5ml of 1.175% of W/V Barium chloride (BaCl<sub>2</sub>.H<sub>2</sub>O). It was mixed and dispensed in 4mls amount into a test tube. The absorbance of 0.5 McFarland standards was obtained spectrophotometrically. The expected bacterial population of 0.5 McFarland standards is 1.5 x 10<sup>8</sup>/ml. Two milliliters (2mls) of sterile 0.9% normal saline (Dana Pharmaceuticals 30346B) was dispensed to each test tube labelled in corresponding with the culture isolate number to be tested. With the aid of sterile wire loop, each of the isolate was introduced into corresponding tubes, emulsified and mixed until homogeneous solutions of the inoculum was formed. The inoculum was standardized by comparing its turbidity with that 0.5 McFarland Standard to ensure uniformity.<sup>[12,13]</sup>

### Antibiotic Susceptibility Testing

One hundred and forty two (142) cultured isolates of *Campylobacter jejuni* were subjected to profile of antibiotics by Agar Diffusion Method, in order to test for their susceptibility. Following the standardization of

inoculum, the Nutrient agar plates were inoculated aseptically with the test organism. With the aid of sterile forcep, commercially prepared standard Antibiotic discs consisting of Augmetin (25µg), Cefuroxime(30µg), Ciprofloxacin (5µg), Cefixime (30µg), Ceftazidime (30µg), Gentamycin (10µg), Nitrofurantoin (30µg) and Ofloxacin (5µg). Discs of antibiotics (Abtek MK06/PB) were placed aseptically on the agar plate inoculated with test organism. The sensitivity plates were incubated under microaerophilic condition at 42°C for 24 hours. This was achieved by placing the plates in anaerobic incubation jar without Catalyst. The gas was produced with a commercially available CO<sub>2</sub> gas generating pack (Lobal Chemie C10067). Incubation at 42°C is to prevent growth of most of the other bacteria present in the foods. After overnight incubation, the plates were examined for zones of inhibition. The zone diameter measured in millimetres (mm) and compared with the Standard interpretative zone diameter provided by National Committee for Clinical and Laboratory Standard <sup>[14]</sup> (NCCLS, 2005). Interpretation was made as sensitive or resistant. The zone diameter measured is roughly proportional to the sensitivity of the test organism to the Drug.<sup>[15]</sup>

### Results

The inhibition of growth appears as a circular zone of inhibition on the agar plate. From the antibiotic susceptibility testing result, Ciprofloxacin with 98.5% activity and Ofloxacin showing 97.2% activity are the most effective drugs for treatment of *C. jejuni* infection, while Nitrofurantoin (19%) and Gentamycin (4.9%) are least effective for treatment of Campylobacteriosis (Table 1).

**Table 1: Antibiotic Susceptibility Profile of *C. jejuni* Isolates**

Antibiotics Profile	Disc concentration (µg)	Sensitive No. (%)	Resistant No. (%)
Augmetin	25	0(0)	142(100)
Cefuroxime	30	0(0)	142(100)
Gentamycin	10	7(4.9)	135(95)
Ciprofloxacin	5	140(98.5)	2(1.4)
Nitrofurantoin	30	27(19)	115(81)
Ofloxacin	5	138(97.2)	4(2.8)
Ceftazidime	30	0 (0)	142(100)
Cefixime	30	0 (0)	142(100)

## Discussion

From the antibiotic susceptibility testing result, Ciprofloxacin with 98.5% activity and Ofloxacin showing 97.2% activity are the most effective drugs for treatment of *C. jejuni* infection, while Nitrofurantoin (19%) and Gentamycin (4.9%) are not effective for treatment of Campylobacteriosis. This is in agreement with the fact that *C. jejuni* is generally susceptible to fluoroquinolones as indicated by Wang<sup>[6]</sup>. Likewise, a study carried out on 125 *C. jejuni* and *C. coli* isolated from poultry in South-East Queensland region, Australia shows that, none of the isolates was resistant to Ciprofloxacin and Nalixidic acid.<sup>[16]</sup> Although, *C. jejuni* infections are self-limiting and do not require treatment, antimicrobial therapy is required for severe infections.<sup>[17]</sup> Therefore, from this research work, Ciprofloxacin and Ofloxacin antibiotics could be used for empirical treatment without waiting for the result of stool culture.<sup>[6]</sup> But the drugs should be used with caution due to their side effects. Moreover, the test Organism showed complete resistance to the remaining four antibiotics comprising of Augmetin, Cefuroxime, Cefixime and Ceftazidime. This implies that none of the aforementioned drugs would

be suitable for treatment. Resistant to Cephalosporins have been established in previous related studies, an incidence of such was reported at Canada by Karmali<sup>[18]</sup> in which the drugs showed little activity against *C. jejuni* isolates. It was also discovered that 92% of the same isolates produced beta-lactamase enzymes<sup>[18]</sup>. Similarly, it was observed in a study of *in-vitro* activities of 12 beta-lactam agents against 100 thermophilic *Campylobacter* strains that beta-lactamase production was detected in 88% of all the strains tested.<sup>[19]</sup>

## Conclusion

The antibiotic susceptibility testing result, 98.5% of the *C. jejuni* isolates were sensitive to Ciprofloxacin, while 97.2% of the isolates were sensitive to Ofloxacin thereby indicating that, they are the most effective drugs for treatment of *C. jejuni* infection. The isolation of *C. jejuni* from roasted chicken meat is of serious public health importance, because consumption this type of food has been implicated as the cause of sporadic infections and outbreaks of Campylobacteriosis worldwide.

## Recommendations

Based on findings of this study, it is recommended that;

- i) *C. jejuni* testing should be included as part of routine analysis to be carried out in cases of gastroenteritis.
- ii) Ciprofloxacin and Ofloxacin should be the drugs of choice in cases of gastroenteritis due to *C. jejuni*.

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