

Detection and viability of *Campylobacter* species isolates from different species of poultry and humans in Sokoto State, Nigeria

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Abstract

Aim: The study was conducted to determine the prevalence and viability of *Campylobacter* species isolates from different species of poultry and humans in Sokoto State, Nigeria.

Materials and Methods: A cross-sectional study was performed in the live birds markets, humans on admission and at outpatient clinics in the randomly selected hospitals in Sokoto State. Isolation and characterization of *Campylobacter* species were performed using standard culture isolation techniques and biochemical characterization. A total of 798 (506 cloacal and 292 fecal) swabs from poultry and humans, respectively, were collected and analyzed. The viability of 307 isolates stored in 15% glycerol and 85% tryptone broth at -20°C was determined after 7-13 months.

Results: A total of 312 (39%) were positive for *Campylobacter* species which comprises 119 (30%), 20 (30%), 3 (14%), 9 (56%), 1 (50%), and 160 (55%) in chicken, guinea fowls, pigeons, ducks, turkey, and humans, respectively. The total of 38 (24%), 63 (39%), and 59 (37%) humans and 29 (19%), 79 (52%), and 44 (29%) poultry isolates were positive for *Campylobacter jejuni*, *Campylobacter Coli*, and *Campylobacter Lari*, respectively. A total of 261 (85%) of the stored isolates were still viable on re-isolation with the viability rates of 41 (95%), 67 (85%), and 17 (59%) at 7, 9, and 13 months of storage, respectively. There was a negative correlation between months of storage and viability rates. However, there was no significant statistical association ($p>0.05$) between prevalence rate and species of poultry.

Conclusion: *Campylobacter* species have been detected with varying degree of prevalence in both poultry and humans and their ability to survive freezing at -20°C (95%) for up to 7 months has been revealed in the study. This is not only a concern to food and livestock industries but also a concern to the public health at large, especially, in view of the study area being considered one of the largest livestock producers in Nigeria. *Campylobacteriosis* is known to be associated with the cost of gastroenteritis management, antimicrobial resistance, food contamination, and complications such as a paralytic condition called Guillain-Barre syndrome.

Keywords: *Campylobacter* species, humans, poultry, Sokoto, Nigeria.

Introduction

Campylobacter species are microaerophilic, non-fermentative, non-spore forming, Gram-negative, and oxidase-positive organism [1]. They are thought to be non-pathogenic to older birds and warm-blooded mammals as they serve as reservoirs of infection to other animals and humans [2]. They are found on the mucous membrane of the reproductive and gastrointestinal tracts of different species of birds [3]. Turkey is commonly colonized followed by geese [4,5], ducks, ostriches, quails, and wildbirds, especially migratory

ones such as cranes [6]. Ingestion of feed contaminated with fecal materials and ecological factors has been implicated in the infection in birds [7]. Infection may induce transient diarrhea, distention, or focal hemorrhage in the jejunum and mortality in day old chick [8]. *Campylobacter* spp. appear in rod shape on culture but can change to coccoid form which has been described as "viable but non-culturable" (VBNC) under unfavorable growth conditions [9]. This form cannot grow on normal medium, but the report has shown that they can infect animals and humans [9]. *Campylobacter* species are widely believed to be among the most common causes of acute bacterial enteritis in human worldwide [10]. There has been an increased frequency of isolation in many developed and developing countries [11,12]. Most of the infections have been linked to handling and consumption of contaminated water and food, which includes unpasteurized milk, meat, poultry, shellfish, fruits, and vegetable [13-15].

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Campylobacter species can survive in food at refrigerated temperature for 1-3 weeks, especially if foods are in air tight containers and for 2-4 weeks under moist, reduced oxygen condition at 4°C often outlasting the shelf life of the stored product [16]. Clinical features of infection through contaminated foods or drinks are usually similar across the different species with an incubation period of 2-10 days post-infection [17]. Fever, diarrhea, abdominal cramping, acute appendicitis, and inflammation of some parts of the ileum and jejunum with mesenteric adenitis may occur especially in teenagers or adults [18]. The most serious complication of *Campylobacter* infection is that of a paralytic condition called Guillain-Barre syndrome followed by Reiter's syndrome which includes arthritis, redness of the eye, and urinary tract signs [18]. The absence of biosecurity and sharing of the same environment with birds by humans, especially in the live bird markets, as observed in the study area, can contribute to *Campylobacter* infections in humans. Study elsewhere has revealed genetic similarity in *Campylobacter* isolates from pets, human clinical cases, and retail food isolates [19]. The ability of *Campylobacter* species to survive in more diverse environmental conditions underscores the importance of this type of study. Although Sokoto state is one of the largest livestock producers in Nigeria there is limited data on viability of *Campylobacter* species isolates from poultry species and humans.

This study was therefore conducted to determine the prevalence and viability of *Campylobacter* species isolates from different species of poultry and humans in Sokoto State, Nigeria.

Materials and Methods

Ethical approval

Ethical approval was obtained from the Research Ethical Committee of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto and the Ministry of Health, Usman Farouk Secretariat Sokoto before this study.

Study area

The study was carried out in Sokoto State, which is geographically located in the extreme northwest of Nigeria. It lies between the latitudes 12°N-58°N and longitudes 4.8°E-6.54°E with annual average temperature of 28.3°C. It has 4 agricultural zones and 23 local government areas (LGAs). It shares boundaries with Zamfara State to the East, the Republic of Niger to the North, and Kebbi State to the West. The State had a human population of 3.7 million people with a population density of 97.7 persons/km² and ranked second to Borno State in livestock production with estimated population of indigenous chicken at 3.4 million [20,21].

Visits to live bird markets

Visits were made to live bird market in each of the selected LGAs in the four agricultural zones

of state to seek approval and cooperation from the authorities of the market union and estimate the number of birds during the market days. For each of the live bird market, sampling was done once every 2 weeks to avoid repeat sampling as birds for sales are usually transported from one live bird market to another. Two out of every five birds counted were randomly sampled at each visit. In live bird market that has slaughtered slab/processing points, cloacal swabs were collected outside the 2 weeks that samples were routinely collected from the market to avoid sampling same birds twice at both sales and slaughter. Convenient sampling technique was used in fecal sample collection in humans with the use of labeled swab sticks which were given to volunteers indicating their sex and ages.

Sample size determination

The minimum sample size for this study was determined by the formula $N = Z^2 p(1-p)/d^2$ [22], where N=Sample size; Z=The score for a given interval which is 1.96 (S.E) at 95% confidence interval; p=known or estimated prevalence; and d=(5%) level of precision. Previous work in the study area obtained *Campylobacter* prevalence rate of 38.8% in birds [23] and 20% estimated in humans. These values were used for birds and humans, respectively. With the known prevalence, the minimum calculated sample size (N) required for the study in birds was $1.96^2 \times 0.39 \times 0.61 / 0.05^2 = 365$, and for humans was $1.96^2 \times 0.20 \times 0.80 / 0.05^2 = 245$. However, because of the low sample size and the increasing population of poultry and humans in the state, a total of 506 and 292 cloacal and human fecal swabs were taken, respectively.

Transportation and processing of samples

The samples were placed in Amies transport media (CMO425, Oxoid), kept cold with the use of ice block [17] and were transported to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto for analysis. Screening of samples for *Campylobacter* species was done using standard culture isolation and biochemical characterization. Samples were plated directly onto selective media; modified charcoal cefoperazone deoxycholate agar (mCCDA) (OxoidCM0739) and incubated at 42°C for 48 h under microaerophilic condition generated by Campygen® (Oxoid, BR0056) in the anaerobic jar [17].

Identification of *Campylobacter* species

Identification of colonies was based on characteristic features on mCCDA plates as creamy or white, moist, flat or slightly raised, extending along the streak line, or regular circular discrete colony [24]. A pure colony selected per sample was Gram-stained and different biochemical tests such as oxidase test, catalase test, and hydrogen sulfide (H₂S) production tests were used. These tests are described in the following sections.

Oxidase test

Oxidase papers were used to touch the isolates. A dark purple along the contact portion of the paper after few seconds of contact indicates a positive reaction. *Campylobacter* species are oxidase positive.

Catalase test

A loop full of pure culture was transferred from the agar onto the surface of a clean, dry glass slide. A drop of 3% hydrogen peroxide was immediately placed onto the colony on the slide. Effervescence indicates a positive reaction. *Campylobacter* species such as *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* and *C. hyointestinalis* are catalase positive while *C. upsailensis* is catalase negative.

Hydrogen sulfide (H₂S) production test

Hydrogen sulfide (H₂S) production test, characteristic colony from the selective medium, was touched with a straight inoculating wire. A tube of triple sugar iron (TSI) agar (LAB 53, Lab M. Limited) was inoculated by stabbing the middle of the agar to within 5 mm from the bottom of the tube and incubated at 37°C for 48 h with a loose cap on the TSI agar. Blackening of the medium indicates hydrogen sulfide production. *C. jejuni* and *C. lari* do not produce H₂S while *C. coli* produces H₂S. *C. jejuni* among the confirmed isolates was identified using the Hippurate hydrolysis test. A pure culture of the isolate was inoculated in 0.4 ml of 1% sodium hippurate substrate (1 g of sodium hippurate (Sigma) and 99 ml of distilled water) in a tube. The tube was then incubated for 2 h at 37°C and 0.2 ml of 2% ninhydrin solution (Sigma) were added and further incubated at 37°C for an additional 15 min. Color change from pale purple to deep purple or violet indicates hippurate hydrolysis. Sensitivity to cephalothin, nalidixic acid, and metronidazole was done using agar disc diffusion method on mCCDA to further differentiate among species [25]. Three to four loopful of the isolates in the culture were transferred into the storage medium (15% glycerol and 85% tryptone broth) and kept at -20°C for a period of 7-13 months before re-isolation.

Statistical analysis

The results obtained were presented in tables and percentages. Chi-square test was used to determine any significant statistical association between *Campylobacter* infection and species of poultry while

descriptive statistics was used to analyze the viability rates.

Results

Out of the 798 samples, a total of 312 (39%) were positive for *Campylobacter*. Among the different species of birds, 119 (30%), 20 (30%), 3 (14%), 9 (56%), and 1 (50%) were positive for chicken, guinea fowls, pigeons, ducks, and turkey, respectively while 160 (55%) were positive for humans (Table-1). In humans, 38 (24%), 63 (39%), and 59 (37%) were *C. jejuni*, *C. coli*, and *C. lari*, respectively. In chicken, the prevalence rates of 23 (19%), 62 (50%), and 34 (29%) were revealed for *C. jejuni*, *C. coli*, and *C. lari*, respectively. *C. coli* also had higher prevalence rate of 10 (50%) and 7 (78%) in guinea fowl and ducks, respectively, than other species such as *C. lari* which has 7 (35%) and 2 (22.2%) in the same species, respectively while *C. jejuni* had the prevalence rates of 3 (15%) and 1 (50%) in guinea fowl and turkey, respectively. In pigeon, 2 (67%) were *C. jejuni* and 1 (33%) was *C. lari* while *C. coli* was neither isolated from pigeon nor turkey (Table-1). There was no significant statistical association ($p > 0.05$) between infection rate and species of poultry. The total of 261 (85%) out of 307 isolates were still viable on re-isolation with the viability rates of 41 (95%), 67 (85%), and 17 (59%) after 7, 8, and 13 months of storage, respectively (Table-2).

Discussion

Campylobacter infection rates as found in this study revealed that duck, which is a waterfowl and turkey are the most colonized among the different species of poultry. Although duck and turkey showed to have a high prevalence, the sample size was small in this study. Another study has also revealed high prevalence rate in turkey [4]. The high prevalence rate as observed in duck can be attributed to their high affinity to water as some tip-up on the surface of shallow water for forage while others submerge completely and swim under the water in search of food. They can get infected especially when the ground water is contaminated with *Campylobacter* species [26]. The low infection rate found in chicken could be linked to a common management system operational in Sokoto and most parts of Nigeria. Free-range management system predominates in the study area, and this does

Table-1: Prevalence of *Campylobacter* species in different species of poultry and humans in Sokoto State.

Sample source	Total sampled	Total (%)	<i>C. jejuni</i> (%)	<i>C. coli</i> (%)	<i>C. lari</i> (%)
Humans	292	160 (55)	38 (24)	63 (39)	59 (37)
Chicken	400	119 (30)	23 (19.3)	62 (50.4)	34 (28.6)
G/fowl	67	20 (30)	3 (15)	10 (50)	7 (35)
Pigeon	21	3 (14)	2 (66.6)	0	1 (33.3)
Duck	16	9 (56)	0	7 (77.8)	2 (22.2)
Turkey	2	1 (50)	1 (100)	0	0
Total	798	312 (39)	67 (21)	142 (46)	103 (33)

G/fowl=Guinea fowl, *C. jejuni*=*Campylobacter jejuni*, *C. coli*=*Campylobacter coli*, *C. lari*=*Campylobacter lari*

Table-2: Recovery rate of *Campylobacter* isolates within 7-13 months of storage at -20°C.

Duration of storage (months)	Number of poultry isolates stored	Viable poultry isolates (%)	Number of human isolates stored	Viable human isolates (%)	Total viable isolates (%)
13	29	17 (59)	0	0	17 (59)
12	21	20 (95)	34	28 (82)	48 (87)
11	0	0	21	19 (90)	19 (90)
9	30	22 (73)	48	45 (94)	67 (85)
8	22	15 (68)	59	54 (92)	69 (85)
7	43	41 (95)	0	0	41 (95)
Total	145	115	162	146	261

not encourage coprophagy in the chicken population as opposed to the practice among birds kept in deep litter system. This is supported by the findings that recorded the prevalence rate of 78.4% in intensively reared poultry and 18.3% in small-scale rural poultry farming [27]. However, the lower prevalence rate can be due to the population size as other studies have revealed higher prevalence in free-range poultry. The prevalence rates in turkey, pigeon and guinea fowl have revealed the possibilities of infection through feeds since these categories of poultry usually feeds on insects, fruits, seeds, and flowers, and these have been suggested as potential routes of infection in poultry [28]. However, the small sample size used for pigeon and turkey might have contributed to the low prevalence rates. There is wide interaction among these species of birds and human surroundings in the study area where birds are caged together in the same cage and even transported from one live bird markets to another until they are sold. This practice will enhance the possibilities of infection spread and transmission to humans [7].

The prevalence rate of *C. coli* was higher than that of *C. jejuni* and *C. lari* in both poultry and humans. This was in disagreement with the usual higher isolation rate of *C. jejuni* than other species [23]. However, it has remained the most isolated species in swine as a major cause of swine dysentery [29]. Both *C. coli* and *C. jejuni* have been revealed as the most common bacteriological cause of gastroenteritis in humans [30]. On the other hand, *C. lari*, which is mostly found in wild birds, usually have low prevalence in humans and poultry, but the high detection rates in this study suggest the possibility of transmission from wild birds [31].

The 85% viability rate of *Campylobacter* isolates after 7 months of storage was in agreement with the work that revealed all isolates viable after the same period [32]. Frozen poultry can serve as a reservoir of *Campylobacter* as well as fresh meat. However, freezing meat has been proven to reduce contamination. Furthermore, the ability of *Campylobacter* to change from viable rod form to VBNC coccoid form that fails to grow on subculture can lead to false negative result on testing for food contamination [9]. Therefore, strict sanitary measures in handling and processing both fresh meat and frozen poultry should be encouraged. This would reduce food contamination and further

reduce the spread of *Campylobacter* infection and development of resistant strains [33].

Authors' Contributions

The manuscript was drafted by ION. MDS and JG contributed substantially to the conception and design of the study. OOF, AAM, and UM supervised the study and approved the experimental protocol. ION and EBI performed the data and statistical analysis. The manuscript was critically reviewed by ION, JG, OOF, and EBI. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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