Antibiotic Susceptibility Profiles of *Campylobacter coli* Isolated from Poultry Farms in Lagos Nigeria – A Pilot Study

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Campylobacter jejuni and *Campylobacter coli* are among the leading causes of gastroenteritis in humans worldwide, particularly in Africa. Poultry remains a major source of *Campylobacter* species and a vector of transmission to humans.

This pilot study was aimed at isolating and determining the antibiotic susceptibility profiles of *Campylobacter* spp. from fresh poultry droppings collected from poultry farms in Lagos State, Nigeria. Susceptibility was assessed using the CLSI standards.

Standard microbiological methods were used in isolation, identification, and characterization of *Campylobacter* spp. Isolates were subjected to antibiotic susceptibility testing by the disk diffusion method.

Of the 150 poultry droppings analyzed, 8 (5.3%) harbored *Campylobacter spp*. All isolates proved to be *C. coli* since they were all negative for the *hip* gene. A percentage of 100% showed resistance to nalidixic acid, chloramphenicol, cloxacillin, and streptomycin. While 87.5% were susceptible to amoxicillin and amoxicillin/clavulanic acid, 62.5% were susceptible to tetracycline. Surprisingly, 62.5% of *C. coli* had decreased (intermediate) susceptibility to erythromycin.

Although there was a low prevalence of *C. coli* from poultry in this study, the presence of antibiotic resistant strains circulating the food chain could result in treatment failures and difficulty in case management if involved in infections of humans.

Keywords: Keywords: *Campylobacter coli*, poultry, antibiotic susceptibility, Nigeria, Africa

Introduction

Campylobacter is one of the leading causes of gastroenteritis in humans worldwide, hence an important public health pathogen [1, 2]. The symptoms of campylobacteriosis include watery and sometimes bloody diarrhea, fever, and abdominal pain [3]. Several weeks after acute campylobacteriosis post-infectious sequelae like the Guillain-Barré syndrome may follow [4]. Domestic animals, including farm animals and pets, are known primary reservoirs of *Campylobacter* [5, 6]. *Campylobacter* infections have been associated with the consumption of improperly cooked and cross-contaminated or inadequately processed foods, including poultry, beef, shellfish, unpasteurized milk, vegetables, and fruits [7]. Antibiotic resistance in *Campylobacter* is on the rise, especially in Africa. Smith et al. reported increased rates of streptomycin and cloxacillin resistance among *Campylobacter* spp. isolated from animals and humans in Nigeria [8]. However, there seem to be a paradigm shift as *Campylobacter* species are now multiple resistant. This means that the isolates are unsusceptible to a wider range of antibiotics; in particular, the high level resistance to tetracycline and fluoroquinolones is of importance [9–12].

Since poultry are largely consumed food animals in Nigeria, and the prevalence of *Campylobacter* in Nigerian poultry was rated high [2, 13–15], this study therefore seeks to classify, evaluate the isolation rate, and the antibiotic susceptibility pattern of *Campylobacter* spp. isolated from poultry in Lagos Nigeria based on a broader test panel of 13 antimicrobial substances. In particular, antibiotic resistance may cause therapy failure in complicated campylobacteriosis, especially in children and immunosuppressed patients.

Materials and Methods

Sample Collection. Fresh poultry droppings were randomly collected immediately after defecation from 116 (77.3%) chickens and 34 (22.7%) guinea fowls in various farms in Lagos State, Nigeria. Sterile spoons attached to universal bottles were used to collect the fecal samples and streaked immediately on site onto 5% sheep blood agar plates containing *Campylobacter* supplement (Oxoid, Butzler SR85) [13]. Inoculated plates were transported to the laboratory in gas jars using microaerophilic atmosphere generating sachets (CampyGen Oxoid CN35A, UK) to produce conditions suitable for the growth of *Campylobacter*.

Isolation of *Campylobacter*. Isolation of *Campylobacter* was done according to the method of Zhao et al., with slight modifications [16]. The blood agar plates inoculated with fecal samples were incubated at 42 °C for 48 h. Suspected colonies with phenotypic characteristics of *Campylobacter*

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were subcultured onto blood agar plates and incubated for 24 h at 42 °C in a candle jar under microaerophilic conditions. Suspected colonies were Gram-stained and subjected to biochemical characterization using Campy API-systems (bioMérieux, Marcy-l'Étoile, France).

Antibiotic Susceptibility Testing. Isolates were subjected to antimicrobial susceptibility testing using the disk diffusion method as described by Olabode et al. [17]. Thirteen antibiotics (Oxoid, Hampshire, UK) were used including amoxicillin/clavulanic acid (30 μg), amoxicillin (25 μg), tetracycline (25 μg), erythromycin (5 μg), nitrofurantoin (200 μg), gentamicin (10 μg), ofloxacin (5 μg), clarithromycin (10 μg), cotrimoxazole (25 μg), nalidixic acid (30 μg), chloramphenicol (10 μg), cloxacillin (5 μg), and streptomycin (10 μg).

The antibiotic-impregnated disks were placed on the inoculated blood agar plates using sterile forceps and incubated at 42 °C for 48 h. The clear zones of inhibition were measured to the nearest millimeter using a transparent millimeter ruler and analyzed as described by Clinical and Laboratory Standard Institute [18].

Polymerase Chain Reaction (PCR) for Suspect *Campylobacter* Spp. DNA extraction of *Campylobacter* spp. was done as described by Samosornsuk et al. [19]. The hippurate hydrolase (*hip*) gene was detected by PCR using the primers: Forward 5′-GAA-GAG-GGT-TTG-GGT-GGT-G-3′ and Reverse 5'-AGC-TAG-CTT-CGC-ATA-ATA-ACT-TG-3′. A 25-μL reaction was used, which contained 5-μL template DNA and 0.2 μM of each primer [20]. PCR was carried out in a thermocycler (Eppendorf AG, Hamburg, Germany) with cycling parameter as follows: denaturation at 94 °C for 1 min, annealing at 66 °C for 1 min, and extension at 72 °C for 1 min. PCR amplicons were electrophoretically separated in 1% agarose gels (Sigma type II, medium EEO), stained with ethidium bromide and visualized under ultraviolet (UV) light.

Ethical Approval. The study did not require an ethical approval, since faecal samples were collected after the birds defecated. Hence there was no direct contact with the birds. However all organizational and institutional ethics were strictly adhered to.

Results

Campylobacter spp. were isolated from 8 (5.3%) of the total 150 fresh poultry droppings analyzed. Isolates were obtained from 6/8 (75%) of chicken and 2/8 (25%) of guinea fowl droppings. All 8 *Campylobacter* isolates were tested negative for the *hip* gene and thus classified as *Campylobacter coli* with a prevalence of 5.3% (8/150).

Table 1. Antibiotic susceptibility pattern of *Campylobacter coli* to test antibiotics

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxicillin/clavulanic acid (AUG)	7/8 (87.5)	1/8 (12.5)	0/8 (0.0)
Amoxicillin (AMX)	7/8 (87.5)	1/8 (12.5)	0/8 (0.0)
Tetracycline (TET)	5/8 (62.5)	1/8 (12.5)	2/8 (25.0)
Erythromycin (ERY)	1/8 (12.5)	5/8 (62.5)	2/8 (25.0)
Nitrofurantoin (NIT)	3/8 (37.5)	1/8 (12.5)	4/8 (50.0)
Gentamicin (GEN)	2/8 (25.0)	0/8 (0.0)	6/8 (75.0)
Ofloxacin (OFL)	2/8 (25.0)	1/8 (12.5)	5/8 (62.5)
Clarithromycin (CLR)	1/8 (12.5)	0/8 (0.0)	7/8 (87.5)
Cotrimoxazole (COT)	0/8 (0.0)	1/8 (12.5)	7/8 (87.5)
Nalidixic acid (NAL)	0/8 (0.0)	0/8 (0.0)	8/8 (100)
Chloramphenicol (CHL)	0/8 (0.0)	0/8 (0.0)	8/8 (100)
Cloxacillin (OXL)	0/8 (0.0)	0/8 (0.0)	8/8 (100)
Streptomycin (STR)	0/8 (0.0)	0/8 (0.0)	8/8 (100)

All *C. coli* isolates were resistant to one or more of the antibiotics tested as shown in Table 1. A vast majority of isolates were resistant to nitrofurantoin 4/8 (50.0%), gentamycin 6/8 (75.0%), ofloxacin 5/8 (62.5%), clarithromycin 7/8 (87.5%), cotrimoxazole 7/8 (87.5%), nalidixic acid 8/8 (100%), chloramphenicol 8/8 (100%), cloxacillin 8/8 (100%), and streptomycin 8/8 (100%). Five (62.5%) of the isolates showed decreased susceptibility (i.e., were intermediate tested) to erythromycin. Seven (87.5%) of the isolates showed susceptibility to amoxicillin/clavulanic acid and amoxicillin, while 5/8 (62.5%) were tested susceptible to tetracycline.

Discussion

Poultry has been reported to be a major reservoir of *Campylobacter* spp. with high levels of up to 9.0 $\log_{10}$ CFU/g (colony-forming units per gram) in the gut and cecal content of colonized birds which are majorly asymptomatic, hence promoting transmission among the flock [21]. The 5.3% prevalence of *Campylobacter coli* isolated from poultry droppings in this study is relatively low compared to the 47.3% and 20% in 1988 and 2005, respectively, reported in the same Nigerian state [13]. Similarly studies conducted in the northern part of Nigeria, i.e., in Sokoto State, showed a prevalence of 30% and 77.6% [7, 22]. The low isolation rate may be due to the indiscriminate use of antibiotics by poultry farmers in Nigeria as observed by Amaechi et al. in another study, which showed that erythromycin and tetracycline were the commonly used antibiotics by farmers in Nigeria in recent times [23]. Viable but non-culturable (VBNC) state of isolates in the bird feces, which is a survival response to stress, could be another reason for the low isolation rate [13, 24].

Globally antimicrobial resistance of bacterial isolates of food, animal and human origin is on the rise [1, 25]. *Campylobacter* isolates demonstrated varied antimicrobial susceptibility. Approximately 85.7% showed sensitivity to amoxicillin/clavulanic acid and amoxicillin, while 57.14% showed sensitivity to tetracycline. However, all the isolates were resistant to gentamicin, nalidixic acid, cloxacillin, chloramphenicol, and streptomycin and demonstrated decreased sensitivity to erythromycin. This is in line with the studies of Biswas et al., which reported a high level of resistance to nalidixic acid and susceptibility to erythromycin [26]. Additionally, Elhadidy et al. reported 40.7% multidrug-resistant *C. coli* isolates cultured from broiler carcasses and feces of diarrheal patients in Belgium [27]. Similarly, Wei and Kang reported the resistance profile of 8 *Campylobacter* isolates from avian sources that were resistant to fluoroquinolones, ampicillin, tetracycline, and macrolides [28]. The persistence of antibiotic resistant *Campylobacter* strains in the food chain remains a public health risk.

The limitations of this study are the geographical coverage area, the number of samples considered, and, consecutively, the number of bacterial isolates and microbial species tested. These shortcomings will be addressed in the main study based on this pilot study in the following way: sampling will take place in 20–30 households from each of 6 areas in Nigeria, namely, Northwest, Northcentral, Northeast, Southwest, Southcentral, and Southeast Nigeria. A much larger number of samples will be targeted as cross-sectional studies will be conducted to detect and isolate *Campylobacter* and *Arcobacter* from feces and/or cloacal/anal swabs from domesticated and neighboring wild animals, as well as water reservoirs and food if present. Cross-sectional and case control studies will be used for humans with diarrhea and those without diarrhea as well as malnourished and non-malnourished children. In addition to *C. coli*, all other microbial species of the genera

Campylobacter and *Arcobacter* will be included in the susceptibility testing.

However being a pilot study, it has provided a basis for design and execution of a larger study.

Conclusion

Poultry being a major source of animal protein to man remain one major vector for the transmission of *Campylobacter* and, in particular, antibiotic resistant strains. Although there was a low prevalence of *C. coli* isolated from chicken and guinea fowl in this study, the presence of strains with resistance to multiple antibiotics was significant. Such multidrug-resistant strains circulating the food chain could result in treatment failures and difficulty in case management if involved in human infection. Therefore, an extensive study is advocated that would provide data needed to inform policy for surveillance and monitoring of campylobacteriosis.

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Authors' Contributions

S.I.S. conceptualized and supervised the study, O.O. collected the samples and carried out laboratory work, A.A. and A.E.Z. analyzed the results and drafted the manuscript, and S. I.S. and A.E.Z. reviewed and edited the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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