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Multidrug resistance and virulence genes in *Campylobacter* isolates from poultry waste in Ibadan, Nigeria

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ABSTRACT

Objective: This study aims to determine antibiotic susceptibility profile and detect virulence genes in *Campylobacter* spp. isolated from poultry waste in Ibadan, Nigeria.

Methods: Poultry droppings were collected from ten poultry farms, and Total Bacteria Count (TBC) and Total Anaerobic Count (TAC) of the samples were determined. *Campylobacter* species were isolated using modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA). The isolates were identified using conventional biochemical techniques, and antibiotics susceptibility testing was performed using Kirby Bauer disk diffusion assay on Mueller Hinton agar. Detection of virulence genes in selected multidrug resistant (MDR) isolates was carried out using polymerase chain reaction.

Results: *Campylobacter* species (240) were isolated from Moniya (44.6%) and Igbo-Oloyin (55.4%) farms. *Campylobacter* (*C.*) *lari* was the most prevalent isolate (32.5%) while *C. fetus* was the least common. Resistance to streptomycin was observed in 76.7% of the isolates, while 3.3% exhibited resistance to ertapenem. Specifically, *C. jejuni* isolates from Igbo-Oloyin farms showed resistance rates of 86% to cephalothin and 71% to cefuroxime. In comparison, *C. jejuni* isolates from Moniya farms showed even higher resistance rates of 95% to cephalothin and 81% to cefuroxime. The antibiotypes of the selected isolates showed that four *Campylobacter* spp. were resistant to a combination of eight antibiotics. Furthermore, the detection of virulence genes from the selected MDR isolates showed that *cadF* and *hipO* genes were detected in 70.0% and 50.0% of cases, respectively.

Conclusions: MDR strains with virulent genes were found in *Campylobacter* species isolated from poultry wastes, posing a significant health challenge. Therefore, prudent use of antibiotics in animal husbandry should be ensured in addition to preventing indiscriminate discharge of untreated poultry waste into the environment.

KEYWORDS: Poultry farms; *Campylobacter* species; Antibiotics resistant bacteria; Virulence genes

1. Introduction

Due to high demand, Nigerian poultry has been recognized as one of the fastest growing livestock sectors in the West African sub region[1]. Poultry products are commonly consumed across every strata of Nigerian society without any religious or ethnic restrictions. Bacterial pathogens have been implicated in food safety problems both in occurrence and number of affected individuals. Among the common bacterial pathogens that are associated with poultry products are *Campylobacter* species, *Salmonella* species, *Escherichia coli O157:H7* and *Listeria monocytogenes*.

Significance

Campylobacteriosis is a significant public concern as it is one of the leading causes of diarrhea worldwide, with *Campylobacter* species being the primary agents of gastroenteritis. This study enhances understanding of the epidemiology, pathogenesis and transmission of *Campylobacter* infections from poultry sources and suggests implications for public health policies, including the regulation of antibiotic use and the advancements of surveillance.

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According to the WHO report, *Campylobacter* is the leading cause of zoonotic enteric human infections in most developed and developing countries and one out of the four key global causes of diarrhoeal diseases[2]. *Campylobacter* species are Gram-negative, curved, motile, microaerophilic bacilli that do not form spores. The bacteria typically colonize the gastrointestinal tracts of chickens, turkeys, cattle, sheep, goat, pigs and wild birds[3]. Since the body temperature of birds are close to the optimum temperature for growth of thermophilic *Campylobacters*, handling of raw poultry meat and consuming poultry products may serve as important risk factors for sporadic Campylobacteriosis[4].

Molecular studies have given insights into some of the essential virulence genes associated with the pathogenesis of *Campylobacter* infections. These include the ability of *Campylobacter* to adhere to cells with the aid of *pldA*, *cadF* and *capA* genes, invade the intestinal epithelial cells with the aid of the *CiaB* and *CiaC* genes, produce toxins with the aid of *cdtA* gene, and survive in the host cells[5]. In addition to pathogenesis, the increasing antibiotic resistance of *Campylobacter* species isolated from humans, animals and the environments is rapidly becoming a significant public health concern[6,7]. Salihu *et al*.[8] have reported a high prevalence (81.9%) of contamination with thermotolerant *Campylobacter* in poultry meat in Sokoto, a city in the northern part of Nigeria. This study was designed to determine the antibiotics susceptibility profile and presence of some virulence genes in *Campylobacter* species isolated from poultry waste in different farms in Ibadan, Nigeria.

2. Materials and methods

2.1. Study site and sample collection

The study site was Akinleye Local Government Area of Oyo state, Nigeria. It is one of the eleven local government areas in the ancient city of Ibadan, created in 1976 and located between the geographical coordinates 7°31’42” North and 3°54’43” East. It shares boundaries with Afijio Local Government to the north, Ibadan North Local Government to the south, Ido Local Government to the west, and Lagelu Local Government to the east. The total land area is 464.892 square kilometers, and has a population density of about 516 persons per square kilometer. Samples of poultry droppings were collected from pens of 10 different farms in Moniya and Igbo-oloyin from July to August, 2019. The samples were collected into sterile containers with sterile spatula, properly labeled and transported in ice packs to the Pathogenic Laboratory of the University of Ibadan, for immediate processing.

2.2. Determination of Total Bacteria Count and Total Anaerobic Count

Total Bacteria Count (TBC) was done using the standard pour plate method on Nutrient Agar plates and were incubated aerobically, while for Total Anaerobic Count (TAC), the culture plates were incubated under anaerobic conditions.

2.3. Isolation and identification of the Campylobacter species

The isolation of *Campylobacter* spp. was performed using standard pour plate technique on a freshly prepared Modified Charcoal Cefoperazone Deoxychocolate Agar (mCCDA) from instead of form Oxoid, which was supplemented with Oxoid (SR0155) CCDA supplements (cefoperazone and amphotericin B) and incubated under microaerophilic condition with Campygen (3A) according to standard method. Isolates that appeared grey, cream, or white, with or without metallic sheen against a black background were sub cultured and characterised using standard biochemical tests such as Gram staining, the motility test, the catalase test, the oxidase test, the hippurate hydrolysis test, the sulphide reduction test, and growth at 25 ℃ under microaerophilic condition.

2.4. Antibiotic susceptibility test

The antibiotics susceptibility testing of the isolates were carried out according to the guidelines by Clinical Laboratory Standards Institute[9] against nalidixic acid (30 μg/mL), cephalothin (30 μg/mL), ofloxacin (5 μg/mL), amoxicillin/clavulanic (30 μg/mL), ertapenem (10 μg/mL), amikacin (30 μg/mL), cefuroxime (30 μg/mL), chloramphenicol (30 μg/mL), streptomycin (10 μg/mL) and azithromycin (15 μg/mL). The test organisms were standardized to conform to 0.5 MacFarland standards and inoculated on Mueller Hinton agar plates. The antibiotics disks were aseptically placed on the inoculated plates and incubated under microaerophilic conditions for 24 h. Zones of inhibition were measured and interpreted according to Clinical & Laboratory Standards Insistitute (CLSI) standards[9]. Isolates that showed resistance to at least three different classes of antibiotics were recorded as multidrug resistant[10].

2.5. Detection of virulence genes in selected multidrug–resistant Campylobacter species

The gene sequence information in the GenBank database, in addition to previously published studies[11, 12] formed the basis for designing the forward and reverse primers specific for the virulence genes. The primers used for the gene detection is shown in Table 1. The PCR reaction mixture consisted of 12.5 μL 5× Red load Taq Master Mix, 1 μL each for the forward *cadF* and *hipO* primers, 1μL each for the reverse *cadF* and *hipO* primers, 3 μL of DNA template and 5.5 μL nuclease-free water, making up a total reaction of 25 μL. The mixture were subjected to initial denaturalization at 95 ℃ for 3 min, followed by 45 cycles at 94 ℃ for 30 s, annealing temperature of 49 ℃ for 30 s and 72 ℃ for 1 min

and a final extension at 72 ℃ for 5 min. The PCR products were electrophoresed on 1.2% agarose gel stained with ethidium bromide and was viewed using a gel imaging system.

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 20 and Microsoft Excel 2016. The *Chi*-square test was used to test for significant difference in the occurrence of *Campylobacter* spp. from Moniya and Igbo-Oloyin. One-way analysis of variance (ANOVA) was used to test for significant differences in the occurrence of multidrug resistant *Campylobacter* species between the study locations.

3. Results

3.1. Total heterotrophic count and anaerobic bacteria count

The 10 farms studied comprised of small (4), medium (4) and big (2) ones. The farms with the highest mean Total Hetertrophic Count (THC) were A3 (Igbo-Oloyin) and D1 (Moniya) with 6.43±5.91 log CFU/g and 6.34±5.95 log CFU/g, respectively. The highest anaerobic bacteria count (TAC) were 6.39±6.06 log CFU/g and 6.09±5.62 log CFU/g obtained from samples in A4 (Igbo-Oloyin) and D3 (Moniya), respectively (Table 2).

3.2. Occurrence and distribution of Campylobacter species in the poultry samples

A total of 240 isolates were obtained, with 107 (44.6%) from Moniya farms and 133 (55.4%) from Igbo-Oloyin farms. Among the isolates, consisted of *C. jejuni* (28 from Igbo-Oloyin, 21 from Moniya), *C. coli* (22 from Igbo-Oloyin and 17 from Moniya), *C. fetus* (6 fromIgbo-Oloyin and 6 from Moniya), *C. upsaliensis* (29 from Igbo-Oloyin and 16 from Moniya), *C. lari* (40 from Igbo-Oloyin and 38 from Moniya), and *C. hyointestinalis* (8 from Igbo-Oloyin and 9 from Moniya) (Table 3). However, there was no statistically significant difference in the occurrence of the isolates across the study locations $P>0.05$)

3.3. Antibiotic resistance patters of Campylobacter species in the poultry samples

The patterns of antibiotic resistance of the *Campylobacter* spp. showed that 76.7% were resistant to streptomycin, 74.2% to azithromycin, while resitance to ertapenem (3.3%) and amikacin (9.2%) was low. Among the isolates obtained from Igbo-Oloyin farms, 86% C. *jejuni* showed resistance to cephalothin and 71% showed resistance to cefuroxime, respectively, while none exhibited resistance to nalidixic acid and ertapenem. It was also observed that the C. *coli* from the same farms showed resistance to cephalothin (100%), cefuroxime (86%) azithromycin (95%), ofloxacin (5%) and amikacin (5%). Furthermore, all C. *fetus* and C. *hyointestinalis* were resistant to streptomycin, while they were all sensitive to cephalothin and ertapenem. Among the isolates from Moniya farms, the resistance rates of C. *jejuni* to cefuroxime and azithromycin were 81% and 76%, repectively, while none showed resistance to nalidixic acid and ertapenem. However, all C. *coli* were resistant to azithromycin and cephalothin, while resistance rates of the isolates to ertapenem and amikacin were 6%. It was also observed that all C. *fetus* were fully susceptible to cephalothin, ofloxacin and ertapenem, while 92%, 84% and 5% of the C. *lari* showed resistance to nalidixic acid, cephalothin and ertapenem, respectively (Table 4).

3.4. Antibiotypes and multiple antibiotic resistance index of Campylobacter species selected for detection of virulence genes

The antibiotypes of the twenty-nine selected MDR isolates showed that four *Campylobacter* spp. were resistant to eight of the ten tested antibiotics. The twenty-nine isolates comprised of fourteen *C. lari*, twelve *C. jejuni*, three *C. coli*, and one *C. upsaliensis*. The resistance profiles indicated that two *C. lari* isolates were resistant to a combination of eight different antibotics, while three other *C. lari* isolates showed resistance to a different set of eight antibiotics. Additionally, four *C. jejuni* isolates were resistant to a combination of five different antibiotics and another two *C. jejuni* showed resistance to seven different antibiotics.

3.5. Occurrence of virulence genes in multidrug–resistant Campylobacter isolates

Detection of the virulence genes in the selected MDR isolates is shown in Figure 1 and Supplementary Figure. It was observed that *cadF* and *hipO* genes were detected in 70.0% and 50.0% of the isolates, respectively.

Table 1. Primers used for the virulence gene detection.

Target gene	Primer sequence (5′-3′)	Product size	References
hipO	F-GAAGAGGGTTTGGGTGGT R-AGCTAGCTTCGCATAATAACTTG	735	[11]
cadF	F-TTGAAGGTAATTTAGATATG R-CTAATACCTAAAGTTGAAAC	400	[12]

F: Forward; R: Reverse.

Table 2. Mean Total Bacteria Count and mean Total Anaerobic Count of samples in the studied farms (Log CFU/g).

Igbo-Oloyin			Moniya		
Farms	TBC	TAC	Farms	TBC	TAC
A1	6.36±6.02	6.02±5.93	D1	6.34±5.95	5.95±5.93
A2	6.42±5.73	6.13±5.96	D2	6.20±5.75	5.97±5.88
A3	6.43±5.91	6.17±5.93	D3	6.31±5.91	6.09±5.62
A4	6.42±6.23	6.39±6.06	D4	6.11±5.73	5.99±5.30
A5	6.36±5.99	6.11±5.79	D5	6.20±5.82	6.03±5.82

Table 3. Occurrence of *Campylobacter* spp. isolates from Igbo-Oloyin and Moniya [*n* (%)].

Farms	Locations	*C. jejuni*	*C. coli*	*C. fetus*	*C. upsaliensis*	*C. lari*	*C. hyoitestinalis*
A1 (*n*=31)	Igbo-Oloyin	6(19)	5(16)	0(0)	5(16)	11(35)	4(13)
A2 (*n*=20)	Igbo-Oloyin	5(25)	2(10)	1(5)	3(15)	7(35)	2(10)
A3 (*n*=23)	Igbo-Oloyin	3(13)	2(9)	1(4)	6(26)	11(48)	0(0)
A4 (*n*=30)	Igbo-Oloyin	6(20)	6(20)	2(7)	8(27)	7(23)	1(3)
A5 (*n*=29)	Igbo-Oloyin	8(28)	6(21)	2(7)	8(28)	4(14)	1(3)
D1 (*n*=24)	Moniya	7(29)	5(21)	0(0)	5(21)	7(29)	0(0)
D2 (n=24)	Moniya	3(13)	2(8)	5(21)	2(8)	11(46)	1(4)
D3 (*n*=16)	Moniya	3(19)	2(13)	0(0)	5(31)	3(19)	3(19)
D4 (*n*=19)	Moniya	7(37)	5(26)	0(0)	2(11)	3(16)	2(11)
D5 (*n*=24)	Moniya	1(4)	4(17)	1(4)	1(4)	14(58)	3(13)
Total (*n*=240)		49(20.4)	39(16.3)	12(5.0)	45(18.8)	78(32.5)	17(7.1)

$P>0.05$; $f = 1.36$; $\chi^2 = 3.57$.

Table 4. Antibiotic resistance pattern of *Campylobacter* species isolated from the poultry waste samples [*n* (%)].

Antibiotics	*C. jejuni*		*C. coli*		*C. fetus*		*C. upsaliensis*		*C. lari*		*C. hyointestinalis*		TR
	Igbo-Oloyin *n*=28	Moniya *n*=21	Igbo-Oloyin *n*=22	Moniya *n*=17	Igbo-Oloyin *n*=6	Moniya *n*=6	Igbo-Oloyin *n*=29	Moniya *n*=16	Igbo-Oloyin *n*=40	Moniya *n*=38	Igbo-Oloyin *n*=8	Moniya *n*=9	*n*=240
NAL	0(0)	0(0)	2(9)	4(24)	5(83)	4(67)	1(3)	0(0)	38(95)	35(92)	8(100)	9(100)	106 (44.2)
CEP	24(86)	20(95)	22(100)	17(100)	0(0)	0(0)	0(0)	0(0)	37(93)	32(84)	0(0)	0(0)	152 (63.3)
OFX	3(11)	1(5)	1(5)	4(24)	1(17)	0(0)	8(28)	4(25)	6(15)	8(21)	5(63)	4(44)	45 (18.8)
AMC	6(21)	6(29)	2(9)	6(35)	0(0)	2(33)	6(21)	5(31)	12(30)	12(32)	2(25)	2(22)	61 (25.4)
ETP	0(0)	0(0)	2(9)	1(6)	0(0)	0(0)	0(0)	0(0)	2(5)	2(5)	0(0)	1(11)	8 (3.3)
AK	3(11)	1(5)	1(5)	1(6)	1(17)	1(17)	2(7)	1(6)	3(8)	6(16)	1(13)	1(11)	22 (9.2)
S	19(68)	15(71)	15(68)	16(94)	6(100)	2(33)	25(86)	10(63)	35(88)	26(68)	8(100)	7(78)	184 (76.7)
CXM	20(71)	17(81)	19(86)	14(82)	4(67)	4(67)	15(52)	7(44)	25(63)	27(71)	2(25)	6(67)	160 (66.7)
C	13(46)	12(57)	13(59)	15(88)	5(83)	3(50)	4(14)	8(50)	20(50)	22(58)	6(75)	6(67)	127 (52.9)
AZM	17(61)	16(76)	21(95)	17(100)	2(33)	3(50)	19(66)	8(50)	33(83)	29(76)	7(88)	6(67)	178(74.2)

Note: TR: Total resistance; AZM: Azithromycin (15 µg); AMC: Amoxicillin/Clavulanate (30 µg); CXM: Cefuroxime (30 µg); OFX: Ofloxacin (5 µg); ETP: Ertapenem (10 µg); S: Streptomycin (10 µg); AK: Amikacin (30 µg); C: Chloramphenicol (30 µg); NAL: Nalidixic acid (30 µg); CEP: Cephalothin (30 µg).

Table 5. Antibiotypes of multidrug resistant isolates selected for virulence genes detection.

Antibiotypes	Classes of antibiotics	*C. jejuni*	*C. coli*	*C. lari*	*C. upsaliensis*	Total	MAR index
C-S-CXM-AZM-KF	5	4	0	0	0	4	0.5
S-AMC-CXM-AZM-KF	5	2	0	0	0	2	0.5
S-OFX-AMC-CXM-AZM-KF	6	2	0	0	0	2	0.5
ETP-NA-S-OFX-CXM-AZM	6	0	0	0	1	1	0.5
NA-C-OFX-CXM-AZM-KF	6	0	0	1	0	1	0.5
ETP-NA-S-AMC-CXM-AZM-KF	7	0	0	2	0	2	0.5
AK-NA-C-S-AMC-CXM-AZM-KF	8	0	0	1	0	1	0.6
AK-NA-C-S-OFX-CXM-AZM-KF	8	0	0	1	0	1	0.6
NA-S -AMC-CXM-AZM-KF	6	0	0	3	0	3	0.6
C-S-OFX-AMC-CXM-AZM-KF	7	2	0	0	0	2	0.6
C-S-AMC-CXM-AZM-KF	6	2	0	0	0	2	0.6
AK-NA-C-S-CXM-AZM-KF	7	0	1	1	0	2	0.7
ETP-NA-C-S-OFX-CXM-AZM-KF	8	0	1	2	0	3	0.8
ETP-AK-NA-C-S-CXM-AZM-KF	8	0	1	3	0	4	0.9

Note: AZM: Azithromycin (15 µg); AMC: Amoxicillin/Clavulanate (30 µg); CXM: Cefuroxime (30 µg); OFX: Ofloxacin (5 µg); ETP: Ertapenem (10 µg); S: Streptomycin (10 µg); AK: Amikacin (30 µg); C: Chloramphenicol (30 µg); NAL: Nalidixic acid (30 µg); KF: Cephalothin (30 µg); MAR: Multidrug antibiotic resistance.

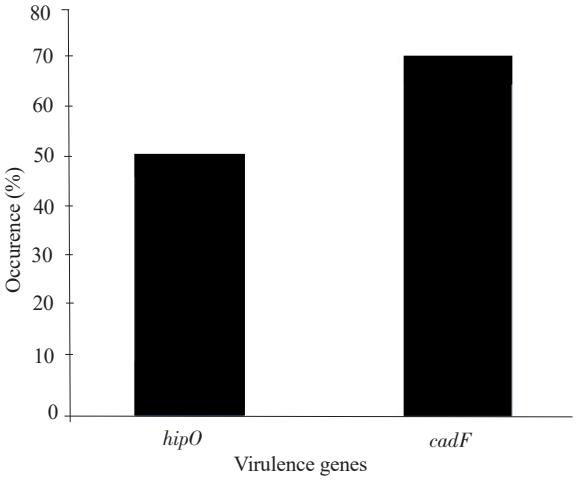

Figure 1. Occurrence of virulence genes in *Campylobacter* spp. isolated from poultry wastes collected in Moniya and Igbo-Oloyin.

4. Dicussion

Thermotolerant species of *Campylobacter* are becoming increasingly important in public health challenges, particularly as agents of infectious diarrhoea in humans. The isolation of *Campylobacter* spp. in poultry droppings of selected farms in this present study confirmed the presence of the bacteria species in poultry droppings[13, 14]. The occurrence of *Campylobacter* spp. in the poultry droppings of the selected farms was lower compared to previous reports of a similar study[13,14]. Factors such as stock density, season, feeding regimen and geographical location have been proposed to account for significant differences and similarities in the isolation rate of the bacteria[15].

The observed occurrence (32.5%) of *C. lari* in this study is comparable to the occurrence of 37.0% from humans and 29.0% from poultry samples in the previous report in the northern part of Nigeria[16]. The lower occurrence obtained from the poultry samples in the latter study might be linked to the free-range system which was common in the study area, since coprophagy could constrain bird to bird transmission. Moreover, it has also been previously reported from a study carried out in Italy that *Campylobacter* spp. prevalence was observed to be higher (78.4%) in intensively reared poultry compared to (18.3%) small scale rural poultry farms[17]. The observed occurrence rate (20.5%) of *C. jejuni* in this study is however much lower compared with 40.0% and 65.0% occurrence rate on similar isolate from studies in Poland and French, respectively[18, 19]. The reason for this disparity might be related to variation in the studied samples and the geographical locations of the study sites.

Furthermore, *C. coli* occurrence rate (16.3%) in this study is similar to the 14.2% previously reported from a study in Lagos metropolis on domestic animal meat samples[20].However, the rate was lower compared to 45.0% and 74.2% reported on similar isolate from Sokoto and Kebbi, respectively in the northern part of the country[16, 21].

The observation from this study revealed a low occurrence rate (7.1%) of *C. hyoitestinalis* which is comparable to the low occurrence rate (2.8%) reported from a study on groundwater samples[22]. Generally, the recovery of this species of *Campylobacter* is low as the isolate was not even detected in a study on human and poultry samples[16]. The absent or low occurrence of this species in poultry might be due to the high temperature of birds, which may reduce the survival chances of the species[23, 24].

However, the prevalence of *Campylobacter* spp. and the sample locations in this study was not statistically significant ($P>0.05$), which was in disagreement with the prevalence study of *Campylobacter* spp. in poultry and humans in four agricultural zones in Sokoto state, Nigeria, where a statistically significant association between the isolates prevalence and agricultural zones were found ($P<0.05$)[25].

The massive use of antibiotics in animal husbandry exacerbates the emergence and spread of drug resistant bacteria, leading to their transmission from animals to humans through the food chain. The direct consequences of infection with antibiotic resistant *Campylobacter* can be severe, including increased mortality, prolonged illnesses and hospital stay[26]. While most cases of *Campylobacter* enteritis are self-limiting, either erythromycin or ciprofloxacin are the drugs of choice when antibiotic therapy is necessary[27]. However, previous reports had shown an upward trend of antibiotics resistant *Campylobacter* with varying patterns in different countries and regions[28]. The resistance (21.4%) of *C. jejuni* to amoxicillin/clavulanic acid in this study is lower compared to the observed 52.7% of *C. jejuni* isolated from broiler chickens in Tunisia to the same antibiotics. The *C. coli* (35.3%) resistance to amoxicillin/clavulanic acid in this study is comparable to the 34.1% obtained from the latter study[25].

In the present study, the observation revealed that none of the isolated *C. jejuni* resisted nalidixic acid, this is a direct opposite of the total (100%) resistance reported from a study in the same city on retail chicken as well as the 52.7% observed in another study on broiler chicken in Tunisia by similar isolates to the same antibiotics[1,25]. Fluoroquinolones are among the recommended drugs for the treatment of campylobacteriosis. However, treatment with fluoroquinolones has become quite a challenge, as some *Campylobacter* spp. have been found to develop resistance to this

class of antibiotics. Infections due to fluoroquinolone-resistant *Campylobacter* strains are usually severe and last longer[29]. In this study, the low resistance of *C. jejuni* (10.7%) to ofloxacin (a member of the fluoroquinolones) is a bit lower but comparable to the 14.2% reported from a study on raw milk in Poland[30].

The observation showed that almost all the *C. coli* were resistant to cephalothin and azithromycin, and all *C. hyoitestinalis* were resistant to nalidixic acid in this present study could be attributed to the persistent use of these antibiotics in the studied farms. This resistance could encourage horizontal transfer of resistant genes on mobile genetic elements among *Campylobacter* spp.[31]. Carbapenems such as imipenem, ertapenem and meropenem, were once considered the last-resort to treat complicated infections of campylobacteriosis. However, with the growing rise of antibiotics resistance, these drugs are gradually being replaced with colistin[32]. In the present study, few of *C. hyoitestinalis* exhibited resistance to ertapenem, this agrees with previous report on similar study[33]. However, the resistance of *C. coli* to ertapenem (7.70%) is lower than that to imipenem (22.0%) for isolates from paediatric stool samples, as reported by Chukwu *et al*.[33].

Furthermore, a high level of multidrug resistant *Campylobacter* spp. was observed in this study, which is consistent with the findings of a previous study on broiler chickens in Tunisia, where all the *C. jejuni* and *C. coli* isolated were MDR[25]. Similar observation of MDR has been made in several countries. For instance, 100% MDR *Campylobacter* spp. was reported from a previous study on broiler chicken and slaughter houses in Algeria[34], as well as poultry production systems and slaughter houses in Italy[31]. Similarly, 90.4% MDR *Campylobacter* spp. from another study on retail meat in Pakistan[35].

The presence and expression of virulence genes in *Campylobacter* are among the factors responsible for the pathogenesis of the bacteria. The *hipO* and *cadF* genes are required for adhesion to the host and hydrolysis of sodium hippurate, respectively. The detection of *hipO* gene in 50.0% of the isolates tested in this study is not in agreement with previous studies in which detection of this gene was in 95.0%[36], 100%[37], and 70.0%[38] of *Campylobacter* species isolated from human and poultry wastes. The c*adF* gene encodes a protein that interacts with a host extracellular matrix protein and fibronectin, which is required for *Campylobacter* adherence and colonization of the host cell surface[39]. This implifies that many isolates from poultry faeces have the potencial of pathogenic to humans. The 70.0% occurrence of the *cadF* gene in the *Campylobacter* isolates of the present study is in agreement with the 71.0% detected in the isolates from human and chicken stool sample[11], but lower compared to the 100.0% detected in commercial chickens and human clinical cases[38], as well as 100.0% detected in *C. jejuni* and *C. coli* from hospitalised children and chicken carcasses in Poland[40].

5. Conclusions

Among the *Campylobacter* species isolated from poultry wastes of the studied farms, *C. jejuni* was the most occurring. The antibiotic resistance patterns showed that the isolates exhibited high resistance to streptomycin and azithromycin, while showing lower levels of resistance to ofloxacin, amikacin and ertapenem. However, the high resistance of multidrug resistance in the isolates is alarming. The *cadF* and *hipO* virulence genes were detected in selected multidrug resistant isolates where all *hipO* positive isolates also appeared positive for the *cadF* gene. These findings showed that poultry wastes harbour virulent and multidrug resistant *Campylobacter* species which can contaminate food and water, leading to serious foodborne illness once released into the environment. Therefore, the prudent use of antibiotics in poultry farms, the implementation of specific control procedures for antimicrobial resistance surveillance and the adoption of Hazard Analysis Critical Control Points (HACCP) are advocated to prevent the spread of resistant *Campylobacter* strains.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Falodun OI conceived, designed, and supervised the study, as

well as revised the draft. Ameh AA performed the experiment and analysed the data, did literature search, and wrote the first draft. Both authors approved the final draft.

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