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Antibiotic resistance and virulence genes in *Campylobacter* species from pig and cattle samples in Ibadan, Nigeria

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ABSTRACT: *Campylobacter* species are the leading cause of gastroenteritis worldwide with incidence cases higher than those caused by *Salmonella*. This study was designed to determine the antibiotic resistance patterns and virulence genes detection in selected Multi-Drug Resistant (MDR) strains of *Campylobacter* species isolated from pigs and cattle dungs. Stool samples were collected from pigs and cattle at the teaching and research farm of the University of Ibadan, Nigeria. Isolation and identification of *Campylobacter* species were made using modified charcoal cefoperazone deoxycholate agar and standard biochemical tests. Antibiotic susceptibility test was carried out using the disk diffusion technique. The *hipO* and *cadF* virulence genes were detected using a multiplex-polymerase chain reaction. The occurrence of *Campylobacter* species in pigs and cattle was 90.0% and 95.0%, respectively. In pigs, *C. coli* had the highest occurrence, while it was *C. jejuni* in cattle. The antibiotic resistance patterns showed that 1.3% and 1.2% of the isolates from pigs and cattle, respectively were resistant to all the antibiotics tested. Among the selected MDR strains, *cadF* genes were detected in 76.5% (pigs) and 75.0% (cattle). However, *hipO* genes were detected in 11.8% (pigs) and 50.0% (cattle) isolates. The *cadF* gene was detected in all the *Campylobacter* species, while *hipO* gene was detected only in *C. jejuni*. In conclusion, the pigs and cattle faecal wastes harbored virulent and multidrug-resistant *Campylobacter* species. Hence, the indiscriminate discharge of untreated animal faecal wastes into the environment and water bodies should be discouraged.

Keywords: Campylobacteriosis; cadF genes; hipO genes.

1. INTRODUCTION

Campylobacteriosis is one of the most common bacterial diseases of humans caused by species of the genus *Campylobacter*, particularly *C. jejuni* and *C. coli*. These organisms are major leading causes of gastroenteritis disease globally, with incidence rates that are higher than those caused by *Salmonella* in low-, middle- and high-income countries [1, 2, 3]. The most common species that have been isolated in human infections are *C. jejuni and C. coli*. The former is the most commonly isolated of all the species, causing over 80% of cases of human campylobacteriosis [4, 5].

The members of the genus *Campylobacter* are known to colonize a wide range of hosts, especially the intestinal tract of healthy birds, pigs and cattle. Raw carcasses and meats from these animals can be

contaminated with faeces during slaughter processes which could be a source of infection when their undercooked forms are consumed [2]. Infection can also occur via the consumption of raw and inadequately pasteurized milk, contaminated water supplies, contact with pets with diarrhea, and occupation exposure when processing animals that harbor this organism in abattoirs [6]. Most cases of human campylobacteriosis are self-limiting, infections may however develop into invasive diseases such as Guillain-Barré syndrome, meningitis, peritonitis, pancreatitis, and reactive arthritis [7]. In cases where treatment is required, antibiotics, usually macrolides and quinolones/fluoroquinolones, are used [8]. Intravenous aminoglycosides are also considered the treatment of choice, especially for bacteremia and other systemic infections due to *Campylobacter* [9].

Antibiotic resistance in some species of *Campylobacter* has been reported, and like other cases of antimicrobial resistance, it has become a major public health concern worldwide. Resistance of *Campylobacter* species to a number of different classes of antibiotics has been reported [10, 11]. In a previous study on antibiotic-resistant Campylobacter species carried out in North India, an incidence of 2.2% resistance was reported between 1989 and 1993. However in the same region, it has increased to 30.6% by 2008 [12, 13]. Similarly, in another study on antimicrobial resistance in *Campylobacter coli* isolated from pigs in two provinces of China, 76.8% multidrug resistance *C. coli* was reported [14].

Infections by *Campylobacter* species are made possible by some virulence genes that are present in the virulent strains of the organism. For example, adherence to, colonization and invasion of the intestinal wall to produce toxins are all important virulence properties that are encoded for by the *flaA, cadF, racR, dnaJ, virB11, ciaB*, *iam, hipO* and *pldA* genes [15, 16]. The *cadF* gene is an important gene present in most *Campylobacter* spp. as a successful invasion of the host cell is dependent on it. Literature searches have also shown the importance of the cadF gene in the molecular identification of *Campylobacter* species [17]. On the other hand, *hipO* gene is *C. jejuni* specific and has not been detected in any other *Campylobacter* species; it is the gene responsible for the hydrolysis of hippurate [18]. Most of the studies in Nigeria on the occurrence and antibiotic resistance of *Campylobacter* species have focused on chicken and beef meats, but there is a dearth of information on the detection of virulence genes in *Campylobacter* species, especially the environmental samples. This present study was designed to determine the antibiotic resistance patterns of *Campylobacter* species from cattle and pig dung samples and detect virulence genes in selected multidrug strains.

2. MATERIALS AND METHOD

2.1. Study site and sample collection

The study site was the piggery and cattle units of the teaching and research farm of the University of Ibadan, Nigeria with the coordinates 7º27’09.8”N 3º53’55.0”E and 7°27’27.1”N 3°53’46.4”E, respectively. A total of forty samples of dung were collected (twenty each for pigs and cattle). It was ensured that all samples were taken at the same period, in the morning between 7:30-8:30 am after cleaning the paddocks/pen where the animals were kept. The locations of the young and adult pigs were 30-40 metres apart, while those of the young and adult cattle were 40-50 metres apart. The sample size and numbers of time sampling were done is shown in Table 1. The samples were collected between the months of June and July 2019. All samples were preserved in ice packs after collection and transported to the Pathogenic Laboratory, Department of Microbiology, University of Ibadan for immediate analyses.

Table 1. Sample size and number of animals sampled.

Animal	Adult	No of times sampled	Total samples	Young	No of times sampled	Total samples
Pig[a]	3	4 times	12	2	4 times	8
Cattle[b]	3	4 times	12	2	4 times	8
Total			24			16

2.2. Isolation and identification of *Campylobacter* species

Isolation of the bacteria was carried out using the standard pour plate technique on Modified Charcoal Cefoperazone Deoxychocolate Agar (mCCDA) (Oxoid) prepared with Oxoid (SR0155) CCDA supplements (cefoperazone and amphotericin B). The plates were incubated under microaerobic conditions using Campygen (Oxoid) in an anaerobic jar. After incubation, white, grey or cream colonies typical of *Campylobacter* species were subcultured first unto fresh agar plates of Deoxychocolate Agar (Oxoid) and later on Nutrient Agar (Oxoid) to obtain a pure culture. The pure colonies were subjected to biochemical tests as described by the United Kingdom Standard for Microbiology Investigations [19], such as Gram staining, motility test catalase, oxidase, hippurate hydrolysis, and sulfide reduction. growth at 25ºC, hydrogen sulfide reduction, and resistance to nalidixic acid and cephalothin. The isolates were then stored in 20% glycerol broth (Nutrient broth and glycerol) for further studies. Specifically, the hippurate hydrolysis test was used to differentiate between *C. jejuni* and *C. coli*.

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing was done using the Kirby-Bauer disc diffusion method against discs of azithromycin (15 µg), amoxicillin/clavulanate (30 µg), cefuroxime (30 µg), ertapenem (10 µg), streptomycin (10 µg), amikacin (30 µg), ofloxacin (5 µg) and chloramphenicol (30 µg). The antibiotic disks were obtained from Oxoid. The test organisms were standardized to conform with 0.5 MacFarland standard and inoculated unto Mueller Hinton agar plates (Oxoid). The antibiotics disks were aseptically placed on the inoculated plates and incubated under microaerophilic conditions for 24 hours. Zones of inhibition were measured and interpreted according to CLSI and EUCAST standards [20, 21]. Isolates that showed resistance to at least three different classes of antibiotics were recorded as Multi-Drug Resistant (MDR). According to the method described by Ogbomo et al. [22], the Multiple Antibiotic Resistance (MAR) index was calculated as the ratio of the number of antibiotic isolates that were resistant to the total number of antibiotics the isolates were exposed to. A MAR index greater than 0.2 indicates that the isolate is from a source where antibiotics are often used.

2.4. Detection of the virulence genes

The DNA of test isolates were extracted using the conventional boiling method as described by Kalantar et al. [23], and the *cadF* and *hipO* were amplified using multiplex polymerase chain reaction as previously described by Al Amri et al. [18] with some modifications. The specific primers used in the detection of the genes are shown in Table 2. The PCR reaction mixture consisted of 12.5 µl 5x Red load Taq Master Mix (RedTaq), 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 mixtures were subjected to initial denaturalization at 95ºC for 3 minutes, followed by 45 cycles at 94ºC for 30 seconds, an annealing temperature of 49ºC for 30 seconds, and 72ºC for 1 minute, and a final extension at 72ºC for 5 minutes. The PCR was carried out in a thermocycler (Applied Biosystem 2720). The PCR products were

electrophoresed on 1.2% agarose gel stained with ethidium bromide and were viewed using a gel imaging system.

Table 2. The primers for detection of virulence genes.

Target Gene	Primer sequence (5'-3')	Product size (bp)	Reference
cadF	F-TTGAAGGTAATTTAGATATG	400	[17]
	R-CTAATACCTAAAGTTGAAAC		
hipO	F-GAAGAGGGTTTGGGTGGT	735	
	R-AGCTAGCTTCGCATAATAACTTG		

2.5. Statistical analysis

The relationship between the occurrence of *Campylobacter* species with respect to the category (either adult or young) of cattle was analyzed using Chi-square (χ^2) test on Microsoft Excel 2016.

3. RESULTS

3.1. Occurrence of *Campylobacter* species in pig and cattle dungs

Out of the 40 samples collected (20 each from pigs and cattle), 37 (92.5%) were positive for *Campylobacter* isolation out of which 18 (90.0%) were positive in pigs, while 19 (95.0%) were positive in cattle. Of the 18 from pig dung, 10 (83.3%) were from adults, while 8 (100%) were from young animals. Also, of the 19 from the cattle dung, 11 (91.6%) and 8 (100.0%) were from adults and young animals, respectively. The occurrence of *Campylobacter* spp. was higher (95.0%) in cattle than in pigs (90.0%), while in the samples collected from young animals, occurrence rates were higher compared to those collected from adult animals. However, the Chi-square test showed that there was no statistically significant difference ($p>0.05$) in the occurrence of *Campylobacter* species in relationship to the age of the animals.

3.2. Distribution of *Campylobacter* species isolated from pig and cattle samples

A total of 236 isolates were obtained from the pig and cattle dungs, comprising 154 (65.3%) from pig and 82 (34.7%) from cattle. The biochemical characteristics of the isolates showed that of the 154 pig samples, *C. coli* was the most occurring (43.5%), followed by *C. jejuni* (23.4%) (Table 3). This was also the same when the animals were grouped, *C. coli* was the most occurring isolate in adult (42.4%) and young (45.2%) pigs.

In the cattle samples, *C. jejuni* was the most occurring (40.2%), followed by *C. upsaliensis* (32.9%). The same was observed among adult and young animals with *C. jejuni* (48.9% in adults and 28.6% in young) and *C. upsaliensis* (29.8% in adults and 37.1% in young) (Table 3).

3.3. Antibiotic resistance of *Campylobacter* species in pig dung samples

The antibiotics-resistance pattern of the *Campylobacter* spp. from the pig dung samples showed that 53.9% were resistant to cefuroxime, while 38.3% and 31.2% showed resistance to amoxicillin/clavulanate and streptomycin, respectively. Furthermore, among the *C. jejuni*, the highest resistance (52.8%) was to cefuroxime, this observation was the same for the other species, except for *C. fetus* that showed no resistance to any of the tested antibiotics (Table 4).

Table 3. *Campylobacter* species isolated from pig and cattle dungs n (%).

Sample	Isolates						
	C. coli	*C. jejuni*	*C. upsaliensis*	*C. lari*	*C. fetus*	*C. hyointestinalis*	Total
PIGS							
Adult	39 (42.4)	23 (25.0)	17 (18.5)	12 (13.0)	1 (1.1)	0 (0)	92 (59.7)
Young	28 (45.2)	13 (21.0)	12 (19.4)	9 (14.5)	0 (0.0)	0 (0.0)	62 (40.3)
Total	67 (43.5)	36 (23.4)	29 (18.8)	21 (13.6)	1 (0.6)	0 (0.0)	154 (65.3)
CATTLE							
	C. jejuni	*C. upsaliensis*	*C. lari*	*C. coli*	*C. fetus*	*C. hyointestinalis*	
Adult	23 (48.9)	14 (29.8)	6 (12.8)	3 (6.4)	1 (2.1)	0 (0.0)	47 (57.3)
Young	10 (28.6)	13 (37.1)	3 (8.6)	6 (17.1)	2 (5.7)	1 (2.9)	35 (42.7)
Total	33 (40.2)	27 (32.9)	9 (11)	9 (11)	3 (3.7)	1 (1.2)	82 (34.7)

Table 4. Antibiotic resistance pattern of *Campylobacter* species isolated from the pig dung samples n (%).

	***Campylobacter* spp. n = 154**					
Antibiotics	***C. jejuni***	***C. coli***	***C. lari***	***C. fetus***	***C. upsaliensis***	
	n=36	n=67	n=21	n=1	n=29	TR
AZM	8 (22.2)	19 (28.4)	4 (19.0)	0 (0.0)	1 (3.4)	32 (20.8)
AMC	14 (38.9)	35 (52.2)	6 (28.6)	0 (0.0)	4 (13.8)	59 (38.3)
CXM	19 (52.8)	44 (65.7)	13 (61.9)	0 (0.0)	7 (24.1)	83 (53.9)
OFX	1 (2.8)	3 (4.5)	1 (4.8)	0 (0.0)	1 (3.4)	6 (3.9)
ETP	2 (5.6)	7 (10.4)	2 (9.5)	0 (0.0)	2 (6.9)	13 (8.4)
S	10 (27.8)	22 (32.8)	8 (38.1)	0 (0.0)	4 (13.8)	48 (31.2)
AK	0 (0.0)	2 (3.0)	2 (9.5)	0 (0.0)	1 (3.4)	5 (3.2)
C	4 (11.1)	12 (17.9)	2 (9.5)	0 (0.0)	2 (6.9)	20 (13.0)

KEYS: 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 (10 µg); C: Chloramphenicol (30 µg).

3.4. Antibiotic resistance of *Campylobacter* species in cattle dung samples

The antibiotic resistance of the isolates obtained from the cattle dung samples showed that 42.7%, 18.3%, and 23.2% were resistant to cefuroxime, amoxicillin/clavulanate, and streptomycin, respectively. The highest resistance among the species was to cefuroxime. The only *C. hyointestinalis* was resistant to cefuroxime but susceptible to the other antibiotics tested. Resistance to ertapenem was the lowest resistance, with *C. jejuni* that showed resistance of 2.0%, *C. lari* 22.2% and no resistance by other isolates (Table 5).

3.5. Antibiotypes and multiple antibiotic resistance index of multidrug *Campylobacter* species selected for detection of virulence genes

A total of 25 isolates, selected based on MAR index (between 0.4 and 1.0) comprised 17 from pigs and 8 from cattle. The most occurring resistance pattern observed was AZM-AMC-CXM-C exhibited by *C. coli* (3 isolates) and *C. jejuni* (1 isolate) and AZM-AMC-CXM-S-C exhibited by 2 isolates each of *C. coli* and *C. jejuni*. This observed pattern had a MAR index of 0.5. A MAR index of 1.0 was observed among 2 isolates of *C. coli* and 1 isolates of *C. lari* (Table 6).

Table 5. Antibiotic Resistance Pattern of *Campylobacter* species Isolated from the Cattle Dung samples n (%).

	***Campylobacter* spp. n = 82**						
Antibiotics	***C. jejuni***	***C. coli***	***C. lari***	***C. fetus***	***C. upsaliensis***	***C. hyointestinalis***	
	n=33	n=9	n=9	n=3	n=27	n=1	TR
AZM	7 (21.2)	2 (22.2)	3 (33.3)	0 (0.0)	1 (3.7)	1 (100.0)	14 (17.1)
AMC	6 (18.2)	1 (11.1)	4 (44.4)	0 (0.0)	4 (14.8)	0 (0.0)	15 (18.3)
CXM	13 (39.4)	5 (55.6)	5 (55.6)	3 (100.0)	8 (29.6)	1 (100.0)	35 (42.7)
OFX	1 (3.0)	0 (0.0)	3 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	4 (4.9)
ETP	1 (3.0)	0 (0.0)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.7)
S	10 (30.3)	2 (22.2)	5 (55.6)	1 (33.3)	1 (3.7)	0 (0.0)	19 (23.2)
AK	0 (0.0)	1 (11.1)	5 (55.6)	0 (0.0)	1 (3.7)	0 (0.0)	7 (8.5)
C	5 (15.2)	3 (33.3)	4 (44.4)	0 (0.0)	0 (0.0)	0 (0.0)	12 (14.6)

KEYS: 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 (10 µg); C: Chloramphenicol (30 µg).

Table 6. Antibiotypes of multidrug-resistant strains (n=25).

Antibiotypes	**Classes of antibiotics**	***C. jejuni***	***C. coli***	***C. lari***	***C. upsaliensis***	**Total**	**MAR Index**
OFX-ETP-C	3	0	0	0	1	1	0.4
CXM-OFX-S-AK	3	0	0	1	0	1	0.5
AMC-CXM-S-C	4	0	2	0	0	2	0.5
AZM-AMC-CXM-S	4	1	3	0	0	4	0.5
AZM-AMC-CXM-C	4	1	2	0	0	3	0.5
AZM-AMC-CXM-ETP	4	0	1	0	0	1	0.5
CXM-OFX-ETP-S	4	1	0	0	0	1	0.5
AMC-CXM-S-AK-C	4	0	0	1	0	1	0.6
AZM-AMC-CXM-S-C	5	2	2	0	0	4	0.6
AMC-CXM-OFX-ETP-C	5	0	1	0	0	1	0.6
AMC-CXM-OFX-S-AK-C	5	0	1	0	0	1	0.8
AZM-AMC-CXM-ETP-S-AK-C	6	0	0	1	0	1	0.9
AZM-AMC-CXM-OFX-ETP-S-AK	6	0	0	1	0	1	0.9
AZM-AMC-CXM-OFX-ETP-S-AK-C	7	0	2	1	0	3	1.0

KEY: AZM: Azithromycin; AMC: Amoxicillin/Clavulanate; CXM: Cefuroxime; OFX: Ofloxacin; ETP: Ertapenem; S: Streptomycin; AK: Amikacin; C: Chloramphenicol.

3.6. Occurrence of Virulence Genes in multidrug-resistant *Campylobacter* isolates

Out of the 25 MDR strains selected for the detection of *cadF* and *hipO* virulence genes, 13 (76.5%) and 6 (75.0%) of the isolates from the pig and cattle samples, respectively, possessed both virulence genes (Figure 1). Furthermore, *hipO* gene was detected in 2 (11.8%) of the isolates from pigs and 4 (50.0%) of the isolates from cattle. Also, it was observed that the *hipO* gene was detected only in the isolates that were identified as *C. jejuni*, while the *cadF* gene was detected in all the isolates regardless of their identity. Moreover, all the isolates from the pigs and cattle samples that carried the *hipO* gene also possessed the *cadF* gene.

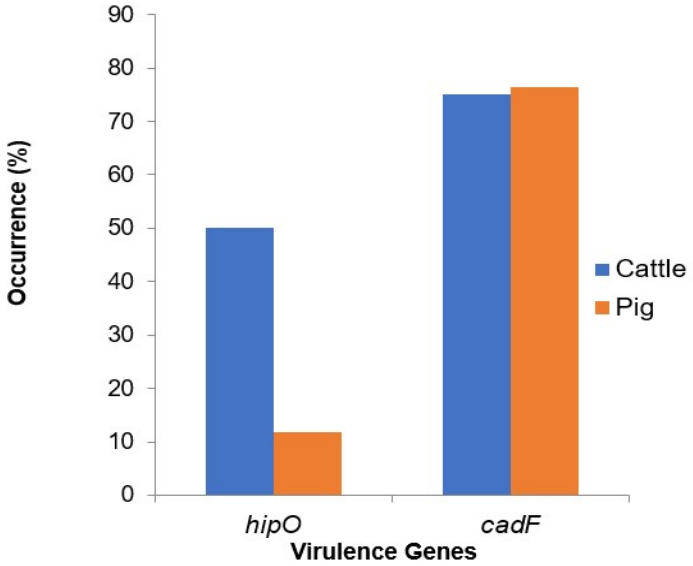

Figure 1. Occurrence of virulence genes in *Campylobacter* species isolated from pigs and cattle dungs. Sample size = 8 cattle and 17 pigs.

4. DISCUSSION

In this present study, patterns of antibiotic resistance and the presence of virulence genes in *Campylobacter* species isolated from pig and cattle dungs in a tertiary institution research farm were examined. The occurrence (90.0%) of *Campylobacter* species in pig samples in this study is in agreement with the previously reported range of its occurrence in pigs which is between 50-100%. This observation is also comparably similar to the rate (92.7%) reported from a similar study in Kebbi, a city in the Northern part of Nigeria. An even higher occurrence (95.0%) was observed in cattle in this present study, Hanon et al. [24] reported a high occurrence of 87.0% in their study on *Campylobacter* prevalence in cattle. These noticeably high occurrence rates of *Campylobacter* species substantiate the assertion that *Campylobacter* species are intestinal commensals of cattle and pigs.

The observation that a higher occurrence rate was obtained from the young animal samples compared to adults in this study is consistent with the findings of Thépault et al. [25], who also reported a higher occurrence of *Campylobacter* species in calves than in adult cattle. The reason for this might be due to an improved level of immunity of the adult animals which is expected to be better compared to the young animals. Secondly, it might also be a result of the fully developed forestomach compartment where a combination of high volatile fatty acid concentration and low pH inhibits the growth of some commensals. However, there was no statistically significant difference in the occurrence of *Campylobacter* species in pigs and cattle.

In the present study, it was observed that *C. coli* was the most prevalent species from the pigs' sample, this is in agreement with the report of previous similar studies [26, 27]. This observation corroborates the established assertion that *C. coli* is commensal to pigs' guts and can, therefore, be easily recovered from their faeces [28 29]. It has also been previously reported that *C. coli* are the main *Campylobacter* species in pigs [30].

Furthermore, *C. jejuni* being the most occurring species in the cattle samples in this study, agrees with a previous study on cattle faecal swab samples in Sokoto, Nigeria, where *C. jejuni* was also reported to be the most prevalent species [31]. Similarly, the observation that *C. jejuni* was the most isolated species in adult cattle is in agreement with the report of another study on adult cattle from a slaughterhouse in France [25]. This may not be a surprise because *C. jejuni* is the most occurring *Campylobacter* species in most carriers. However, the

C. upsaliensis that was observed to be the most occurring species among the young cattle samples in the present study is not in agreement with a study on young cows in Finland where the reported most occurring *Campylobacter* species was found to be *C. hyointestinalis* [32]. This observation is particularly surprising because the high occurrence of *C. upsaliensis* has been mainly found to be associated with cats and dogs. By observation, none of these domestic animals were found to be associated with the sites where the samples were collected. In the present study, one *C. hyointenstinalis* was isolated from the cattle sample, this low number corroborates previous studies where *C. hyointestinalis* were isolated at the lowest rates [27, 33] compared to other reported species, however, unlike the present study, it was isolated in pigs from the study of Gwimi *et al.* [27]. *Campylobacter hyointestinalis* is implicated as a pathogen causing gastroenteritis and diarrhea in humans and has been recovered from human stool samples [27, 33].

The patterns of resistance of *Campylobacter* species isolated from pig samples to cefuroxime and ertapenem in this study are similar to the patterns reported for *Campylobacter* species obtained from human diarrhoeal stool samples in the Vhembe district of South Africa [34]. This high resistance to cefuroxime may be due to the suspected high use of cephalosporin in the pig pens; all efforts to obtain the list of antibiotics used at the sample site were not successful. Our study observed a lower level of resistance among the isolates to azithromycin (20.2%), this should be a great cause for concern, even with a rate this low because macrolides are the drugs of choice for the treatment of campylobacteriosis. Hence the finding from this present study is an indication of increased resistance to the antibiotic. This observation is also consistent with the findings of Marotta et al. [35] and Papadoupolous et al. [36].

The observed lowest level of resistance of the isolates to the fluoroquinolone drug tested in this study (ofloxacin), is not in agreement with most findings among *Campylobacter* in pigs where very high resistant to fluoroquinolones (mostly ciprofloxacin) from the pigs were reported. The reason for this is not well understood; however, a study conducted in Morogoro, Tanzania, reported a low level of resistance to fluoroquinolone with values similar to the one obtained from this study [37].

The observed high antibiotic resistance of *Campylobacter* species isolated from the cattle samples to cefuroxime, azithromycin and streptomycin in this study may be a result of the use of antibiotics in the sample site, we must state that this is clearly a hypothesis of ours due to how most livestock farms are being operated in the area and country where this study was carried out. The high resistance of the isolates to cefuroxime is comparably similar compared with the report of another study in Ghana [38]. The observed resistance of the isolates in this study to chloramphenicol is consistent with a study carried out on *Campylobacter* species in Sub-Saharan Africa [39], it also agrees with the findings of a recent study carried out in Addis Ababa, Ethiopia [40]. The observation that showed a low resistance of the isolates to ertapenem in this study agrees with the report of a previous study where carbapenems were generally found to be effective against *Campylobacter* species. As a result of this, it has been suggested that they could be regarded as the drug of choice for severe and invasive campylobacteriosis [41].

Detection of the *cadF* virulence gene in *Campylobacter* species in this study agrees with the previous assertion that the presence of the *cadF* gene in *Campylobacter* is irrespective of species. This has made it possible for the *cadF* gene to be used as a means of identification of *Campylobacter* spp. [17, 42, 43]. It has been reported that the presence of the *cadF* gene helps the organism to adhere to fibronectin, and it also has been demonstrated *in vivo* using a chicken model that the gene helps the organism to successfully colonize the host [44]. However, the high occurrence (76.5% from pigs and 75.0% from cattle) of *cadF* in this study is lower compared to the 100.0% reported in a study carried out on human and chicken faeces in Durban, KwaZulu-

Natal province of South Africa [17]. Despite the difference, this occurrence shows that *Campylobacter* isolates from pig and cattle feces have pathogenic properties that could be harmful to humans and animals.

Furthermore, the 11.8% and 50.0% *hipO* gene detected in the *C. jejuni* isolated from pigs and cattle, respectively, is lower compared to the 68.0% and 67.0% previously reported in *C. jejuni* isolates from humans and chicken, respectively [17]. The reason for this difference might be studied samples. Although the role of the *hipO* gene in the pathogenesis of *C. jejuni* is still controversial, it has been reported that it is responsible for the production of hippuricase or N-acetyl aminohydrolase which is an enzyme that enables *C. jejuni* to degrade hippuric acid in the large intestine [45, 46]. Moreover, all the isolates that carried the *hipO* gene also possessed the *cadF* gene in the current study, which is similar to the observation from another study on humans and chickens by Reddy and Zishiri [17].

In addition to all that has been discussed above, we do recommend that further testing, such as whole genome sequencing and multi-locus sequence typing, should be carried out to ascertain that strains of the same species are not phylogenetically related. The limitation of the present study is the inability to carry out the whole Genome Sequencing, which would have shown the phylogenetic relatedness of the strains of the same species.

5. CONCLUSION

The overall high occurrence and frequency of *Campylobacter* spp. in the samples of pig and cattle faeces, coupled with the multidrug-resistant species detected among the isolates, is of great concern because of the common practice of indiscriminate discharge of untreated animal faecal wastes into the environment. Therefore, there should be calls for caution in the indiscriminate discharge of untreated animal faecal wastes into the environment and water bodies as these wastes can come in contact with humans and cause a health issue

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REFERENCES

1. Samuel MC, Vugia DJ, Shallow S, Marcus R, Segler S, McGivern T, et al. Epidemiology of sporadic Campylobacter infection in the United States and declining trend in incidence, Food Net 1996-1999. Clin Infect Dis. 2004; 38(3): S165-S174.
2. Nguyen TNM, Hotzel H, El-Adawy H, Tran HT, Hong Le MT, Tomaso H, et al. Genotyping and antiobiotic resistance of thermophilic Campylobacter isolated from chicken and pig meat in Vietnam. Gut Pathol. 2016: 8(19): 1-11.
3. World Health Organisation. Campylobacter: Fact sheet detail 2018. Retrieved July 29, 2018 from https://www.who.int/en/news-room/fact-sheets/detail/campylobacter.
4. Dingle KE, Colles FM, Wareing DR, Ure R, Fox AJ, Bolton FE, et al. Multilocus sequence typing system for Campylobacter jejuni. J Clin Microbiol. 2001; 39: 14-23.

5. Wilson DJ, Gabriel E, Leatherbarrow AJH, Cheesbrough J, Gee S, Bolton E, et al. Tracing the Source of Campylobacteriosis. PLoS Genetics 2008; 4(9): e1000203
6. Lindmark H, Boqvist S, Ljungstrom M, Bjorkholm B, Agren P, Engstrand L. Risk Factors for Campylobacteriosis: an Epidemiological Surveillance Study of Patients and Retail Poultry. J Clin Microbiol. 2009; 47(8): 2616-2619.
7. Wieczorek K, Szewczy KR, Osek J. Prevalence, antimicrobial resistance and molecular characterization of Campylobacter jejuni and Campylobacter coli isolated from retail raw meat in Poland. Vet Med. 2012; 57(6): 293-299.
8. Coker AO, Isokpehi RD, Thomas BN, Amisu KO, Obi CL. Human campylobacteriosis in developing countries. Emerg Infect Dis. 2002; 8: 237-243.
9. Aarestrup FM, Engberg J. Antimicrobial resistance of thermophilic Campylobacter. Vet Res. 2001; 32: 311-321.
10. Padungton P, Kaneene JB. Campylobacter spp. in human, chickens, pigs and their antimicrobial resistance. J Vet Med Sci. 2003; 65: 161-170.
11. Moore JE, Corcoran D, Dooley JSG, Fanning S, Lucey B, Matsuda M, et al. Campylobacter. Vet Res. 2005; 36: 351-382.
12. Prasad KN, Mathur SK, Dhole TN, Ayyagari A. Antimicrobial susceptibility and plasmid analysis of Campylobacter jejuni isolated from diarrhoeal patients and healthy chickens in Northern India. J Diarrh Dis Res. 1994; 12(4): 270-273.
13. Chen X, Naren GW, Wu CM, Wang Y, Dai L, Xia LN, et al. Prevalence and antimicrobial resistance of Campylobacter isolates in broilers from China. Vet Microbiol. 200; 144(1-2): 133-139.
14. Qin SS, Wu CM, Wing Y, Jeon B, Shen ZQ, Wing Y, et al. Antimicrobial resistance in Campylobacter coli isolated from pigs in two provinces of China. Int J Food Microbiol. 2011; 146(1): 94-98.
15. Talukder KA, Aslam M, Isla Azm Dutt DK, Hossain S, Nur-E-Kamal A, Nair GB, et al. Prevalence of virulence genes and cytolethal distending toxin production in Campylobacter jejuni isolates from diarrhoeal patients in Bangladesh. J Clin Microbiol. 2008; 46(4): 1485-1488.
16. Bardon J, Pudova V, Kolackova I, Kapriskova R, Roderova M, Kolar M. Virulence and Antibiotic Resistant Genes in Campylobacter spp. in the Czech Republic. Epidem Microbiol Imunolog. 2017; 66: 59-66.
17. Reddy S, Zishiri OT. Genetic characterisation of virulence genes associated with adherence, invasion and cytotoxicity in Campylobacter spp. isolated from commercial chickens and human clinical cases. Onderstepoort J Vet Res 2018; 85(1): a1507.
18. Al Amri A, Senok AC, Ismaeel AY, Al-Mahmeed AE, Botta GA, Lake R. Human and animal Campylobacteriosis in Tanzania: A review. BMC Microbiol. 2013; 95: 169-174.
19. United Kingdom Standards for Microbiology Investigations. Bacteriology Test Procedures: Motility. Pub Hlth Eng. 2018; 4: 1-16.
20. CLSI - Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S15. Clinical and Laboratory Standards Institute, Wayne 2018.
21. EUCAST - European Committee on Antimicrobial Susceptibility Testing. Antimicrobial wild type distributions of microorganisms 2019. Available from https://mic.eucast.org/Eucast2/.
22. Ogbomon EO, Akpomie OO, Enenya RP, Obanor O, Morka E. Prevalence and Antibiotic Susceptibility Patterns of Campylobacter Species in Locally Pasteurized Milk Product (Nunu) Sold in Zaria Metropolis, Kaduna State, Nigeria. Int J Microbiol Biotech. 2018; 3(3): 89-94.
23. Kalantar M, Soltan Dallal MM, Fallah F, Yektaie F. Monitoring the Virulence Genes in Campylobacter coli Strains Isolated from Chicken Meat in Tehran, Iran. Infect Epidemiol Microbiol. 2017; 3: 12-15.

24. Hannon SJ, Allan B, Waldner C, Russell ML, Potter A, Babiuk LA, Townsend HGG. Prevalence and risk factor investigation of Campylobacter species in beef cattle feces from seven large commercial feedlots in Alberta, Canada. Canad J Vet Res. 2009; 73: 275-282.
25. Thépault A, Poezevara T, Quesne S, Rose V, Chemaly M, Rivoal K. Prevalence of Thermophilic Campylobacter in Cattle Production at Slaughterhouse Level in France and Link Between C. jejuni Bovine Strains and Campylobacteriosis. Front Microbiol. 2018; 9: 1-9.
26. Obeng AS, Rickard H, Sexton M, Pang Y, Peng H, Barton M. Antimicrobial susceptibilities and resistance genes in Campylobacter strains isolated from poultry and pigs in Australia. J App Microbiol. 2012; 113: 294-307.
27. Gwimi PB, Faleke OO, Salihu MD, Magaji AA, Abubakar MB, Nwankwo IO, Ibitoye EB, Prevalence of Campylobacter species in fecal samples of pigs and humans from Zuru Kebbi State, Nigeria. Int J One Hlth, 2015; 1: 1-5.
28. Guévremont E, Higgins R, Quessy S. Characterization of Campylobacter isolates recovered from clinically healthy pigs and from sporadic cases of campylobacteriosis in humans. J Food Protect. 2004; 67(2): 228-234.
29. Horrocks SM, Anderson RC, Nisbet DJ, Ricke SC. Incidence and ecology of Campylobacter jejuni and coli in animals. Anaerob. 2009; 15(1-2): 18-25.
30. Kempf I, Kerouanton A, Bougeard S, Nagard B, Rose V, Mourand G, Osterberg J, Denis M, Bengtsson BO. Campylobacter coli in organic and conventional pig production in France and Sweden: prevalence and antimicrobial resistance. Front Microbiol. 2017; 8: 955.
31. Salihu MD, Junaidu AU, Oboegbulan SI, Egwu GO, Magaji AA, Lawal M, Hassa Y. Isolation and prevalence of Campylobacter species in Cattle from Sokoto State, Nigeria. Vet Italia. 2009; 45(4): 501-505.
32. Hakkinen M, Heiska H, Hänninen ML. Prevalence of Campylobacter spp. in cattle in Finland and antimicrobial susceptibilities of bovine Campylobacter jejuni strains. Appl Environ Microbiol. 2007; 73(10): 3232-3238.
33. Audu BJ, Norval S, Bruno L, Meenakshi R, Marion M, Forbes KJ. Genomic diversity and antimicrobial resistance of Campylobacter spp. from humans and livestock in Nigeria. J Biomed Sci. 2022; 29(1): 7.
34. Samie A, Ramalivhana J, Igumbor EO, Obi CL. Prevalence, haemolytic and haemagglutination activities and antibiotic susceptibility profiles of Campylobacter spp. isolated from human diarrhoeal stools in Vhembe District, South Africa. J Hlth Pop Nutr. 2007; 25(4): 406-413.
35. Marotta F, Di Marcantonio L, Janowicz A, Pedonese F, Di Donato G, Ardelean A, et al. Genotyping and Antibiotic Resistance Traits in Campylobacter jejuni and coli From Pigs and Wild Boars in Italy. Front Cell Infect Microbiol. 2020; 10: 592512.
36. Papadopoulos D, Petridou E, Papageorgiou K, Giantsis IA, Delis G, Economou V, et al. Phenotypic and Molecular Patterns of Resistance among Campylobacter coli and Campylobacter jejuni Isolates, from Pig Farms. Animals. 2021; 11(8): 2394.
37. Komba EV, Mdegela RH, Msoffe PL, Matowo DE, Maro MJ. Occurrence, species distribution and antimicrobial resistance of thermophilic Campylobacter isolates from farm and laboratory animals in Morogoro, Tanzania. Vet World. 2014; 7(8): 559-565.
38. Karikari AB, Obiri-Danso K, Frimpong EH, Krogfelt KA. Antibiotic Resistance of Campylobacter Recovered from Faeces and Carcasses of Healthy Livestock. Biomed Res Int. 2017: 1-9.
39. Hlashwayo DF, Sigaúque B, Bila CG. Epidemiology and antimicrobial resistance of Campylobacter spp. in animals in Sub-Saharan Africa: A systematic review. Heliyon, 2020; 6(3): e03537.

40. Chala G, Eguale T, Abunna F, Asrat D, Stringer A. Identification and Characterization of Campylobacter Species in Livestock, Humans, and Water in Livestock Owning Households of Peri-urban Addis Ababa, Ethiopia: A One Health Approach. Front Pub Hlth. 2021; 9: 750551.
41. Fernandez-Cruz A, Munoz P, Mohedano R, Valerio M, Marin M, Alcala L, et al. Campylobacter bacteraemia: Clinical characteristics, incidence and outcome over 23 years. Medicine. 2010; 89(5): 319-330.
42. Rozynek E, Dzierzanowska-Fangrat K, Jozwiak P, Popowski J, Korsak D, Dzierzanowska D. Prevalence of potential virulence markers in Polish Campylobacter jejuni and Campylobacter coli isolates obtained from hospitalized children and from chicken carcasses. J Med Microbiol. 2005; 54: 615-619.
43. Al Amri A, Senok C, Ismaeel AY, Al-Mahmeed AE, Botta GA. Multiplex PCR for direct identification of Campylobacter spp. in human and chicken stools. J Med Microbiol. 2007; 56: 1350-1355.
44. Thibodeau A, Fravalo P, Taboada EN, Laurent-Lewandowski S, Guévremont E, Quessy S, Letellier A. Extensive characterization of Campylobacter jejuni chicken isolates to uncover genes involved in the ability to compete for gut colonization. BMC Microbiol. 2015; 15: 97.
45. Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, et al. Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. Int J Food Microbiol. 2010; 139: 3-15.
46. Nejabat M, Bazargani A, Nazari N. The frequency of virulence genes: flaA, hipO and wlan among Campylobacter jejuni isolates obtained from clinical specimens in shiraz teaching hospitals. Revista Publicando, 2018; 5(17): 140-148.