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Occurrence of antibiotic resistant *Campylobacter* in wild birds and poultry

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ABSTRACT

Aims: *Campylobacter* is a major cause of gastroenteritis in humans worldwide, particularly in developed countries and is reported to show an increased trend in antibiotic resistance. The purpose of this study was to determine the occurrence of *Campylobacter* in wild birds, poultry and in poultry environments in Selangor, Malaysia as well as to determine the rate of antibiotic resistance among *Campylobacter* isolates from poultry and wild birds.
Methodology and results: The wild birds were trapped near poultry farm areas and in open areas which were more than 5 km away from poultry farms (refered to as open environment). Of 57 wild birds trapped near the farm environment, 17.5% were positive for *Campylobacter* and out of these, 90% were *Campylobacter jejuni*. Of a total of 77 birds in the open environment, 22.1% were positive for *Campylobacter* and of these 88.7% were *C. jejuni*. The poultry farms consisted of 3 chicken and 2 duck farms. About 60% of the chickens and 44.8% of the ducks were positive for *Campylobacter* of which 80% were *C. jejuni*, while 20% were *Campylobacter coli*. The *Campylobacter* isolates were subjected to antibiotic susceptibility test using disk diffusion method against 12 antibiotics. All the isolates (100%) from wild birds around poultry houses were resistant to at least one antibiotic.
Conclusion, significance and impact of study: The findings showed 93% of the isolates from wild birds were resistant to at least two antibiotics. *Campylobacter* isolates from poultry in the farms were resistant to at least one antibiotic. The antibiotic resistant *Campylobacter* is of public health importance.

Keywords: antibiotic, resistance, *Campylobacter*, poultry, wild birds

INTRODUCTION

Thermophilic *Campylobacter* is one of the most common causes of human gastroenteritis worldwide particularly in developed countries. *Campylobacter jejuni* is reported to be the most frequent *Campylobacter* species isolated from humans and animals (Haruna *et al.*, 2013; Komba *et al.*, 2015). Most *Campylobacter* cause acute but usually self-limiting illness characterized by diarrhea, fever and abdominal cramps. However, in some instances severe infections can occur (Shin *et al.*, 2015). Antimicrobial treatment can shorten the duration of illness and may be lifesaving in serious infections (Komba *et al.*, 2015).

Poultry is recognized as an important reservoir for *Campylobacter* and poultry meat contaminated with this pathogen may results in high percentage of human campylobacteriosis (Colles *et al.*, 2016). The presence of *Campylobacter* in poultry farms mainly results from contaminated water, contaminated air from adjacent poultry houses, poultry litter, contamination during transportation, presence of other infected livestock on the farm, mechanical transmission such as via insects, and infected wild birds (Bull *et al.*, 2006; Cox *et al.*, 2012). There is strong evidence supported by reports highlighting that wild birds in countries where studies have been carried are reservoirs and potential sources of *Campylobacter* infection in farm animals and humans.

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Several studies had shown that *Campylobacter* were isolated from humans, animals, as well as the environment and that the reservoirs for *Campylobacter* are typically domestic animals in particular poultry, pigs and wild birds. These birds have great mobility that they can spread these pathogens in the environment (Saleha *et al.*, 2001; Sensale *et al.*, 2006; Tsiodras *et al.*, 2008; Rahimi *et al.*, 2011). The presence of these birds around recreational areas, areas of food processing plants, farms, pastures, water reservoirs and residential areas can threaten human health. Among the wild birds that were found to carry *Campylobacter* include wild fowls, shore birds, gulls (Waldenström *et al.*, 2002), pigeons (Rahimi *et al.*, 2011) and crows (Matsuda *et al.*, 2003). Owing to the excessive mobility of the wild birds, it is reported that they might work as effectual spreaders of disease through fecal contamination of poultry farms environment (equipment, soil, water, and feed) (Bull *et al.*, 2006; Patriarchi *et al.*, 2011).

For clinical therapy of campylobacteriosis, erythromycin (a macrolide) is considered as the drug of choice, but fluoroquinolones (e.g., ciprofloxacin) are also commonly used owing to their expansive continuum of activity against enteric pathogens (Luangtongkum *et al.*, 2009). The results from a study on *Campylobacter* (Chen *et al.*, 2010) showed that a vast majority of the isolates from birds appeared to be resistant to fluoroquinolones by more than 98%. In the same study, *C. jejuni* was resistant to gentamicin at 27.2%. Importantly, a previous study from The Netherlands revealed a substantial increase in fluoroquinolone resistance among human cases since the use of enrofloxacin in veterinary medicine (Endtz *et al.*, 1991). According to Zendehbad *et al.* (2013), *Campylobacter* spp. isolates from chicken, quail, and turkey meat in Mashhad (Iran) showed low resistance to streptomycin at 4.9%, 7.4% and 5.4%, respectively. Alternative drugs used in cases of systemic infection with *Campylobacter* include tetracycline and gentamicin (Ge *et al.*, 2002). However, the growing resistance to *Campylobacter* is of a serious concern globally. *Campylobacter* is continuously exposed to antibiotics used in animal production and human medicine; thus, causing it to become resistant to antibiotics which can create limited therapeutic options (Luangtongkum *et al.*, 2009).

In an attempt to reduce vaccination or combat respiratory problems due to *E. coli*, fluoroquinolone (enrofloxacin) is administered in broiler chickens in the first week of life. However, such use of antibiotics can lead to the development of antibiotic resistance (Jacobs-Reitsma *et al.*, 1994; Landoni and Albarellos, 2015). In New Zealand and Iran, high percentage of wild birds in children playgrounds were found positive for *C. jejuni* (French *et al.*, 2009; Abdollahpour *et al.*, 2015). Thus, the objectives of this study were to determine the occurrence of *Campylobacter* in wild birds and poultry and the antibiotic resistance profile of *Campylobacter* isolated from these wild birds and poultry.

MATERIALS AND METHODS

Samples collection

Wild birds

The wild birds were collected from five selected locations which were more than 5 km away from poultry farms (referred as open environment) and in five poultry farms. In open environment, three locations were near forest areas, one was a residential area and the remaining one was in a wetland. The wild birds were trapped using a mist net which was set up in the morning and placed for 6 h. Every 20 minutes the trap was checked, and the captured birds were photographed, marked and sampled by taking a cloacal swab before their release. Similarly, the trap was set up between the poultry houses in poultry farms. Seventy-seven birds in the open environment and 57 birds in poultry farms were sampled.

Poultry

Five farms were visited which consisted of three chicken and two duck farms. The cloacal swab samples were collected from 31-37 chickens or ducks per farm. A total of 101 chickens and 103 ducks were sampled.

Isolation and identification of *Campylobacter* species

Each cloacal swab was streaked onto a plate of Modified *Campylobacter* Blood Free Selective Agar (mCCDA) (Oxoid) supplemented with cefoperazone, amphotericin and teicoplanin (CAT) selective supplement. The plates were then incubated for 48 h at 42 °C in gas jars under microaerophilic conditions generated by gas packs (BD CampyPakTM; Becton, Dickinson and Company, UK). Presumptive *Campylobacter* colonies were subjected to Gram staining for cellular morphology observation and hanging drop method under phase contrast microscopy to observe the motility characteristics and were then subcultured onto a freshly prepared Columbia blood agar (CBA) (Oxoid) supplemented with 5% defribinated horse blood and incubated at 42 °C for 24-48 h. All suspected *Campylobacter* isolates were further identified by oxidase, catalase production, hippurate hydrolysis and indoxyl acetate hydrolysis tests.

Confirmation of *Campylobacter* isolates using multiplex Polymerase Chain Reaction (mPCR) assay

DNA Extraction

A suspension of each fresh overnight culture was prepared in a 1.5 mL Eppendorf tube containing 1 mL of Brucella broth (Oxoid). The suspension was centrifuged at 16000× *g* for 2 min. The extraction of the genomic DNA was conducted using Wizard Purification Systems (Promega) according to the manufacturer's protocol. A total of 600 µL of nuclei lysis solution was added to the suspension and pipetted gently to mix. The suspension

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was incubated at 80 °C for 5 min and then allowed to cool at room temperature. Three microliters of RNase solution were added, mixed well and incubated at 37 °C for 60 min. Then 200 µL of the Protein Precipitate was added and vortexed. The suspension was then incubated on ice for 5 min. Subsequently, it was centrifuged at 16,000× *g* for 3 min. The supernatant was transferred into a clean 1.5 mL Eppendorf tube containing 600 µL of isopropanol kept at 25 °C. The mixture was centrifuged for 2 min at 16000× *g*. Then, an aliquot of 600 µL 70% ethanol was added and mixed. The suspension was further centrifuged for 2 min at 16000× *g*. The supernatant was discarded and was allowed to air-dry for 15 min. Finally, the DNA pellet was rehydrated in 100 µL of Rehydration Solution for 1 h at 65 °C.

Multiplex Polymerase Chain Reaction (mPCR) assay

The mPCR was performed in a 50 µL reaction volume. The reaction mixture consisted of 2 µL, 25 µL of PCR Master Mix (Promega), 18 µL of RNase free water.of genomic DNA and 5 µL of forward and reverse primers. Table 1 shows the primers (Bio Basic Inc., Canada) used in PCR assay for *Campylobacter* isolates confirmation. The mPCR assay procedure was performed in a Veriti™ 96-Well Eppendorf Thermal Cycler as described by Yamazaki-Matsune *et al*. (2007) as follows: initial activation step at 95 °C for 15 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, annealing at 58 °C for 90 sec and extension of 72 °C for 60 sec and final extension at 72 °C for 7 min. The following well characterized reference strains, namely *Campylobacter jejuni* (LMG 8841T), *Campylobacter coli* (JCM 2529T), *Campylobacter upsaliensis* (ATCC 43954T) and *Campylobacter lari* (JCM 2530T) were used as positive controls. Equal volume of RNase free water was used as negative control.

Agarose gel electrophoresis

Amplified PCR products were resolved in a 2% agarose gel (Agarose, LE Analytical Grade) prepared in a 1× Tris-Borate-EDTA (TBE) buffer (40 mM Tris-Borate, 2 mM EDTA, PH 7.5) and Gel-red (3 µL/mL) at 75V for 90 min. The electrophoresed gel was then observed under ultraviolet (UV) transilluminator using a gel documentation system Alpha Imager (BIO-RAD).

Antibiotic susceptibility test

Confirmed *Campylobacter* isolates were kept at –20 °C for future use. The frozen isolates were thawed at room temperature. Each isolate was subcultured on Colombia blood agar (Oxoid) and then incubated at 42 °C for 48 h under microaerophilic condition. Susceptibility antibiotic test was performed using the disc diffusion method as described by CLSI (2010) The isolates were tested against twelve antibiotics: ampicillin (A), 20 µg; gentamicin (Cn), 10 µg; cefotaxime (Ctx) 30 µg; ciprofloxacin (Cip) 5 µg; nalidixic acid (Na) 30 µg; erythromycin (E), 15 µg; streptomycin (S) 10 µg; tetracycline (Te), 30 µg; enrofloxacin (Enr) 5 µg; clindamycin (Da) 2 µg; sulfamethoxazole trimethoprim (Sxt) 25 µg; chloramphenicol (C) 30 µg. which were selected from the lists of Critically Important Antimicrobial for Human Medicine (WHO, 2011) and Antimicrobial Agents of Veterinary Importance (OIE, 2015).

Multidrug drug resistance (MDR) is defined as resistance to at least one antibiotic in four or more classes of antibiotic as reported in several European countries (Kumarasamy *et al.*, 2010).

Data analysis

Data for the occurrence of *Campylobacter* in wild birds from different locations were analyzed by Chi square test. The statistical significance was considered at $P< 0.05$.

RESULTS

Isolation and identification of *Campylobacter* in the birds and poultry

Wild birds in open and poultry farms environment

The occurrence of *Campylobacter* in wild birds from open environment and in poultry farms is shown in Table 2. There was no significant difference in the occurrence of *Campylobacter* in wild birds from the two locations (22.1 vs 17.5%). From the open environment, four species of birds were identified which consisted mainly of 31 Rock Pigeons (*Columba livia*), 24 Spotted Doves (*Streptopelia chinesis*), 14 Zebra Dove (*Geopelia striata*) and 8 Eurasian Tree Sparrow (*Passer montanus*) (Table 3). Out of the 77 birds trapped and sampled from the open environment, 22.1% were positive for *Campylobacter* (Table 2). In poultry farm locations, 57 wild birds were sampled with only three species identified which made up of 25 Eurasian Tree Sparrows, 23 Rock Pigeons and 9 Zebra Dove (Table 3) and of these, 17.5% were positive for *Campylobacter* (Table 2).

Table 4 shows the occurrence of *Campylobacter* spp., *C. coli* and *C. jejuni* in wild birds' species. The location had no significant ($P>0.05$) effect on the occurrences in Eurasian Tree Sparrow and Zebra Dove. However, the location showed a significant ($P<0.05$) effect in Rock Pigeon as the occurrence of *Campylobacter* spp. was high in these birds in the open environment compared to those in poultry farms.

Poultry

The prevalence of *Campylobacter* in chickens was 60.3% and all the isolates (100%) were identified as *C. jejuni* (Table 4). In ducks, the prevalence of *Campylobacter* was 44.8% with 80% of the isolates found to be *C. jejuni* and 20% *C. coli* (Table 4).

Moreover, mPCR assay was used for the confirmation of *Campylobacter*, *C. jejuni* and *C. coli* isolated from poultry and wild birds (Figure 1).

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Table 1: The primers used in PCR assay to confirm *Campylobacter* isolates.

Species	Primer	Oligonucleotide sequence	Size
Genus *Campylobacter*	CH412F	5'-GGA TGA CAC TTT TCG GAGC-3'	816bp
	C1228R	5'-CAT TGT AGC ACG TCT GTC-3'	
C. jejuni	C-1	5'-CCATAAGCACTAGCTAGCTGAT-3'	161bp
	C-3	5'-CCATAAGCACTAGCTAGCTGAT-3'	
C. coli	CC18FR	5'-GGTATGATTTCTACAAAGCGAG-3'	502bp
	CC519R	5'-ATAAAAGACTATCGTCGCGTG-3'	
C. lari	CLF	5'-TAG AGA GAT AGC AAA AGA GA-3'	251bp
	CLR	5'-TAC ACA TAA TAA TCC CAC CC-3'	
C. upsaliensis	CU61F	5'-CGA TGA TGT GCA AAT TGA AGC-3'	86bp
	CU146R	5'-TTC TAG CCC CTT GCT TGA TG-3'	

Source: Yamazaki-Matsune *et al.* (2007)

Table 2: Occurrence of *Campylobacter* in wild birds in open environment and near poultry farms.

Open environment	No. of wild birds	No. of birds positive	Occurrence of *Campylobacter* (%)
A	2	1	
B	16	11	
C	18	2	
D	17	0	
E	24	3	
Total	77	17	22.1
Poultry farms			
F	7	1	
G	10	0	
H	5	5	
I	24	4	
J	11	0	
Total	57	10	17.5
P-value			P>0.05

Table 3: Occurrence of *Campylobacter*, *C. coli* and *C. jejuni* in wild birds, chickens and ducks.

Wild birds in open environment				
Wild birds species (Scientific name) (No.)	No. of birds positive	No. of *Campylobacter* isolates	No. of *C. coli* isolates	No. of *C. jejuni* isolates
Eurasian Tree Sparrow (*Passer montanus*) (8)	5	0	1	4
Rock Pigeon (*Columba livia*) (31)	8	0	0	8
Zebra Dove (*Geopelia striata*) (14)	1	1	0	0
Spotted Dove (*Streptopelia chinesis*) (24)	3	0	0	3
Total 77	17 (22.1%)	1	1	15
Wild birds in poultry farms				
Eurasian Tree Sparrow (*Passer montanus*) (25)	9	1	0	8
Rock Pigeon (*Columba livia*) (23)	1	0	0	1
Zebra Dove (*Geopelia striata*) (9)	0	0	0	0
Total 57	10 (18.2%)	1	0	9
Poultry in farms	No. of poultry positive			
Chicken (101)	61 (60.4%)	0	0	61
Ducks (67)	30 (44.8%)	0	6	24
Total 168	91	0	6	85

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Table 4: Occurrence (%) of *Campylobacter*, *C. coli* and *C. jejuni* in wild birds, chickens and ducks.

	Wild birds' species			
	Eurasian Tree Sparrow (%)	Rock Pigeon (%)	Zebra Dove (%)	Spotted Dove (%)
No. of positive samples				
Open environment	62.5	25.8	7.1	12.5
In poultry farms	36.0	4.3	0	-
P-value	>0.05	*	>0.05	NA
Campylobacter isolated				
Open environment	0	0	100.0	0
In poultry farms	11.0	0	0	-
P-value	>0.05	>0.05	>0.05	NA
C. jejuni				
Open environment	80.0	100.0	0	100.0
In poultry farms	88.9	100.0	0	-
P-value	>0.05	>0.05	>0.05	NA
C. coli				
Open environment	1.0	0	0	0
In poultry farms	0	0	0	-
P-value	>0.05	>0.05	>0.05	NA

	Poultry	
	Chickens (%)	Ducks (%)
Campylobacter isolated	61 (60.3)	30 (44.8)
C. jejuni	61 (100.0)	24 (80.0)
C. coli	0 (0)	6 (20.0)

NA= not applicable
*= *P* <0.05

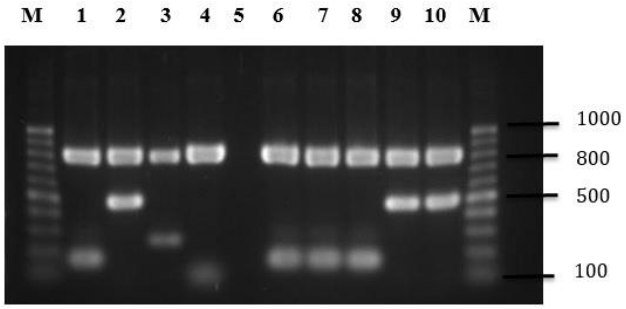

Figure 1: mPCR amplification of representative *Campylobacter* isolated from poultry and wild birds.
Lane: M 100-bp molecular DNA, Lane 1: *C. jejuni* reference strain (LMG 884IT), Lane 2: *C. coli* reference strain (JCM 2529T), Lane 3: *C. lari* reference strain (JCM 2530T), Lane 4: *C. upsaliensis* (ATCC 43954T), Lane 5: negative control, Lanes 6,7,8: *C. jejuni isolates*, Lanes 9, 10: *C. coli* isolates.

Antibiotic resistance

Data on the antibiotic resistance of *Campylobacter* isolated from wild birds and poultry is shown in Table 5. A total of 84 isolates (15 and 3 isolates from wild birds in open environment and in poultry farm, respectively, and 66 isolates from poultry) were subjected to antibiotic susceptibility test. The *Campylobacter* isolates for birds in the open environment showed resistance to all antibiotics except to ciprofloxacin. The highest resistance was to clindamycin and cefotaxime (92.2% each) and lowest to enrofloxacin (14.3%).

It is unfortunate that not all *Campylobacter* isolates were able grow despite several attempts. This is particularly so for the isolates from wild birds near poultry farms as only 3 out of 9 isolates were viable and culturable, and they showed resistance to clindamycin (66.6%). *Campylobacter* isolates from chicken and ducks showed resistance to all antibiotics. Higher resistance was observed to clindamycin (87.7%) and erythromycin (69.2%) and lowest resistance was to chloramphenicol (10.8%).

The resistance profile of isolates obtained from wild birds in the open environment and around poultry houses revealed that 42.9%, 64.3%, 21.4% and 92.2% were resistant to ampicillin, tetracycline, gentamicin and cefotaxime, respectively. However, none of the isolates obtained from birds around poultry houses were resistant to ampicillin, tetracycline, gentamicin, cefotaxime, and clindamycin. Similarly, none of the isolates obtained from birds around poultry houses were resistant to ciprofloxacin, enrofloxacin, sulfamethoxazole-trimethoprim, and nalidixic acid. However, 33% were resistant to cefotaxime, sulfamethoxazole-trimethoprim, chloramphenicol and streptomycin. Isolates obtained from birds in the open environment were also resistant to clindamycin (92.9%), erythromycin (50%), ciprofloxacin (0%), enrofloxacin (14.3%), and nalidixic acid (18.6%), sulfamethoxazole-trimethoprim (42.9%), chloramphenicol (57.1%) and streptomycin (78.6%).

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Table 5: Antibiotic resistance of *Campylobacter* isolated from wild birds and poultry.

Antibiotics	Wild birds from open environment *(n=15)	Percentage of antibiotic resistance	Wild birds near poultry farms *(n=3)	Percentage of antibiotic resistance	Poultry *(n=66)	Percentage of antibiotic resistance
Ampicillin (A)	6**	42.9%	0**	0	27**	41%
Streptomycin (S)	11	78.6%	1	33.0%	28	42.4%
Enrofloxacin (Enr)	2	14.3%	0	0	14	21.2%
Gentamicin (Cn)	3	21.4%	0	0	18	27.2%
Cefotaxime (Ctx)	13	92.9%	1	33.0%	32	48.5%
Erythromycin (E)	7	50.0%	0	0	45	68.2%
Ciprofloxacin (Cip)	0	0	0	0	13	19.7%
Nalidixic Acid (Na)	11	78.6%	0	0	16	24.2%
Clindamycin (Da)	13	92.9%	2	66.6%	57	86.4%
Tetracycline (Te)	9	64.3%	0	0	41	62.1%
Sulfamethoxazole Trimethoprim (Sxt)	6	42.9%	1	33.0%	12	18.2%
Chloramphenicol (C)	8	57.1%	1	33.0%	7	10.6%

* No. of *Campylobacter* isolates.
**No. of isolates resistant to antibiotics

It was found that the MDR *Campylobacter* isolates in birds ranged from 33.3 to 87.5%, while *Campylobacter* isolates from chicken and duck varied according to farms, with the highest rate at 94.7 % and lowest at 11.1%.

DISCUSSION

In this study, the prevalence of *Campylobacter* in all the wild birds was 18%, with the occurrence of *Campylobacter* in wild birds in open environment was numerically but not statistically higher (22.1%) compared to those in poultry farms (17.5%). Other studies showed prevalence of *Campylobacter* in birds varied from low to high. A study on wild birds in the United States reported an occurrence of 21.6% (Waldenström *et al.*, 2002). In another study conducted in the United Kingdom by Colles *et al.* (2008) showed out of 331 geese that were sampled, 50.2% were positive for *C. jejuni* and 0.3% were positive for *C. coli*. In their study, Adhikari *et al.* (2002) detected *C. jejuni* from urban sparrows (39.6%) and farm sparrows (37.7%). In another study, Rahimi *et al.* (2011) isolated 16.7% *C. jejuni* from pigeons. Fernandez *et al.* (1996) isolated 24.2% *Campylobacter* spp. from waterfowl and *C. jejuni* was found to be most frequently isolated at 69.5% followed by *C. coli* at 23.1%. As in most studies, C*. jejuni* was largely isolated at 60% with *C. coli* only at 1%. The reports from similar studies on other species of birds also indicated the presence of various species of *Campylobacter* (Saleha *et al.*, 2001; Adhikari *et al.*, 2002; Waldenström *et al.*, 2002; Colles *et al.*, 2008; Ibrahim *et al*., 2018). In Malaysia, Saleha *et al*. (2001) reported the isolation of 18.1% *Campylobacter* spp. from flying birds near poultry farms. Also, Ganapathy *et al.* (2007) isolated 57.3% *Campylobacter* spp. from crows. Apart from *Campylobacter,* birds are known to be healthy carriers of many types of zoonotic viruses, bacteria, fungi and protozoa (Dhama *et al.*, 2008). Considering their capability to fly without restrictions covering great distances such as migratory birds, these birds have the potential to disperse these pathogens in the environment such as grazing pastures, park areas, surface water and particularly to the animals and the farm environment (Abulreesh *et al.*, 2006). In a study in Georgia (USA) it was discovered that birds caught near chicken houses carry *Salmonella* spp., *C. jejuni* and *Clostridium perfringens* and the study suggested that by gaining access to poultry houses, the birds have the capability to spread these pathogens to poultry.

According to a study on *Salmonella* by Andrés *et al.* (2013) who compared the presence of *Salmonella* in wild birds trapped in and near pig farms and in areas far (>2 km) from the pig farms, they found the group of birds trapped in and around the pig farms were reported as 16 times more of being *Salmonella* positive than the other group of birds that were far from pig farms. Among the many sources of *Salmonella* in the pig farms, were the insects which could be carrying *Salmonella*. When the wild birds feed on these insects, they can get infected with *Salmonella*. Such a similar situation could possibly occur with *Campylobacter* in poultry farms environment. Insects such as house flies and beetles are attracted to the litter, water and feed. Cokal *et al.* (2011) and Hald *et al.* (2004) had identified likely sources of *Campylobacter* in poultry houses which included flies and drinking water.

In this study, the occurrence of *Campylobacter* was higher in open environment compared to those in poultry farms in Rock Pigeon but not in other wild birds' species. The occurrence of *Campylobacter* in wild birds in open environment could be caused by a number of factors, among which are environmental factors associated with feeding habits of these birds. This may give rise to the bacterial infection in the birds (Waldenström *et al.*, 2002). Different feeding habits influence the presence of *Campylobacter* in wild birds, as reported by some surveys

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(Waldenström *et al.*, 2002; Sensale *et al.*, 2006; Antilles *et al.*, 2015). From this present study, it was found that the birds sampled near the housing area exhibited higher prevalence of *Campylobacter.* This could be due to the fact that these groups of birds had the feed which ranged from vegetation to human garbage that were probably contaminated with *Campylobacter*. Some studies suggested that wild birds can get infected with *Campylobacter* from the river water and other surface water. According to Van Dyke *et al.* (2010), the study showed 30% *C. jejuni*, 9% *C. coli* and 66% *C. lari* were found in river water samples. Also, infected farm animals excrete *Campylobacter* spp. in feces thereby contaminating the farms environment, which can be the source of *Campylobacter* to the wild birds. According to numerous studies in different animal farms, such as pigs (Thakur and Gebreyes, 2010; Carrique-Mas *et al.*, 2014), cattle (Sanad *et al.*, 2011), sheep (Salihu *et al.*, 2009), goats (Rapp and Ross, 2012) and poultry (Hermans *et al.*, 2012) they showed that these farm animals play an important role in contamination of the environment.

The *Campylobacter* from wild birds showed more than 50% resistance to six antibiotics (50-93%) with highest resistance to clindamycin and cefotaxime. While isolates from poultry showed more than 50% resistance to three antibiotics (63-88%) with highest resistance to clindamycin. This is possible because the birds may have been exposed to antibiotic resistant *Campylobacter* in the open environment, poultry or other animals in the farm. The use of antibiotics in the farm may provide the selective pressure for development of antibiotic resistance.

Poultry isolates showed low resistance to fluoroquinolones (ciprofloxcin and enrofloxcin) (20-21.5%) and from wild birds at 14%, which may be due to the lesser use of such drugs as recommended by World Organization for Animal health (OIE). According to Adzitey *et al.* (2012), *C. jejuni* showed high resistance to tetracycline (96%), sulfamethoxazole-trimethoprim (96%), nalidixic acid (84%) and ampicillin (81%). In this study, the poultry isolates showed more than 50% resistance to tetracycline but low resistance to ampicillin, nalidixic acid and sulfamethoxazole-trimethoprim. The low level of resistance may be attributed in part, to the fact that these antibiotics are less used in the poultry industry, either prophylactically or therapeutically, due to its intramuscular route of administration, which may be impracticable for large scale application on poultry farms. Wild birds in the open environment of which one location is near the residential area, were probably exposed to antibiotic resistant *Campylobacter* in the area, such as contaminated human garbage, pests and litter. The rate of resistance to chloramphenicol was reported to be high in wild birds in the open environment (57.1%) and among the birds in poultry farms (30%), but resistance was low in poultry (10.8%). In poultry, the reason may be that they were not exposed to the drug.

It is interesting to note that while the chickens and ducks showed low resistance to chloramphenicol and sulfamethoxazole trimethoprim the birds showed otherwise. None of the isolates from bird were resistant to ciprofloxacin compared to those isolated from poultry.

The very small number of antibiotic resistant *Campylobacter* isolates in wild birds in poultry farms limits the discussion. However, it could be possible that the *Campylobacter* isolates, which were not culturable showed resistance to antibiotics. It has been reported that *Campylobacter* has several mechanisms to resist antibiotics; it could be possible that each *Campylobacter* may have more than one mechanism of resistance or may carry several resistant genes (Martinez, 2009).

CONCLUSION

In conclusion, wild birds may play an important role in the spread of antibiotic resistant *Campylobacter* to the environment and poultry farms.

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REFERENCES

Abdollahpour, N., Zendehbad, B., Alipour, A. and Khayatzadeh, J. (2015). Wild-bird feces as a source of *Campylobacter jejuni* infection in children's playgrounds in Iran. *Food Control* **50, 378-381.**

Abulreesh, H. H., Paget, T. A. and Goulder, R. (2006). *Campylobacter* in waterfowl and aquatic environments: Incidence and methods of detection. *Environmental Science and Technology* **40(23), 7122-7131.**

Adhikari, B., Madie, P., Connolly, J., Davies, P., Layland, M. and Rogers, L. (2002). Wild birds, flies, and rodents as reservoirs of *Campylobacter* spp. on dairy farm: New Zealand Ministry of Agriculture and Forestry Technical Paper NO: 2002/18. https://www.researchgate.net/profile/Joanne_Connolly2/publication/237617689_Wild_Birds_Flies_and_Rodents_as_Reservoirs_of_Campylobacter_spp_on_Dairy_Farm/links/54d429860cf25013d02914aa/Wild-Birds-Flies-and-Rodents-as-Reservoirs-of-Campylobacter-spp-on-Dairy-Farm.pdf. [Retrieved on 10 November 2017]

Adzitey, F., Rusul, G., Huda, N., Cogan, T. and Corry, J. (2012). Prevalence, antibiotic resistance and RAPD typing of *Campylobacter* species isolated from ducks, their rearing and processing environments in Penang, Malaysia. *International Journal of Food Microbiology* **154(3), 197-205.**

Andrés, S., Vico, J. P., Garrido, V., Grilló, M. J., Samper, S., Gavín, P., Herrera-León, S. and Mainar-Jaime, R. C. (2013). Epidemiology of subclinical salmonellosis in wild birds from an area of high prevalence of pig salmonellosis: Phenotypic and

genetic profiles of *Salmonella* isolates. *Zoonoses and Public Health* **60(5), 355-365.**

Antilles, N., Sanglas, A. and Cerdà-Cuéllar, M. (2015). Free-living waterfowl as a source of zoonotic bacteria in a dense wild bird population area in northeastern Spain. *Transboundary and Emerging Diseases* **62(5), 516-521.**

Bull, S. A., Allen, V. M., Domingue, G., Jørgensen, F., Frost, J. A., Ure, R., Whyte, R., Tinker, D., Corry, J. E. L. and Gillard-King, J. T. J. H. (2006). Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. *Applied and Environmental Microbiology* **72(1), 645-652.**

Carrique-Mas, J. J., Bryant, J. E., Cuong, N. V., Hoang, N. V. M., Campbell, J., Hoang, N. V., Dung, T. T. N., Duy, D. T., Hoa, N. T. and Thompson, C. (2014). An epidemiological investigation of *Campylobacter* in pig and poultry farms in the Mekong delta of Vietnam. *Epidemiology and Infection* **142(7), 1425-1436.**

Chen, X., Naren, G.-W., Wu, C.-M., Wang, Y., Dai, L., Xia, L.-N., Luo, P.-J., Zhang, Q. and Shen, J.-Z. (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. *Veterinary Microbiology* **144(1-2), 133-139.**

CLSI (2010). Performance standards for antimicrobial susceptibility testing: Twentieth information Supplement M100-S20 Wayne. PA. USA.

Cokal, Y., Caner, V., Sen, A., Cetin, C. and Telli, M. (2011). The presence of *Campylobacter* jejuni in broiler houses: Results of a longitudinal study. *African Journal of Microbiology Research* **5(4), 389-393.**

Colles, F. M., Cain, R. J., Nickson, T., Smith, A. L., Roberts, S. J., Maiden, M. C. J., Lunn, D. and Dawkins, M. S. (2016). Monitoring chicken flock behaviour provides early warning of infection by human pathogen *Campylobacter*. *Proceedings of the Royal Society B* **283(1822), 1-6.**

Colles, F. M., Dingle, K. E., Cody, A. J. and Maiden, M. C. J. (2008). Comparison of *Campylobacter* populations in wild geese with those in starlings and free-range poultry on the same farm. *Applied and Environmental Microbiology* **74(11), 3583-3590.**

Cox, N., Richardson, L. J., Maurer, J. J., Berrang, M. E., Fedorka-Cray, P. J., Buhr, R. J., Byrd, J. A., Lee, M. D., Hofacre, C. L. and O'Kane, P. M. (2012). Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. *Journal of Food Protection* **75(10), 1896-1902.**

Dhama, K., Mahendran, M. and Tomar, S. (2008). Pathogens transmitted by migratory birds: Threat perceptions to poultry health and production. *International Journal of Poultry Science* **7(6), 516-525.**

Endtz, H. P., Ruijs, G. J., van Klingeren, B., Jansen, W. H., van der Reyden, T. and Mouton, R. P. (1991). Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *Journal of Antimicrobial Chemotherapy* **27(2), 199-208.**

Fernandez, H., Gesche, W., Montefusco, A. and Schlatter, R. (1996). Wild birds as reservoir of thermophilic enteropathogenic *Campylobacter* species in Southern Chile. *Memórias do Instituto Oswaldo Cruz* **91(6), 699-700.**

French, N. P., Midwinter, A., Holland, B., Collins-Emerson, J., Pattison, R., Colles, F. and Carter, P. (2009). Molecular epidemiology of *Campylobacter jejuni* isolates from wild-bird fecal material in children's playgrounds. *Applied and Environmental Microbiology* **75(3), 779-783.**

Ganapathy, K., Saleha, A. A., Jaganathan, M., Tan, C. G., Chong, C. T., Tang, S. C., Ideris, A., Dare, C. M. and Bradbury, J. M. (2007). Survey of *Campylobacter*, *Salmonella* and mycoplasmas in house crows (*Corvus splendens*) in Malaysia. *The Veterinary Record* **160(18), 622-624.**

Ge, B., Bodeis, S., Walker, R. D., White, D. G., Zhao, S., McDermott, P. F. and Meng, J. (2002). Comparison of the Etest and agar dilution for *in vitro* antimicrobial susceptibility testing of *Campylobacter*. *Journal of Antimicrobial Chemotherapy* **50(4), 487-494.**

Hald, B., Skovgård, H., Bang, D. D., Pedersen, K., Dybdahl, J., Jespersen, J. B. and Madsen, M. (2004). Flies and *Campylobacter* infection of broiler flocks. *Emerging Infectious Diseases* **10(8), 1490-1492.**

Haruna, M., Sasaki, Y., Murakami, M., Mori, T., Asai, T., Ito, K. and Yamada, Y. (2013). Prevalence and antimicrobial resistance of *Campylobacter* isolates from beef cattle and pigs in Japan. *Journal of Veterinary Medical Science* **75(5), 625-628.**

Hermans, D., Pasmans, F., Messens, W., Martel, A., Van Immerseel, F., Rasschaert, G., Heyndrickx, M., Van Deun, K. and Haesebrouck, F. (2012). Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. *Vector-Borne and Zoonotic Diseases* **12(2), 89-98.**

Ibrahim, M. J., Abdul-Aziz, S., Bitrus, A. A., Mohammed, D. G., Abu, J., Bejo, S. K., Mohamed, M. A and Mohamed, M. Y. I. (2018). Occurrence of multidrug resistant (MDR) Campylobacter species isolated from retail chicken meats in Selangor, Malaysia and their associated risk factors. *Malaysian Journal of Microbiology* **14(3), 272-281.**

Jacobs-Reitsma, W. F., Kan, C. A. and Bolder, N. M. (1994). The induction of quinolone resistance in *Campylobacter* bacteria in broilers by quinolone treatment. *Letters in Applied Microbiology* **19(4), 228-231.**

Komba, E. V. G., Mdegela, R. H., Msoffe, P. L. M., Nielsen, L. N. and Ingmer, H. (2015). Prevalence, antimicrobial resistance and risk factors for thermophilic *Campylobacter* infections in symptomatic and asymptomatic humans in Tanzania. *Zoonoses and Public Health* **62(7), 557-568.**

Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C. G. and Irfan, S. (2010). Emergence of a new antibiotic resistance mechanism

in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *The Lancet Infectious Diseases* **10(9), 597-602.**

Landoni, M. F. and Albarellos, G. (2015). The use of antimicrobial agents in broiler chickens. *The Veterinary Journal* **205(1), 21-27.**

Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C. M. and Zhang, Q. (2009). Antibiotic resistance in *Campylobacter*: Emergence, transmission and persistence. *Future Microbiology*. **4(2), 189-200.**

Martinez, J. L. (2009). The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proceedings of the Royal Society of London B: Biological Sciences* **276(1667), 2521-2530.**

Matsuda, M., Kaneko, A., Stanley, T., Millar, B. C., Miyajima, M., Murphy, P. G. and Moore, J. E. (2003). Characterization of urease-positive thermophilic *Campylobacter* subspecies by multilocus enzyme electrophoresis typing. *Applied and Environmental Microbiology* **69(6), 3308-3310.**

OIE (2015). OIE list of antimicrobial agents of veterinary importance.http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/Eng_OIE_List_antimicrobials_May2015.pdf [Retrieved on 17 September 2017].

Patriarchi, A., Fox, Á., Maunsell, B., Fanning, S. and Bolton, D. (2011). Molecular characterization and environmental mapping of *Campylobacter* isolates in a subset of intensive poultry flocks in Ireland. *Foodborne Pathogens and Disease* **8(1), 99-108.**

Rahimi, E., Chakeri, A. and Tajbakhsh, E. (2011). Detection of *Campylobacter* species in feces of Persian sheepdogs, pigeons and squirrels. *Global Veterinaria* **7(4), 365-369.**

Rapp, D. and Ross, C. M. (2012). Prevalence of six *Campylobacter* species in a New Zealand dairy goat herd. *New Zealand Journal of Agricultural Research* **55(3), 235-240.**

Saleha, A. A., Christopher, J. R. G., Aini, I. and Ganapathy, K. (2001). Occurrence of *Campylobacter* in free flying birds. *In: Proceeding of 2nd International Conference 13th VAM conference and CVA Australasia/Oceania regional Symposium.* 27-30 August, Kuala Lumpur, Malaysia. **pp. 70-71.**

Salihu, M. D., Junaidu, A. U., Oboegbulem, S. I. and Egwu, G. O. (2009). Prevalence and biotypes of *Campylobacter* species isolated from sheep in Sokoto State, Nigeria. *International Journal of Animal and Veterinary Advances* **1(1), 6-9.**

Sanad, Y. M., Kassem, I. I., Abley, M., Gebreyes, W., LeJeune, J. T. and Rajashekara, G. (2011). Genotypic and phenotypic properties of cattle-associated *Campylobacter* and their implications to public health in the USA. *PloS ONE* **6(10), e25778.**

Sensale, M., Cuomo, A., Dipineto, L., Santaniello, A., Calabria, M., Menna, L. F. and Fioretti, A. (2006). Survey of *Campylobacter jejuni* and *Campylobacter coli* in different taxa and ecological guilds of migratory birds. *Italian Journal of Animal Science* **5(3), 291-294.**

Shin, E., Hong, H., Oh, Y. and Lee, Y. (2015). First report and molecular characterization of a *Campylobacter jejuni* isolate with extensive drug resistance from a travel-associated human case. *Antimicrobial Agents and Chemotherapy* **59(10), 6670-6672.**

Thakur, S. and Gebreyes, W. A. (2010). Phenotypic and genotypic heterogeneity of *Campylobacter coli* within individual pigs at farm and slaughter in the US. *Zoonoses and Public Health* **57(s1), 100-106.**

Tsiodras, S., Kelesidis, T., Kelesidis, I., Bauchinger, U. and Falagas, M. E. (2008). Human infections associated with wild birds. *Journal of Infection* **56(2), 83-98.**

Van Dyke, M. I., Morton, V. K., McLellan, N. L. and Huck, P. M. (2010). The occurrence of *Campylobacter* in river water and waterfowl within a watershed in southern Ontario, Canada. *Journal of Applied Microbiology* **109(3), 1053-1066.**

Waldenström, J., Broman, T., Carlsson, I., Hasselquist, D., Achterberg, R. P., Wagenaar, J. A. and Olsen, B. (2002). Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. *Applied and Environmental Microbiology* **68(12), 5911-5917.**

World Health Organization (WHO) (2011). "Critically Important Antimicrobials for Human Medicine – 3rd rev.," WHO Document Production Services. Geneva, Switzerland. **pp. 9-15**.

Yamazaki-Matsune, W., Taguchi, M., Seto, K., Kawahara, R., Kawatsu, K., Kumeda, Y., Kitazato, M., Nukina, M., Misawa, N. and Tsukamoto, T. (2007). Development of a multiplex PCR assay for identification of *Campylobacter coli, Campylobacter fetus, Campylobacter hyointestinalis subsp. Hyointestinalis, Campylobacter jejuni, Campylobacter lari* and *Campylobacter upsaliensis*. *Journal of Medical Microbiology* **56, 1467-1473.**

Zendehbad, B., Arian, A. A. and Alipour, A. (2013). Identification and antimicrobial resistance of *Campylobacter* species isolated from poultry meat in Khorasan province, Iran. *Food Control* **32(2), 724-727.**

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