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Occurrence of antibiotic resistant *C. jejuni* and *E. coli* in wild birds, chickens, humans, and the environment in Malay villages, Kedah, Malaysia

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Abstract: Foodborne pathogens have become a major concern not only due to the diseases they cause, but also because of the rise of antibiotic resistant strains in human and animals. The purposes of this study were to determine the occurrence of *Campylobacter jejuni* and *Escherichia coli* and their antibiotic resistance profiles in wild birds, chickens, humans, and the environment in Malay villages in Malaysia. Three Malay villages in Kota Setar, Kedah were chosen. Three hundred nine (309) samples were collected in this study including wild birds (38), chickens (71), humans (47), and the environment (153). Subsequently, the *C. jejuni* and *E coli* isolates were tested against antibiotics using the disc diffusion method. *Campylobacter jejuni* was found positive in 17 (37.8%) flies and 8 (11.3%) chickens. Also, *E. coli* was found positive in 89.4% of human, 47.4% of bird, 44 62% of chicken and in 71.2% of the environmental samples. Ten antibiotics were used to determine the susceptibility of the isolates. Eighty four percent (84%) of *C. jejuni* and 100% of *E. coli* isolates were found to show resistance towards at least one antibiotic. The isolates showed high resistance to cefpodoxime and tetracycline.

Keywords: biochemical tests; faeces; flies; identification; PCR assay; soil; water

The widespread use of antimicrobial drugs is a serious problem in terms of increasing resistance in many bacteria, and, consequently, the probable public health threat. Antibiotics are commonly used in prophylactic, therapeutic, and metaphylactic ways in modern food animal husbandry (Habib 2021; Mohamed 2021a). Apart from the animal population, the human population has also been

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identified as a threat element for the spread and increase of antibiotic resistance (Zaman et al. 2017).

In recent years, foodborne pathogens have become a public health concern not only because of the disease they caused, but also due to the rise in the isolation frequency of antibiotic resistant bacteria in human and animals (Liao et al. 2020). The increasing resistance of bacteria to antibiotics is of significant concern in the animal population, as animals may become carriers of resistant agents, which may, in turn, transfer these resistance agents to the pathogenic bacteria affecting humans (Gregova and Kmet 2020).

The emergence of resistant bacteria in chickens has been reported to be on steep rise, mainly because of the increasing numbers of resistant pathogens isolated from human infections. *Campylobacter* has been reported to be resistant to macrolides, fluoroquinolones, aminoglycosides, and tetracycline (Mohamed 2021a).

Wild birds or chickens harbouring resistant *C. jejuni* and *E. coli* can transfer the genes to microorganisms in their gastrointestinal tracts in several ways, which they then act as a vehicle for the contamination of the environment, areas around human houses, and eatery areas as well as for the introduction of multidrug resistant (MDR) *C. jejuni* and *E. coli* (Mohamed 2021b). Also, food of animal origin (including chicken meat) may act as a vehicle for the transmission of resistant *C. jejuni* and *E. coli* to humans via the food chain. A report on antibiotic resistance would enable a better understanding of the trends in their patterns over time to ensure the long-term efficiency of antibiotics (Mohamed 2021c).

In Malaysia, the population is made up mainly of a city or urban population, a rural or village population, and the Orang Asli population (Al-Delaimy et al. 2014). The main ethnic groups in the Malaysian population consist of Malay, Chinese and Indian. Malay villages are away from cities, with people working on agricultural farms, rearing livestock, and trading. The majority of the villages are served with electricity and the villagers use treated water (Fadaeenejad et al. 2014). Common wild birds in these villages are Eurasian tree sparrows (*Passer montanus*), white-vented myna (*Acridotheres javanicus*), the jungle myna (*Acridotheres fuscus*), the rock pigeon (*Columba livia*), peaceful doves (*Geopelia placida*), and Oriental magpie robins (*Copsychus saularis*).

The antibiotic resistance of *C. jejuni* and *E. coli* isolated from wild birds, chickens, humans, and the environment in Malay villages has not been reported and there is no information on the multidrug resistance pattern. Thus, the study aimed to determine the occurrence of *C. jejuni* and *E. coli* in wild birds, chickens, humans, and the environment in Malay villages as well as determining the antibiotic resistance profiles of the isolates.

MATERIAL AND METHODS

This study was executed as an observational cross-sectional study to examine the presence or absence of the organisms under study in the Malay villages which are situated in the outskirts of Kota Setar, Kedah.

There were limitations in this study, in particular the sampling of the Malay villages. The unwillingness of the villagers to participate affected the study. However, Prof. Abdul Rashid Khan (co-author) had a good rapport with the head villages, so that we managed to include three Malay villages in this study. Thus, the sample collection from the humans, wild birds, chickens and the environment was carried out through convenient sampling.

The study was approved for animals by Institution Animal Care and Use Committee (IACUC) (AUP No. R089/2016), and for human by Ethics Committee for Research Involving Human Subjects [Ref No. FPV(EXP16)P168].

Description of the villages sampled

The Malay villages are situated in rural areas, near rice fields, where treated water is available. In general, the hygiene is good. They are situated near to a town area, schools and clinics. The education is high, but low at a tertiary level. In Malay villages, the villagers are generally involved in agricultural farming. The villagers kept chickens near their houses (backyard chickens) and fed them with food leftovers.

It was observed that, in these villages, some chickens were let out from the chicken houses and ate in the open environment or from human garbage, and, in doing so, they contaminate the environment with their faeces. In these villages, people do not keep dogs.

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Sample collection

Wild birds: The locations identified for capturing birds were among the houses in the villages or not more than 5 km away from the villages. In each location, a trap (mist net) was set up and placed for six hours. This was undertaken in the morning. Every twenty minutes, the trap was checked for birds. A photograph of the bird was taken for identification and each bird was marked by a red band around one of its legs to avoid being resampled. A cloacal swab was taken before the bird was released. A total of thirty-eight (38) birds were sampled.

Chickens, healthy humans, and the environment: Cloacal swabs, fresh stools, or environmental samples which included drinking water, the soil and flies were collected. A total of 71 chickens and 47 humans were sampled and an additional 153 environmental samples were collected.

Isolation of *C. jejuni*

(a) Wild birds, chickens and human samples:

Each cloacal swab or fresh stool was streaked directly onto a *Campylobacter* blood free selective agar base [mCCDA modified Charcoal Cefoperazone Deoxycholate Agar (Oxoid, London, United Kingdom)] with a CCDA supplement containing cefoperazone and amphotericin (Oxoid London, United Kingdom).

(b) Environmental samples:

1) Water: A 50 ml water sample was placed into a sterile bottle for the *C. jejuni* isolation. The method followed that of Hudson et al. (1999), 100 ml of each water sample was filtered through a sterile 0.45 µm pore-size cellulose nitrate membrane filter (47 mm diameter) (Milipore Sigma, Milwaukee, WI, USA). Each membrane filter was put in a sterile bottle containing 100 ml Bolton selective enrichment broth (Oxoid, London, United Kingdom) and incubated at 42 °C under microaerophilic conditions for 48 hours. Following incubation, a loopful of each enriched culture was streaked onto the mCCDA.

2) Pooled flies: Each pooled sample of flies (3 flies per pooled sample) was placed in 10 ml of a Bolton selective enrichment broth and was then crushed using a sterile cotton swab, mixed and then streaked onto the mCCDA plate.

3) Soil: 10 g soil samples were taken using a sterile spoon and placed in a bottle containing 19 ml of the Bolton selective enrichment broth; all the bottles were incubated at 42 °C under microaerophilic conditions for 48 hours. Subsequently, a few loopfuls of each enriched culture were streaked onto the mCCDA plate.

After streaking, all the plates were placed in anaerobic jars in which a microaerophilic atmosphere was generated by the use of a gas generating sachet CampyGen (Oxoid, London, United Kingdom) and incubated at 42 °C for 48 hours.

Plates with no growth were re-incubated under the same conditions for an additional 48 hours.

Identification of *C. jejuni*

All the small and grey colonies suspected as being *C. jejuni* colonies were examined by Gram staining. *Campylobacter jejuni* is gram-negative, with slightly curved, slender rods appearing as spiral, S or seagull-wing shapes. Phase contrast microscopy of a wet mount prepared from a plate culture was carried out to observe the motility of each isolate. *Campylobacter jejuni* is motile, showing spiral, fast darting corkscrew movement.

Biochemical tests namely: oxidase, hippurate hydrolysis, catalase and indoxyl-acetate hydrolysis tests were conducted to identify the isolates.

Isolation of *E. coli*

(a) Enrichment of samples:

1) Wild bird, chicken and human samples: The bottles containing buffered peptone water (BPW) (Oxoid, London, United Kingdom) and cloacal swabs or fresh stool were aerobically incubated for 24 h at 37 °C.

2) Water: 50 ml of the water sample were placed into a sterile bottle containing 100 ml of BPW for *E. coli* isolation and aerobically incubated for 24 h at 37 °C.

3) Pooled flies: The samples were crushed in a bottle containing 10 ml of BPW and aerobically incubated for 24 h at 37 °C.

4) Soil: Each soil sample (10 g) was taken using a sterile spoon and placed directly into a sterile bottle containing 10 ml of BPW for *E. coli* isolation and was aerobically incubated for 24 h at 37 °C.

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(b) Culture of enriched samples:

After incubation, the enriched cloacal swab, fresh stool, fly, water, and soil cultures were streaked onto Brilliance *E. coli*/coliform selective media (Oxoid, London, United Kingdom) and aerobically incubated at 37 °C for 18–24 hours. Suspected *E. coli* colonies appeared dark blue to violet and were sub-cultured on Brilliance *E. coli*/coliform selective media and aerobically incubated at 37 °C for 18–24 hours.

Identification of *E. coli*

On the Gram staining, the *E. coli* appeared as small, gram-negative, rod-shaped bacteria. The biochemical tests carried out were namely: tryptone sugar iron (TSI), sulfide-indole motility (SIM), citrate, urease, methyl red and Voges-Proskauer (MR-VP) tests, which were performed on the purified cultures.

Confirmation of *C. jejuni* and *E. coli* isolates using multiplex polymerase chain reaction (mPCR) assay

DNA extraction method: The DNA was extracted using the conventional boiling method. Bacterial DNA was extracted by boiling a bacterial suspension in sterile distilled water. A loop full or a single colony was added to 1 000 µl sterile distilled water in a 1.5 ml Eppendorf tube (Eppendorf, Sydney, Australia), and incubated for 10–15 min in a dry water bath at 94 °C, and allowed to cool at room temperature (37 °C). Each bacterial suspension was centrifuged for 3 min at 13 000 × *g*. Each supernatant from the centrifuge tube was transferred to a new 1.5 ml Eppendorf tube to be used as the template DNA while the pellet was discarded.

PCR assay for *C. jejuni*: Fifty microliters of the PCR mixture was used, which encompassed 2 µl of the bacterial DNA extract, 5 µl of a primer mix as well as 18 µl of RNase free H_2O (Qiagen, Wuppertal, Germany) and 25 µl of an m-PCR Master Mix (Qiagen, Wuppertal, Germany). The PCR was optimised using known *C. jejuni* (LMG 8841T).

The bacterial DNA extract was replaced with the equivalent amount of sterile distilled water for the negative control. The PCR amplification procedure was performed following the procedure in Mohamed et al. (2019a). The initial activation step was at 95 °C for 2 min, followed by 30 cycles at 95 °C for 60 s and an extension of 72 °C for 40 seconds. The annealing temperature was 59 °C for *C. jejuni*. The PCR assay was completed with a final extension at 72 °C for 3 minutes. This was performed in a Veriti™ 96-Well Eppendorf Thermal Cycler (Hamburg, Germany).

PCR assay for *E. coli*: The *E. coli* isolates were recovered and subjected to a final confirmation using PCR. DNA was amplified in a 50 µl reaction volume containing 25 µl of the Master Mix, 1 µl (10 mM) of the primer set, as described by Mohamed et al. (2019a), using the internal control targeted gene *E. coli* 16s rRNA (Table 1), 4 µl of DNA templates and 19 µl of RNase-free water. The volume of this mix was adjusted to 50 µl with sterile water. The reaction mixtures were amplified in a DNA thermal cycler (Eppendorf) with the following cycling parameters; an initial denaturation step at 95 °C for 90 s, followed by 30 cycles of amplification with denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s, ending with a final extension at 72 °C for 7 minutes.

Agarose gel electrophoresis

The agarose (Agarose, LE Analytical Grade, Melbourne, Australia) was dissolved in a 1 × TBE buffer. Two microliters of loading dye were mixed with 5 µl of the PCR mixture. Subsequently, the PCR mixture was meticulously loaded into the wells of the submerged gel. Then, the PCR mixture was electrophoresed through a 2% agarose gel contain-

Table 1. The primers used in the PCR assay to confirm the *C. jejuni* and *E. coli* isolates (Mohamedet al. 2019a)

Species	Primer	Oligonucleotide sequence	Size
C. jejuni	C-1 C-3	5'-CCATAAGCACTAGCTAGCTGAT-3' 5'-CCA TAAGCA CTA GCT AGCTGAT-3'	161 bp
E. coli	E16S-a E16S-b	CCCCCTGGACGAAGACTGAC ACCGCTGGCAACAAAGGATA	401 bp

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ing a tris-borate-EDTA (TBA) buffer (40 mM tris-borate, 2 mM EDTA, pH 7.5) and gel red (3 µl/ml) in a 1 × TBA buffer at 75 V for 90 minutes. The gel was then observed under UV transillumination by using the Alpha Imager (Bio-Rad, Richmond, CA, USA) gel documentation system.

Antibiotic susceptibility test

Bacterial isolates and growth condition: All the *E. coli* and *C. jejuni* isolates that were obtained earlier from the chickens, wild birds, humans and the environment were subjected to antibiotic susceptibility. The *C. jejuni* isolates that were stored at –80 °C were revived on a Columbia blood agar base (CBA) (Oxoid, London, United Kingdom) supplemented with 5% defibrinated horse blood and incubated at 42 °C for 48 h under microaerophilic conditions using a CampyGen gas pack (Oxoid, Basingstoke, UK). The *E. coli* isolates on nutrient slant agar (Oxoid, London, United Kingdom) were recovered on the Brilliance *E. coli*/coliform selective media (Oxoid, London, United Kingdom).

The antibiotic susceptibility test was performed on 25 *C. jejuni* and 213 *E. coli* isolates using the disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI 2016). *Escherichia coli* and *C. jejuni* isolates were tested against ten antibiotics from eight different classes of antibiotics. The ten antibiotics were chosen from the World Organisation for Animal Health (OIE) Recommendation for Veterinary Critically Important Antibiotic (2012) and the World Health Organization (WHO) Recommendation for the Critically Important Antimicrobials for Humans (2011) lists. The used antibiotic discs (Oxoid, London, United Kingdom) included ampicillin-sulbactam (Sam), 10 µg; tetracycline (Te), 30 µg; gentamicin (Cn), 10 µg; erythromycin (E), 15 µg; ciprofloxacin (Cip), 5 µg; nalidixic acid (Na), 30 µg; enrofloxacin (Enr), 5 µg; sulfamethoxazole-trimethoprim (Sxt), 25 µg; cefpodoxime (Cpd), 10 µg; streptomycin (S), 10 µg. A suspension of each *E. coli* and *C. jejuni* isolate was prepared by adding a loopful of the colonies into a 2 ml tube containing 0.9% NaCl. The turbidity of each inoculum was adjusted to the 0.5 McFarland standard (1.5×10^8 cfu/ml). Then using a sterile cotton swab, the bacterial suspension was spread gently in three different directions over the surface of a Mueller-Hinton agar plate (Oxoid, London, United Kingdom) supplemented with 5% defibrinated horse blood for the *C. jejuni* isolates. For the *E. coli*, the Mueller-Hinton (Oxoid, London, United Kingdom) agar plate was used without any supplements. Before dispensing the antibiotic discs onto the agar, any excess moisture was absorbed by allowing the agar plates to dry for 3–5 minutes. At least two (2) Mueller-Hinton agar plates were required for each isolate and five (5) antibiotic discs were placed onto each inoculated plate using a disc dispenser.

The *E. coli* isolates were aerobically incubated at 37 °C for 24 h, while for the *C. jejuni* isolates, the plates were micro-aerobically incubated at 42 °C for 48 hours. Upon completion of the incubation period, the diameter of each inhibition zone was measured using digital callipers. For quality control, reference strains of *E. coli* (ATCC 25922) and *C. jejuni* (ATCC 33560) were used. The isolates were classified as sensitive, intermediate, and resistant using zone diameter breakpoints based on the CLSI system (CLSI 2016).

According to many EU countries, resistance to at least one antibiotic in three or more classes of antibiotic is termed as multidrug drug resistant (MDR) (Magiorakos et al. 2012).

RESULTS

Occurrence of *C. jejuni* and *E. coli* in test samples

Wild birds: A total of thirty-eight (38) birds were trapped, cloacal swabs were taken, and then the birds were released. Three wild bird species were identified, which consisted mainly of 23 Eurasian tree sparrows (*Passer montanus*), nine jungle mynas (*Acridotheres fuscus*) and six white-vented mynas (*Acridotheres javanicus*). All the birds were negative for *C. jejuni* while 18 (47.4%) were positive for *E. coli*. Table 2 shows the incidence of *E. coli* in the various species of wild birds.

Chickens: Seventy-one (71) cloacal swabs were collected. *E. coli* was found to be prevalent in 44 (62%) chickens while *C. jejuni* was found to be prevalent in 8 (11.3%) chickens.

Humans: Of the collected 47 fresh stool samples, 42 (89.4%) were positive for *E. coli*, whereas all of them were negative for *C. jejuni*.

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Table 2. *E. coli* isolated from the different species of wild birds

Bird species (Scientific name) (No.)	Location	*C. jejuni*	*E. coli*
Eurasian tree sparrow (*Passer montanus*) (23)	A (10), B (6), C (7)	0	14 (60.9%)
Jungle myna (*Acridotheres fuscus*) (9)	B (3), C (6)	0	3 (33.3%)
White-vented myna (*Acridotheres javanicus*) (6)	A (5), C (1)	0	1 (16.7%)
Total (38)	38	0 (%)	18 (47.4%)

Environment: Of the 153 samples that were collected, 109 (71.2%) were found positive for *E. coli* with samples from the water, flies, and soil positive at 39 (65%), 40 (88.9%), and 30 (62.5%), respectively, while only 17 (37.8%) were positive for *C. jejuni*.

Table 3 shows the incidence of *C. jejuni* and *E. coli* that were isolated from all the sources. Figures 1 and 2 represent the PCR assay used to confirm the *C. jejuni* and *E. coli*, respectively.

Antibiotic resistance of *C. jejuni* and *E. coli* isolates

The antibiotic susceptibility test was performed for 213 *E. coli* isolates which were made up from 44, 18, 42, 39, 40, and 30 isolates from the chickens, wild birds, human, water, flies, and soil, respectively, and 25 *C. jejuni* isolates (8 isolates from chickens and 17 from flies). All the *E. coli* isolates were found to be resistant to at least one antibiotic and four *C. jejuni* isolates (two from chickens and two from flies) were sensitive towards all antibiotics.

In the humans, all the *E. coli* isolates showed resistance to erythromycin, followed by tetracycline (45%) with a low percentage of isolates found to show resistance to cefpodoxime (4.8%) and none to ampicillin-sulbactam.

In the chickens, 98% of the *E. coli* isolates were resistant to erythromycin, 84% were resistant to tetracycline (84%), and 6.8% were resistant to ampicillin-sulbactam and gentamicin.

The *Escherichia coli* isolates from wild birds also showed similar profiles, with a high percentage resistant to erythromycin (100%) and tetracycline (56%), whereas they were all susceptible to ciprofloxacin, enrofloxacin, gentamicin, nalidixic acid, and cefpodoxime (Table 4).

Similar results were shown by the *E. coli* isolates from flies, water, and soil with 100% of the isolates were resistant to erythromycin and 97% to 57% to tetracycline with none-resistant to ampicillin-sulbactam for the fly isolates and gentamicin for the soil isolates and a low percentage at 10.3% and 3.3% were shown to be resistant by the water and soil isolates, respectively, as shown in Table 5.

The *Campylobacter jejuni* isolated from the chickens were resistant to eight antibiotics ranging from 50% to 12.5% and were sensitive to ampicillin-sulbactam and gentamicin, while the isolates from

Table 3. *C. jejuni* and *E. coli* positive sources in the villages

Location	Humans No./+ (%)	Chickens No./+ (%)	Wild birds No./+ (%)	Flies No./+ (%)	Water No./+ (%)	Soil No./+ (%)
E. coli						
A	24/21 (87.5)	21/16 (76.2)	15/9 (60)	30/28 (93.3)	20/8 (40)	20/14 (70)
B	10/10 (100)	20/15 (75)	9/3 (33.3)	3/3 (100)	20/16 (80)	18/10 (55.6)
C	13/11 (84.6)	30/13 (43.3)	14/6 (42.9)	12/9 (75)	20/15 (75)	10/6 (60)
Total	47/42 (89.4)	71/40 (62)	38/18 (47.4)	45/40 (88.9)	60/39 (65)	40/30 (62.5)
C. jejuni						
A	24/0 (0)	21/7 (33.3)	15/0 (0)	30/15 (0)	20/0 (0)	20/0 (0)
B	10/0 (0)	20/1 (5)	9/0 (0)	3/0 (0)	20/0 (0)	18/0 (0)
C	13/0 (0)	30/0 (0)	14/0 (0)	12/2 (13.3)	20/0 (0)	10/0 (0)
Total	47/0 (0)	71/8 (11.3)	38/0 (0)	45/17 (37.8)	60/0 (0)	48/0 (0)

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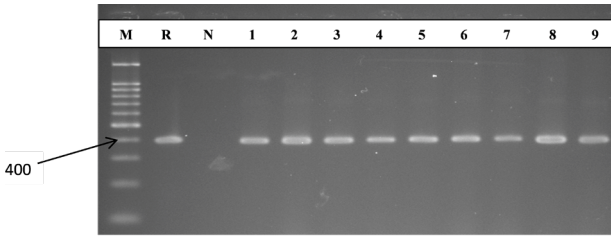

Figure 1. PCR amplification of the representative *E. coli* isolates

Lane M = marker 100 bp ladder; lane R = *E. coli* ATCC 25922 16S rRNA as positive control; lane N = negative control; lanes 1, 9 = *E. coli* isolates

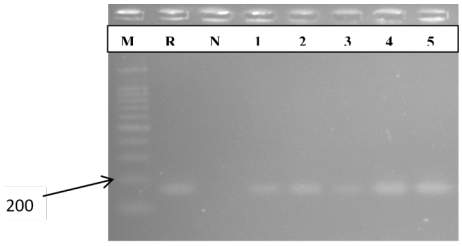

Figure 2. PCR amplification of the representative *C. jejuni* isolates

Lane M = marker 100 bp ladder; lane R = *C. jejuni* ATCC 29428 as positive control; lane N = negative control; lanes 1, 5 = *C. jejuni* isolates

Table 4. Antibiotic resistance of the *E. coli* isolates

Antibiotics	Humans (n = 42)	Antibiotic resistance (%)	Chickens (n = 44)	Antibiotic resistance (%)	Wild birds (n = 18)	Antibiotic resistance (%)	Flies (n = 40)	Antibiotic resistance (%)	Water (n = 39)	Antibiotic resistance (%)	Soil (n = 30)	Antibiotic resistance (%)
Ciprofloxacin (Cip)	6	14.3	6	13.6	0	0	5	12.5	11	28.2	5	16.7
Enrofloxacin (Enr)	9	21.4	13	29.5	0	0	11	27.5	24	61.5	11	36.7
Ampicillin-sulbactam (Sam)	0	0	3	6.8	2	11.1	0	0	4	10.3	1	3.3
Tetracycline (Te)	19	45.2	37	84.1	10	55.6	39	97.5	35	89.7	17	56.7
Gentamicin (Cn)	6	14.3	3	6.8	0	0	6	15	4	10.3	0	0
Streptomycin (S)	7	16.7	18	40.9	5	27.8	20	50	23	59	10	33.3
Erythromycin (E)	42	100	43	97.7	18	100	40	100	39	100	30	100
Nalidixic acid (Na)	10	23.8	15	34.1	0	0	8	20	18	46.2	9	30
Sulfamethoxazole-trimethoprim (Sxt)	7	16.7	23	52.3	3	16.7	18	45	25	64.1	11	36.7
Cefpodoxime (Cpd)	2	4.8	1	2.3	0	0	2	5	6	15.4	6	20

Table 5. Antibiotic resistance of the *C. jejuni* isolated from the chickens and flies

Antibiotics	Chickens (n = 8)	Antibiotic resistance (%)	Flies (n = 17)	Antibiotic resistance (%)
Ciprofloxacin (Cip)	1	12.5	2	11.8
Enrofloxacin (Enr)	1	12.5	4	23.5
Ampicillin-sulbactam (Sam)	0	0	1	5.9
Tetracycline (Te)	3	37.5	11	64.7
Gentamicin (Cn)	0	0	0	0
Streptomycin (S)	2	25	1	5.9
Erythromycin (E)	1	12.5	0	0
Nalidixic acid (Na)	2	25	2	11.8
Sulfamethoxazole-trimethoprim (Sxt)	1	12.5	7	41.2
Cefpodoxime (Cpd)	4	50	12	70.6

the flies showed to be sensitive to erythromycin and gentamicin. The fly isolates demonstrated resistance to all antibiotics with the highest to cefpodoxime (70.6%) and lowest to ampicillin-sulbactam and streptomycin at (5.9%) as presented in Table 5.

DISCUSSION

Escherichia coli is a considerable common commensal enteric bacteria in animals and humans, and the pathogenic pathotypes are of public health risk and significance (Sarowska et al. 2019; Wallace et al. 2020).

In this study, the occurrence of *E. coli* found in wild birds was 47.4%. An investigation on yellow-legged gulls conducted in France stated a similar prevalence of *E. coli* at 47.1% (Bonnedahl et al. 2009).

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Different feeding habits influence the presence of different species of bacteria in birds, as reported by some surveys (Vittecoq et al. 2017; Sharma et al. 2018). The birds in these villages had fed from human garbage and vegetation that were perhaps contaminated with *E. coli*.

The study showed a high occurrence (62%) of *E. coli* in chickens. Other studies showed a high occurrence of *E. coli* in chickens (Jalo et al. 2018; Mohamed et al. 2019b). A high occurrence of *E. coli* in the chickens in these villages may be because of the poor hygiene management in the chicken houses as well as from the environment where they might pick up *E. coli* from the ground, water, and garbage. They may also spread *E. coli* through their faeces in the villages.

The occurrence of *E. coli* in humans in this study was 89.4%. However, other investigations demonstrated low occurrence (Kolenda et al. 2015; Nielsen et al. 2018). In the current study, *C. jejuni* was only detected in chickens (11.3%) and flies (37.8%). Other studies showed the occurrence of *C. jejuni* in chickens varied from low to high. These findings were similar to those reported by other researchers, such as Nwankwo et al. (2017) and Osbjer et al. (2016). This is probably because the chicks were exposed to *C. jejuni* in the environment, especially to flies which tested positive in this study.

In this study, the flies were found to be a possible source of *C. jejuni* in chickens and the flies may also have spread *C. jejuni* in human houses and the areas around the eateries in the villages. Flies were seen in and near the chicken houses, human houses, and kitchens in the eateries suggesting that they could easily contaminate the feed, food, utensils, and the environment. According to Royden et al. (2016), the possibility of bacterial transmission by flies may be high, especially during the summer when the fly population is highest.

The absence of *C. jejuni* in humans is not surprising with regard to healthy humans. *Campylobacter jejuni* and other pathogens such as *Vibrio cholerae*, *Mycobacterium avium*, and *Salmonella enterica* were not detected in healthy human stools, whereas *E. coli* was present at a high level in the samples as reported by Huttenhower et al. (2012).

The wild birds were negative for *C. jejuni* probably due to the feeding habits of these birds, feeding on insects and vegetables. According to a recent study conducted in Malaysia, insectivorous birds such as the Oriental magpie robin were negative for *C. jejuni*, while other species of birds, such as the rock pigeon and spotted dove, were positive for *C. jejuni* (Mohamed et al. 2019b). Also, Sensale et al. (2006) did not detect *C. jejuni* in insectivorous birds. This study also suggested that insectivorous birds are not a source of *C. jejuni* in the environment.

The absence of *C. jejuni* in the environmental sources (water and soil) might be due to the poor resistance of *C. jejuni* to environmental conditions that would have them formed into a viable-but-non-culturable (VBNC) form (Magajna and Schraft 2015). Numerous studies had detected a low level of *C. jejuni* in the water (Banting et al. 2016; Nilsson et al. 2017) and soil (Guevremont et al. 2017), whereas a high occurrence in flies and poultry has been detected. According to Magajna and Schraft (2015), after the chickens or humans pick up the *C. jejuni* from the water or soil, they remain viable and possibly contagious for a longer period than before.

Commensal *E. coli* may be reservoirs of resistant genes for pathogenic bacteria. It is considered that resistance is a helpful indicator for pressure selection due to the use of antibiotics causing resistance issues in pathogens. The overuse of antibiotics should be discontinued as antibiotics may completely lose their efficacy against pathogens. *Escherichia coli* acquires resistance to antibiotics faster than any other microbes (Mohamed 2021a).

This research found that the resistance against antibiotics exhibited by *E. coli* was high. The *E. coli* found in wild birds demonstrated that they might have picked up resistant bacteria from the environment. The isolates showed resistance of more than 50% against two antibiotics, that is, erythromycin (100%) and tetracycline (55%); however; the resistance was less than 50% for the other three antibiotics. The isolates were sensitive to ciprofloxacin, enrofloxacin, nalidixic acid, gentamycin, and cefpodoxime. The isolates from wild birds in these villages were 44.4% MDR. These results were similar to the work by Nhung et al. (2015), who reported high resistance to sulfamethoxazole-trimethoprim, ampicillin, and tetracycline and low resistance to cefpodoxime.

Chicken isolates showed resistance exceeding 50% to three antibiotics, namely erythromycin (97.7%), tetracycline (84.1%), and sulfamethoxazole-trimethoprim (52.3%), and lower than 50% resistance against seven antibiotics. The isolates

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from chickens in these villages showed a high MDR at 70.5%. The high incidence of MDR *E. coli* might be because these chickens were exposed to these antibiotics. The extent of the resistance to erythromycin and tetracycline reported in these villages was similar to that reported by Luangtongkum et al. (2006) on *Campylobacter* in poultry; this might be because the poultry were similarly exposed to erythromycin and tetracycline.

The isolates from humans were found to be resistant to all the antibiotics. The isolates showed resistance to erythromycin at 100%, and other antibiotics were less than 50% resistant while sensitive to ampicillin-sulbactam. The isolates from humans in these villages showed 26.2% MDR. The humans were exposed to the resistant *E. coli* present in the environment.

The flies, water and soil isolates were found to be highly resistant to erythromycin (100%) tetracycline (97.5% to 56.7%). These results were similar to a study by Milanovic et al. (2016). Nevertheless, environmental isolates showed lower than 50% resistance against the other antibiotics.

The *Campylobacter jejuni* found in chickens showed resistance to eight antibiotics (50% to 12.5%) and were susceptible to two whereas the highest resistance was found to be to cefpodoxime and tetracycline.

On the other hand, the fly isolates showed resistance of more than 50% to cefpodoxime (70.6%) and tetracycline (64.7%) and were susceptible to three antibiotics. It may be because of the exposure of the chickens to *C. jejuni*, which were resistant to antibiotics and found in the open environment, as well as to the garbage or other animals present in the village. Human antibiotics use may be responsible for the selective pressure causing resistance against the antibiotics and it may also be responsible for contaminating the environment.

The isolates from flies showed low susceptibility to gentamicin and erythromycin, and the chicken isolates were susceptible to ampicillin-sulbactam and gentamicin, while the chicken (12.5%) and fly (11.8–23.5%) isolates had low resistance to the fluoroquinolones (ciprofloxacin and enrofloxacin), and also to streptomycin and ampicillin-sulbactam at 2.5–5.9% and 0–5.9%, respectively. In this study, the chicken isolates showed 37.5% resistance to tetracycline, but showed lower resistance to nalidixic acid (25%), sulfamethoxazole-trimethoprim (12.5%) as well as being sensitive to ampicillin-sulbactam. It was reported that, in chickens, the resistance rate to cefpodoxime was high 50%, while for flies, it was 70.6%; however, resistance against streptomycin was found to be low in chickens (25%), and flies (5.9%); all the isolates were found to be susceptible to gentamicin and had low resistance to aminoglycosides (gentamicin) possibly due to them being less used in the poultry industry.

In the Malay villages, *E. coli* was found to be present in wild birds, humans, flies, the water, and soil. However, *C. jejuni* was isolated from chickens and flies, whereas they were found to be negative in the wild bird, human, water, and soil isolates. The *E. coli* isolates showed resistance to all the antibiotics and most of the isolates were resistant to tetracycline and erythromycin. All the *C. jejuni* isolates were sensitive to gentamicin, while most of the isolates showed resistance to other antibiotics. Thus, the infected wild birds might play a part in the incidence of resistance mechanisms in the Malay villages in Kota Setar, Kedah, Malaysia. The occurrence of MDR *E. coli* and *C. jejuni* in several sources in the environment may pose a threat to human health upon exposure to these bacteria. More epidemiology research is obligatory to fully distinguish the role of the environment in *E. coli* and *C. jejuni* epidemiology in villages in Malaysia.

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Conflict of interest

The authors declare no conflict of interest.

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