



***ANTIBIOTIC RESISTANCE OF Escherichia coli AND Campylobacter jejuni
AND MULTILOCUS SEQUENCE TYPING OF PATHOGENIC
Escherichia coli ISOLATED FROM SELECTED ORANG ASLI AND MALAY
VILLAGES IN MALAYSIA***

MOHAMED YOUSIF IBRAHIM MOHAMED

FPV 2019 24



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By

MOHAMED YOUSIF IBRAHIM MOHAMED

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

July 2019

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DEDICATION

This thesis is specially dedicated to:

My beloved parents,
YOUSIF IBRAHIM MOHAMED ELNOHI
and
SIHAM TAHA SALIM HAMED

My beloved siblings,
IHLAM YOUSIF IBRAHIM MOHAMED,
ESRAA YOUSIF IBRAHIM MOHAMED,
OMER YOUSIF IBRAHIM MOHAMED
and
ABEDELNASSER YOUSIF IBRAHIM MOHAMED

My beloved wife,
SAHAR SMAIR YOUSIF ALI

My beloved children,
YOUSIF MOHAMED YOUSIF IBRAHIM
and
SIHAM MOHAMED YOUSIF IBRAHIM

Who always supported and encourage me to do the best.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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MOHAMED YOUSIF IBRAHIM MOHAMED

July 2019

Chairman : Professor Jalila Abu, PhD
Faculty : Veterinary Medicine

Campylobacter jejuni and *Escherichia coli* are prominent bacterial causes of human gastroenteritis in the developing countries and the emergence of antibiotic resistance of these bacteria has been widely reported to be on the increase, particularly because of the increase in the number of resistant *C. jejuni* and *E. coli* isolated from human infections. Poultry, in particular chickens and wild birds are reported to be frequently infected with *C. jejuni* and *E. coli*. Wild birds or chickens harboring resistant *C. jejuni* and *E. coli* can transfer the genes to microorganisms in their gastrointestinal tracts. A number of studies in the country had shown the occurrence of *C. jejuni* and *E. coli* in chickens and chicken meat. Wild birds were observed in abundance at residential areas, market places and farms. It was reported that wild birds harbor and may transmit *C. jejuni* and *E. coli* to residential areas and farms environment. However, very few studies had been carried out on antibiotic resistance of *C. jejuni* and *E. coli* from wild birds, chickens and humans and their environment in Malaysia. Also, very few studies had been conducted to detect the pathogenic *E. coli* genes and no study had been conducted to detect the sequence typing (STs) of pathogenic *E. coli* from different sources in Malaysia. Thus, the objectives of this study were, (i) to determine the presence of *C. jejuni* and *E. coli* in wild birds, chickens, environment and human at selected villages in Perak and Kedah, (ii) to determine the antibiotic resistance profiles among *C. jejuni* and *E. coli* isolates, (iii) to detect pathogenic *E. coli* (EPEC, EIEC) among *E. coli* isolated, (iv) to characterize *E. coli* using Multilocus Sequence Typing (MLST) assay, and (v) to determine the interrelatedness among the pathogenic *E. coli* sequence types (STs) from different sources.

A total of three Orang Asli villages were selected in Perak and three Malay villages in Kota Setar, Kedah. Ninety-nine (99) human stool, cloacal swabs from 113 chickens

and 78 from wild birds were collected and environmental samples included flies (85) water (100) and soil (88). *Campylobacter jejuni* was isolated from 24(21.2%) chickens and 19(22.4%) flies. A total of 92(92.9%) humans were found positive for *E. coli* while no human samples were positive for *C. jejuni*. Fifty-eight (74.4%) of the birds and 84(74.3%) of the chickens were positive for *E. coli*. Eight-four (74.3%) of the chickens were positive for *E. coli*. Out of 273 environmental samples, 220 (80.6%) showed positive for *E. coli* mainly isolated from flies, 77(35%) and water, 74 (33.6%).

The *C. jejuni* and *E. coli* isolates were subjected to antibiotic susceptibility test using disc diffusion method. The isolates were tested against 10 antibiotics, namely ampicillin-sulbactam, tetracycline, gentamicin, erythromycin, ciprofloxacin, nalidixic acid, enrofloxacin, sulfamethoxazole-trimethoprim, cefpodoxime, streptomycin. The *C. jejuni* isolates from chickens showed resistance to 1-7 antibiotics. The highest resistance was to nalidixic acid (40.9%) while all isolates were found sensitive to gentamicin. Flies isolates showed resistance to 2-7 antibiotics. The highest resistance was to cefpodoxime (65%) and isolates were sensitive to gentamicin and erythromycin. The *E. coli* isolates from humans showed resistance to 1-9 antibiotics and the highest resistance was to erythromycin (100%). Chicken isolates showed resistance to 2-9 antibiotics with highest resistance was to erythromycin (98.8%). *Escherichia coli* isolates from wild birds showed resistance to 1-8 antibiotics. Hundred percent (100%) of the wild birds isolates were found resistant to erythromycin followed by tetracycline (87.3%). Flies isolates showed resistance to 2-9 antibiotics. The highest resistance was to erythromycin (97%) followed by tetracycline (57.5%). The isolates from water showed resistance to 3-10 antibiotics. Hundred percent (100%) of the water isolates were found resistant to erythromycin. Soil isolates showed resistance to 1-10 antibiotics. The highest resistance was to erythromycin (100%) followed by tetracycline (60.3%).

The occurrence of enteropathogenic *E. coli* (EPEC) and enteroinvasive (EIEC) was through the detection of *eaeA* and *bfpA*, and *ial* genes respectively. Thirty-eight (45.2%) of the chicken isolates were positive for *eaeA* gene only, six (7.1%) and two (2.4%) were positive for EPEC and EIEC respectively. Seven (12.1%) of the wild bird isolates were positive for *eaeA* gene only. Sixteen (29.6%) of the environmental isolates were positive for *eaeA* gene, EPEC was detected in isolates from water (1.9%) and EIEC was detected in isolates from soil (1.9%).

The typing of pathogenic *E. coli* isolates and interrelatedness among the isolates was done using Multilocus sequence typing (MLST) and eBURST analyses. Multilocus sequence typing (MLST) analysis revealed twenty sequence types (STs) from 42 pathogenic *E. coli*. Seven wild bird isolates revealed six STs namely ST 2705, ST 2491, ST 484, ST 2253, ST 453 and ST295. Thirteen STs were revealed from 19 chicken isolates namely ST 5686, ST 155, ST 10, ST 181, ST 3856, ST 1141, ST 202, ST 542, ST 295, ST 188, ST 453, ST 206 and ST 48 and one undefined ST. Three flies isolates revealed three STs namely ST 295, ST 155 and ST 181. Six water isolates revealed two STs namely ST 162, ST 69 and one undefined ST. Five STs were

revealed from seven soil isolates namely ST 5686, ST 181, ST 46, ST 2419, ST 202 and two undefined STs.

From this study, the overlapping of similarities shown among several of the pathogenic *E. coli* sequence types (STs) isolated from the wild birds and chickens with the environmental isolates in the villages could imply that these animals are main sources of the pathogenic *E. coli* in the villages. Also, the isolates from wild birds in several villages showed high multidrug resistance (MDR) and the overlapping of similarities shown between several of the pathogenic *E. coli* STs isolated from the wild birds (ST 295, ST 2491 and ST 453) with the chicken and environmental isolates in several villages could possibly showed that these birds had been the main sources of the antibiotic resistant *E. coli* and pathogenic *E. coli*. The presence of high MDR in *C. jejuni* and *E. coli* could compromise treatment in humans and in particular, if the bacteria is resistant to the drugs of choice and alternative drugs for treatment and therefore poses a significant public health risk. The chickens and flies could play a role in the dispersal and spread of or may had acquire antibiotic resistant *C. jejuni* in the villages environment. Thus, the presence of *C. jejuni* in chickens and flies, and pathogenic *E. coli* in wild birds, chickens and the environment (flies, water, soil) may cause health hazard to human upon exposure to the organisms.

Keywords: *Campylobacter jejuni*, *Escherichia coli*, wild birds, chickens, environment, human, antibiotic resistance.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KERINTANGAN ANTIBIOTIK *Escherichia coli* DAN *Campylobacter jejuni*
DAN PENGETIPAN URUTAN MULTILOKUS BAGI PATOGEN
Escherichia coli DITERIMA DARIPADA TERPILIH LADANG DI
PERKAMPUNGAN ORANG ASLI DAN PERKAMPUNGAN ORANG
MELAYU DI MALAYSIA**

Oleh

MOHAMED YOUSIF IBRAHIM MOHAMED

Julai 2019

Pengerusi : Profesor Jalila Abu, PhD
Fakulti : Perubatan Veterinar

Campylobacter jejuni dan *Escherichia coli* merupakan penyebab gastroenteritis pada manusia yang ketara di negara membangun dan kemunculan kerintangan antibiotik bagi bakteria tersebut telah dilaporkan secara meluas dan semakin meningkat, terutama kerana peningkatan bilangan *C. jejuni* dan *E. coli* yang rintang antibiotik yang diasingkan daripada jangkitan manusia. Beberapa kajian melaporkan produk asal haiwan yang dicemari menyumbang secara signifikan kepada penyakit tersebut. Unggas, terutama ayam dan burung liar telah kerap dilaporkan dijangkiti dengan *C. jejuni* dan *E. coli*. *Campylobacter jejuni* dan *E. coli* yang rintang antibiotik yang terdapat pada burung liar dan ayam dapat memindahkan gen kepada mikroorganisma dalam saluran gastrousus mereka. Beberapa kajian dalam negara telah menunjukkan kehadiran *C. jejuni* dan *E. coli* pada ayam dan daging ayam. Burung liar telah kelihatan dalam jumlah yang banyak di kawasan perumahan, kawasan pasar dan ladang. Kajian tersebut telah melaporkan bahawa burung liar dapat memindahkan *C. jejuni* dan *E. coli* kepada kawasan perumahan dan persekitaran ladang ternakan. Walau bagaimanapun, sangat sedikit kajian telah dijalankan mengenai *C. jejuni* dan *E. coli* yang kerintangan antibiotik pada burung liar, ayam dan manusia di Malaysia. Juga, sangat sedikit kajian telah dijalankan bagi mengesan gen pada patogen *E. coli* dan tiada kajian telah dijalankan bagi mengesan jenis urutan (ST) bagi patogen *E. coli* daripada berbagai sumber di Malaysia. Oleh sebab itu, objektif kajian ini adalah, (i) untuk menentukan kehadiran *C. jejuni* dan *E. coli* pada burung liar, ayam, persekitaran dan manusia di perkampungan terpilih di Perak dan di Kedah, (ii) untuk menentukan profil kerintangan antibiotik dalam kalangan isolat *C. jejuni* dan *E. coli*, (iii) untuk mengesan patogen *E. coli* (EPEC, EIEC) dalam kalangan isolat *E. coli*, (iv) untuk mencirikan *E. coli* menggunakan asai pengetipan urutan multilokus (MLST), dan

(v) untuk menentukan keterkaitan antara patogen *E. coli* dan jenis urutan (ST) daripada sumber yang berbagai.

Sebanyak tiga buah perkampungan Orang Asli telah dipilih di Perak dan tiga perkampungan orang Melayu di Kota Setar, Kedah. Sembilan puluh sembilan (99) najis manusia, swab kloaka daripada 113 ayam dan 78 burung liar telah dikumpul. Sampel persekitaran termasuk, lalat (85) air (100) dan tanah (88). *Campylobacter jejuni* telah diasingkan daripada 24(21.2%) ayam dan 19(22.4%) lalat. Sejumlah 92(92.9%) manusia didapati positif *E. coli* manakala tiada sampel manusia yang positif *C. jejuni*. Lima puluh lapan (74.4%) burung positif *E. coli*. Lapan puluh empat (74.3%) ayam positif *E. coli* manakala 24(21.2%) positif *C. jejuni*. Daripada 273 sampel persekitaran, 220 (80.6%) menunjukkan positif *E. coli*, kebanyakannya ialah isolat daripada lalat, 77(35%) dan air, 74 (33.6%).

Isolat *C. jejuni* daripada ayam menunjukkan kerintangan pada 1-7 antibiotik. Ketahanan yang paling tinggi adalah pada asid nalidiksik (40.9%) manakala semua isolat didapati sensitif pada gentamisin. Isolat lalat menunjukkan kerintangan pada 2-7 antibiotik. Kerintangan paling tinggi adalah pada sefpodosimin (65%) dan isolat adalah sensitif pada gentamisin dan eritromisin. Isolat *E. coli* daripada manusia menunjukkan kerintangan pada 1-9 antibiotik dan kerintangan tertinggi adalah pada eritromisin (100%). Isolat ayam menunjukkan kerintangan pada 2-9 antibiotik dengan kerintangan tertinggi adalah pada eritromisin (98.8%). Isolat *E. coli* daripada burung liar menunjukkan kerintangan pada 1-8 antibiotik. Seratus peratus (100%) isolat burung liar didapati rintang pada eritromisin diikuti oleh tetrasiklin (87.3%). Isolat lalat menunjukkan kerintangan pada 2-9 antibiotik. Kerintangan tertinggi adalah pada eritromisin (97%) diikuti oleh tetrasiklin (57.5%). Isolat dari air menunjukkan kerintangan pada 3-10 antibiotik. Seratus peratus (100%) isolat air didapati rintang pada eritromisin. Isolat tanah menunjukkan kerintangan pada 1-10 antibiotik. Ketahanan tertinggi adalah pada eritromisin (100%) diikuti oleh tetrasiklin (60.3%).

Tiga puluh lapan (45.2%) isolat ayam adalah positif gen *eaeA*, enam (7.1%) dan dua (2.4%) adalah masing-masing positif EPEC dan EIEC. Tujuh (12.1%) isolat burung liar adalah positif gen *eaeA*. Enam belas (29.6%) isolat persekitaran adalah positif gen *eaeA*, EPEC telah dikesan dalam isolat air (1.9%) dan EIEC telah dikesan dalam isolat tanah (1.9%).

Analisis Pengetipan Urutan Multilokus (MLST) memperlihatkan dua puluh jenis urutan (ST) daripada 42 patogen *E. coli*. Tujuh isolat burung liar memperlihatkan enam ST, iaitu ST 2705, ST 2491, ST 484, ST 2253, ST 453 dan ST295. Tiga belas ST telah dikesan daripada 19 isolat ayam, iaitu ST 5686, ST 155, ST 10, ST 181, ST 3856, ST 1141, ST 202, ST 542, ST 295, ST 188, ST 453, ST 206 dan ST 48 dan satu ST tidak dapat dikesan. Tiga isolat lalat memperlihatkan tiga ST, iaitu ST 295, ST 155 dan ST 181. Enam isolat air memperlihatkan dua ST, iaitu ST 162, ST 69 dan satu ST tidak dapat dikesan. Lima ST telah diperlihatkan daripada tujuh isolat tanah, iaitu ST 5686, ST 181, ST 46, ST 2419, ST 202 dan dua ST tidak dapat dikesan.

Daripada kajian ini, kerintangan dadah majmuk (MDR) yang tinggi ialah isolat daripada burung liar di beberapa perkampungan dan persamaan diperlihatkan antara beberapa *E. coli* ST daripada burung liar (ST295, ST2491 dan ST453) dengan isolat ayam dan persekitaran di beberapa perkampungan mengimplicasikan bahawa burung tersebut merupakan sumber utama bagi kerintangan antibiotik *E. coli* dan patogen *E. coli*. Juga, kajian mendapati bahawa *C. jejuni* terdapat pada ayam dan lalat manakala *E. coli* terdapat pada manusia, ayam, burung liar dan persekitaran di perkampungan tersebut. Walau bagaimanapun, patogen *E. coli* telah dikesan daripada burung liar, ayam dan persekitaran. Kewujudan kerintangan dadah majmuk yang tinggi dalam *C. jejuni* dan *E. coli* akan menjejaskan rawatan pada manusia dan terutama sekali, sekiranya bakteria tersebut rintang pada dadah pilihan dan dadah alternatif bagi rawatan dan oleh itu, secara signifikan boleh mengakibatkan risiko kesihatan awam. Ayam dan lalat memainkan peranan dalam penyebaran atau mungkin memperoleh kerintangan antibiotik *C. jejuni* pada persekitaran perkampungan tersebut. Oleh itu, kehadiran *C. jejuni* dalam ayam dan persekitaran, dan patogen *E. coli* dalam burung liar, ayam dan persekitaran mungkin boleh memudaratkan kesihatan manusia sekiranya terdedah pada organisma tersebut.

Kata kunci: *Campylobacter jejuni*, *Escherichia coli*, burung liar, ayam, persekitaran, manusia, kerintangan antibiotik.

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Signature: _____
Name of Member
of Supervisory
Committee: Professor Dr. Zunita Zakaria

Signature: _____
Name of Member
of Supervisory
Committee: Professor Dr. Abdul Rashid Khan

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
°C	Degree Celsius
CCUG	Culture Collection, University of Goteborg
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EFSA	Community Zoonoses Reports of the European Food Safety Authority
FAO	Food and Agriculture Organization
FoodNet	Foodborne Diseases Active Surveillance Network
km	Kilometer
MDR	Multidrug drug resistance
ml	Milliliter
m-PCR	Multiplex polymerase chain reaction
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
rRNA	Ribosomal ribonucleic acid
V	Volt

CHAPTER 1

INTRODUCTION

Antimicrobial resistance (AMR) is an emerging global threat. According to one estimate, unless appropriate action is taken, the annual number of deaths due to antimicrobial resistance may reach 10 million by 2050 (Jasovský *et al.*, 2016). This estimate, however, is considered high and has been questioned by others. Nevertheless, problems associated with antimicrobial resistance and the urgency to act are recognized by all. The presence of antimicrobial resistant bacteria and related resistant genes in the environment is well recognized for their role in the spread of antimicrobial resistance (Singer *et al.*, 2015). Numerous resistant genes associated with human diseases have an environmental origin (Braykov *et al.*, 2014). Recent studies related to AMR have focused on the monitoring and treatment of resistant genes in different environmental matrices. A focus on environmental occurrence is essential not only from the perspective of water and environmental safety but also to prevent the spread of AMR. Resistant genes in wastewater treatment plants, for example, are being studied from multiple perspectives due to the reuse of wastewater for agricultural purposes often without extensive treatment.

Globally, the increase and spread of antibiotic resistance, especially to foodborne zoonotic bacteria with their reservoirs in healthy food animals, such as poultry, pigs and cattle, have become a public health concern (EFSA, 2009). In developed countries, there is an increasing number of scientific reports regarding the widespread usage of antibiotics in food animal production, which leads to the development of resistant pathogenic organisms in the food chain (Marshall and Levy, 2011; Philips *et al.*, 2004).

The resistant elements reduce the efficiency of antibiotic therapy, which results in an increased morbidity and mortality associated with disease outbreaks (Da Costa *et al.*, 2009). It has been reported by Schwarz and Chaslus-Dancla (2001) that an antibiotic therapy with a specific agent has been either accompanied or followed shortly by the occurrence of resistant bacteria, and, for the past ten years, the effect of antibiotics on the development of resistance has gained much attention (Shin *et al.*, 2007). In developed and developing countries, *Campylobacter* species are among the leading causes of zoonotic infections (EFSA, 2013), and the prevalence rate is increasing. In Europe, campylobacteriosis is the most reported zoonotic enteric disease surpassing salmonellosis (EFSA, 2013; Fosse *et al.*, 2009).

Wild birds are important with regard to antibiotic resistance in several different ways: 1) As sentinels, mirroring human activity and its impact on the environment because of the diverse ecological niches of birds, and as they easily pick up human and environmental bacteria. 2) As a reservoir and melting pot of antibiotic-resistant bacteria and resistant genes. 3) As potential spreaders of antibiotic resistance through the ability to migrate long distances in short periods of time. 4) As a possible source

of antibiotic resistant bacteria colonizing and/or infecting human beings. The first antibiotic resistant bacteria noted in wildlife were in fact from wild bird strains of *Escherichia coli*, which were resistant to multiple antibiotics; for example, chloramphenicol was isolated in pigeons around 1975 (Bonnedahl and Järhult, 2014). Many bird species have been found to carry antibiotic resistant bacteria. Resistant *E. coli* has been isolated from ducks and geese (Huang *et al.*, 2017, Vogt *et al.*, 2018), cormorants (Tausova *et al.*, 2012), birds of prey (Vredenburg *et al.*, 2014), gulls (Atterby *et al.*, 2016), doves, and passerines (Mohamed-Yousif *et al.*, 2018). The results from a study on *Campylobacter* by Chen *et al.* (2010) showed that the vast majority of the isolates from birds appeared to be resistant to fluoroquinolones by more than 98%. In the same study, *Campylobacter 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 advent of enrofloxacin in veterinary medicine (Skov *et al.*, 2015; Shadoud *et al.*, 2015; Kikuchi *et al.*, 2014).

Poultry is regarded as the major source of campylobacteriosis in humans, while, apart from chickens, other animals, such as pigs and cattle, also serve as reservoir hosts (Stanley and Jones, 2003). *Campylobacter* species are also found in various other domestic and wild animals, including goats, horses, cats, rodents, and dogs (Man, 2010), and have been isolated from marine animals, such as shellfish and dolphins. Poultry are considered to be the main reservoir of *Campylobacter*. Among *Campylobacter* and *Campylobacter jejuni* are frequent causes of foodborne diseases in humans (Uaboi-Egbenni *et al.*, 2012). The risk to *C. jejuni* infection in humans includes the consumption of undercooked poultry meat, handling of raw poultry carcasses, drinking untreated water, drinking unpasteurized milk or ingestion of dairy products made from raw unpasteurized milk, and international travel (Danis *et al.*, 2009). The majority of the cases occur intermittently. The consumption of poultry meat, contact with infected animals, drinking contaminated water, and travel are the highest risks associated with the infection (Everest and Ketley, 2002). In poultry, the occurrence of *C. jejuni* varies according to the age and type of farm; they are seldom detected in broiler chickens less than 2-3 weeks of age and in those managed under a closed house system (Sahin *et al.*, 2002).

Campylobacter infection is normally self-limiting, but it may be associated with complications, such as Guillain-Barre Syndrome (neurological) and Reiter's Syndrome (reactive arthritis) (Yan *et al.*, 2005). Once a broiler chicken becomes infected, *Campylobacter* spread rapidly to other broiler chickens in that flock, and up to slaughter age, or, at thinning, the chickens remain colonized. Almost all (100%) of the broiler chickens brought to the slaughterhouses were reported to be colonized with *Campylobacter* (Jacobs-Reitsma *et al.*, 2008), and the contaminated chicken meat acts as a probable risk of human campylobacteriosis. The detection of *C. jejuni*, *C. coli* and *E. coli* on the carcasses is mainly due to contamination from the gastrointestinal contents of slaughtered healthy animals during processing as well as at retail (Nonga *et al.*, 2010). *Campylobacter* has been reported to develop resistance to a number of antibiotics including ciprofloxacin and other fluoroquinolones, macrolides and lincosamides, chloramphenicols, aminoglycosides, tetracyclines, and ampicillins, as

well as β -lactams, cotrimoxazole, and tylosin (Moore *et al.*, 2006; Padungton and Kaneene, 2003).

Escherichia coli is part of the normal enteric microbial flora in humans, poultry, and other animals, and the pathogenic *E. coli* causes disease in both (Amin *et al.*, 2012). The pathogenic *E. coli* causes a number of diseases in both poultry and humans, which include hemorrhagic colitis, hemolytic uremic syndrome, acute and chronic endemic and epidemic diarrhea. Diarrheagenic strains of *E. coli* can be divided into five main categories on the basis of distinct epidemiological and clinical features, specific virulence determinants, and association with certain serotypes: enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enterotoxigenic *E. coli* (ETEC). They are transmitted from person to person via direct contact with animal carriers, feces, contaminated soil and water or via ingestion undercooked meat and other animal products, as well as contaminated vegetables and fruits. As a result of contamination from feces, they are often found in soil, water and food. Commensal *E. coli* flora can be regarded as a rich source of emergence and spreading of antibiotic resistance (Da Costa *et al.*, 2013). Chickens may also be infected with antibiotic resistant *E. coli* or may develop resistance to one or more antibiotics in the gut.

DNA-based methods of typing *C. jejuni* and pathogenic *E. coli* have the potential to resolve the controversy surrounding the origin of infection, but have thus far failed to do so. *Campylobacter jejuni* and pathogenic *E. coli* isolated from humans show considerable genetic overlap with meat and poultry isolates (Manges *et al.*, 2016; Dearlove *et al.*, 2016). Hence, a model-based approach that includes disparate sources is needed. Although *C. jejuni* genotypes and pathogenic *E. coli* genotypes do show some host association, the population is not strongly structured into differentiated clusters, so predicting the host from the genotype is challenging (Kovanen *et al.*, 2016; Riley, 2014). Phylogenetic approaches to tracing the source of infection have suggested that human isolates are more closely related to pathogens found in non-livestock than livestock (Wilson *et al.*, 2008a). However, recombination is frequent in organisms, which means that a single phylogenetic tree is not an appropriate way to represent the ancestral history of a collection of organisms' genomes (Manges *et al.*, 2016; Riley, 2014).

There is an alarming increase in antibiotic resistant bacteria, particularly in terms of MDR, among the human and animal populations globally (Carlet *et al.*, 2012; Marshal and Levy 2011). However, limited data exist concerning the occurrence of multidrug resistant (MDR) *E. coli* and *C. jejuni* in chickens and the environment in the villages in Malaysia.

In Malaysia, the three different populations are made up of city population, Malay villages population and Orang Asli population (Al-Delaimy *et al.*, 2014). Malay villages are away from cities, with people working on farms, rearing chickens and trading. An overwhelming 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*), Jungle Myna (*Acridotheres fuscus*), Rock Pigeon (*Columba livia*), Peaceful Doves (*Geopelia placida*) and Oriental Magpie Robins (*Copsychus saularis*).

The Orang Asli is the indigenous people of Peninsular Malaysia. Orang Asli villages are further away from cities near jungle. People living in these villages are the genus of Malaysian (Al-Delaimy *et al.*, 2014). Depending on the tribe, some are hunter-gatherers while others are farmers and traders. Some of the Orang Asli villages have no access to treated water or electricity. The houses in these villages are primitive and made from wood. Common wild birds in these villages include Oriental Magpie Robins, White-rumped Shama (*Copsychus malabaricus*), Little Spiderhunters (*Arachnothera longirostra*), Blue-tailed Bee-eaters (*Merops philippinus*) and White-throated Kingfishers (*Halcyon smyrnensis*).

The hypotheses of this study were:

- i. There is a low occurrence of antibiotic-resistant *C. jejuni* and *E. coli* in wild birds, chickens, humans, and the environment in Orang Asli and Malay villages.
- ii. There is a low occurrence of multidrug resistant (MDR) *C. jejuni* and *E. coli*.
- iii. Wild birds could play a role in the occurrence of *C. jejuni* and *E. coli* in chickens and the environment in the villages.

Thus, the objectives of this study were:

- i. To detect the occurrence of *C. jejuni* and *E. coli* in wild birds, chickens, humans, and environment.
- ii. To determine the antibiotic resistance profiles among *C. jejuni* and *E. coli* isolates from wild birds, chickens, and humans.
- iii. To detect the occurrence of typical EPEC (*eaeA* and *bfeA*) and atypical EPEC (*eaeA*) and EIEC (*ial*) in *E. coli* isolates.
- iv. To characterize molecularly *C. jejuni* and *E. coli* isolates using multilocus sequence typing (MLST).
- v. To determine the interrelatedness among the *E. coli* genotypes.

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