UNIVERSITI PUTRA MALAYSIA

OCCURRENCE OF MULTIDRUG RESISTANT E.coli AND Campylobacter IN CHICKEN AND CHICKEN MEAT AND THEIR ASSOCIATED RISK FACTORS

IBRAHIM JALO MUHAMMAD

FPV 2015 9

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By

IBRAHIM, JALO MUHAMMAD

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

May 2015

DEDICATION

This thesis is specially dedicated to:

My beloved parents,

ALHAJI IBRAHIM JALO MUHAMMAD

and

HAJIYA FATSUMA IBRAHIM JALO

My beloved wife and children,

HUSSAINA ABUBAKAR LAWAN

FATIMA MUHAMMAD JALO (AMNA)

ABUBAKAR MUHAMMAD JALO (AMMAR)

Who always pray, support and encourage me to do the best

And finally,

In memory of my late sister

HAUWA IBRAHIM JALO

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

OCCURRENCE OF MULTIDRUG RESISTANT *E.coli* AND *Campylobacter* IN CHICKEN AND CHICKEN MEAT AND THEIR ASSOCIATED RISK FACTORS

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IBRAHIM JALO MUHAMMAD

May 2015

Chairperson: Professor Saleha Abdul Aziz, PhD
Faculty: Veterinary Medicine

Antibiotic usage is an important factor that have been widely reported to bring about the emergence and spread of antibiotic resistant microorganisms in both animal and human health. Chickens may be infected with antibiotic resistant *Campylobacter* and *E.coli* which may be resistant to one or more antibiotics. There is an alarming increase of multidrug resistance among bacterial pathogens affecting human and animal populations globally. There is limited data on the occurrence of multidrug resistance (MDR) *E. coli* and *Campylobacter* in poultry and poultry meat in Malaysia. This study was aimed at investigating the occurrence of multidrug resistant *E. coli* and *Campylobacter* in chicken and chicken meat and their associated risk factors. The specific objectives were: to determine the occurrence of *E.coli* and *Campylobacter* in chickens and farm environment and chicken meat retailed in the markets and their associated risk factors and to determine the antibiotic resistance and MDR profiles of the isolates from chicken and chicken meat. Two hundred and ten (210) samples were collected from five farms and seventy (70) samples from seven markets. Five (5) farms were visited three (3) times each during the study period. The first visit was to sample chicken at market age (35-42 days) prior to harvesting (considered as first population) and farm environment (which is included water, feed, flies and chicken house floor). The second visit was to sample farm environment at two (2) weeks after the chicken farms were emptied (all chicken were sold), cleaned and disinfected and the third visit was carried out on a new batch of chickens at market age also prior to harvesting (as second population) and the environment. In each farm, thirty (30) cloacal swab samples and twelve (12) environmental samples which consisted of three (3) samples each of water from the drinker, feed from the feeder, chicken house floor and pooled samples of flies each consisting 5-7 per pooled sample were collected (3). Chicken meat which consisted of breast, thigh and wings including the skin were randomly chosen from stalls in each market. The number of stalls visited in seven markets varied from 4 to 10 per market depending on the number of poultry meat retailers in that market. Cutting boards and weighing scales were also sampled. Fifty five point seven percent (60.0% and 51.3%) of the chickens in the first and second population were colonized with *Campylobacter*. The proportion of *Campylobacter* isolated from environmental samples in the first and third visits was 7.5% (1.7% and 13.3%). Only one water

sample (6.7%) was positive (first visit) and two (13.3%) in the third visit. Flies were found positive for *Campylobacter* only in the third visit (33.3%). Among the chicken house floor samples, only one was positive (6.7%). All feed samples were negative (0%). The most frequently isolated species was *C. jejuni* at 74.9% (82.2% and 67.5%), followed by *C. coli* 25.2% (17.8% and 32.5%). At the markets, the occurrence of *Campylobacter* was 20.0% for chicken meat, and 5.7% for weighing scales and cutting boards. The species identified were 78.6%, 17.9% and 3.6% for *C.jejuni*, *C.coli* and *C.upsaliensis* respectively. The occurrence of *E.coli* in chickens is 53.0% (56.0% and 50.0%). Similarly, the occurrence of *E.coli* in environmental samples at first and third visits were 33.4% (26.7%, 0% and 40.0%). Among the environmental samples, the isolation of *E.coli* in feed was 16.7%, floor 40.0%, water 20.0% and flies 56.7%. The occurrence of *E.coli* in chicken meat at markets was 45.0%. In the farms, the occurrence of the *Campylobacter* and *E.coli* in the first and second population were almost similar; this was probably due to the farm practices were similar and the biosecurity measures practised in the farm may not be sufficient. The presence of flies and birds around the farm and/or the use of contaminated water may transfer these bacteria to the chickens or the workers brought them into the chicken houses from the farm environment. At the markets, cross contamination was a possible factor because during meat handling and cutting, the surfaces of poultry carcasses could become contaminated with *Campylobacter* and *E.coli* from the intestinal content due to accidental rupture of the gut or from contaminated equipment or water. The absence of *Campylobacter* in feed samples may probably be due to poor resistance of *Campylobacter* to atmospheric condition and other environmental pressures during storage that would have converted to viable-but-non-culturable (VBNC) form. It was reported that most flocks were negative until two (2) weeks of age, and once *Campylobacter* colonized a broiler flock, the spread is very rapid and up to 100% of birds within a flock can become colonized within three days. The antibiotic resistance of these *Campylobacter* (n=208) and *E.coli* (n=269) isolates was done using disc diffusion method against 12 different antibiotics. *Campylobacter* isolates from first and second population showed high resistance to penicillins at 75.5% (74.4% and 76.6%) and the least resistance was to amoxycillin_clavulanic acid at 33.7% (32.2% and 35.1%). Sixty two point five percent (62.5%) *Campylobacter* isolated from chicken meat was resistant to ampicillin and the least was to streptomycin, 3.1%. *E.coli* isolated from chicken in first population showed high resistance to penicillin, erythromycin, ampicillin and tetracycline at 98.8%, 96.4%, 94.0% and 92.8% respectively and the least resistance was to amoxycillin_clavulanic at 22.9%. Those isolated from the second population were 100% resistant to erythromycin and tetracycline and the least resistance was to cefotaxime at 20.0%. In chicken meat, the highest resistance was to erythromycin and penicillins at 100% each and the least was to gentamicin at 40%. The high resistance may likely be as a result of imprudent use of these agents for growth promotion and for prophylactic purposes at farm level. Multidrug resistance (MDR) (resistance to 3 or more classes of antibiotics) was high in *Campylobacter* isolated in chicken farms, at 87.5%, chicken meat 71.9%, while in *E.coli* it was 99.5% for chicken, and for chicken meat was 100%. The most common multidrug resistance profile for *Campylobacter* isolates in chicken was to 7 (EPNaCtxSCipAmp) and 10 (EPNaCipTeAmcSxtCnEnrAmp) classes of antibiotics at 13.2% each in the first population. In the second population, the common MDR pattern was to 9 (EPNaCtxSCipAmcEnrAmp) classes at 12.9%. In chicken meat it was to 4 (TeAmcEnrAmp) and 5 (EPTeCtxAmp) classes at 15.6% each. The most common MDR profile for *E. coli* was to 9 (EPNaSCipTeAmcSxtAmp) and 10 (EPNaSCipTeAmcSxtEnrAmp) antibiotic classes at 16.0% each in the first population and in the second population, the most common profile was to 8 (EPNaSCipTeAmcSxtEnrAmp) classes at 19.2%. It was to 8 (EPNaSCipTeEnrAmp) classes of antibiotics at 21.4% in chicken meat. The findings suggested the possible persistence of the resistant organisms in the farm environment even after cleaning and disinfection of the chicken houses. Flies may had play a role in introducing the resistant bacteria to the chicken farm and environment. Imprudent use of antibiotics as growth promoters or for therapeutic purposes not administered professionally could lead to development of resistance by these bacteria. However, health records were not available in the farms and information on antibiotics used could not be obtained making it impossible to make sound deduction except to make assumptions. Risk factors that were significantly ($p<0.05$) associated with *Campylobacter* contamination in chickens meat included; no working attire (OR 2.7, CI 1.144-6.374, $p=0.033$), fair and poor usage of protective equipment (OR 12.6, CI 1.186-133.899, $p=0.036$) and (OR 38.50, CI 2.915-508.463, $p=0.006$) respectively, poor stall hygiene (OR 44.00, CI 2.193-882.66, $p=0.013$) and use of wood counter surface (OR 6.1, CI 1.198-31.164, $p=0.029$). The relevant but not significant factor was poor working hygiene (OR 5.250, CI 0.988-27.895, $p=0.05$). This study identified five risk factors for *Campylobacter* contamination that, if taken together, might account for most sporadic cases. The presence of high MDR *Campylobacter* and *E.coli* species 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. Certainly, at farm level, the prevalence of MDR *Campylobacter* and *E.coli* in broiler flocks should be monitored. From this study it was observed that there is a need to stress the awareness among farmers to observe good hygienic practices and prudent use of antibiotics to reduce the menace of antibiotic resistance.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sains Veterinar

KEHADIRAN *E.coli* DAN *Campylobacter* RINTANG PELBAGAI DRUG PADA AYAM DAN DAGING AYAM SERTA FAKTOR RISIKO YANG BERKAITAN

Oleh

IBRAHIM JALO MUHAMMAD

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Penggunaan antibiotik adalah faktor penting yang membawa kepada kemunculan dan penyebaran mikroorganisma rintang antibiotik pada kesihatan veterinar dan manusia. Ayam boleh dijangkiti *Campylobacter* dan *E. coli* yang rintang terhadap satu atau lebih antibiotik. Terdapat peningkatan yang membimbangkan terhadap kerintangan antibiotik pada patogen bakteria manusia dan haiwan secara global. Terdapat data yang terhad berkaitan kejadian *E.coli* dan *Campylobacter* rintang pelbagai drug (MDR) pada ayam dan daging ayam di Malaysia. Kajian ini dijalankan untuk mengkaji *E.coli* dan *Campylobacter* yang rintang pelbagai drug pada ayam dan daging ayam dan faktor risiko yang berkaitan. Objektif khusus adalah: untuk menentukan kehadiran *E.coli* dan *Campylobacter* pada ayam dan persekitaran ladang dan daging ayam yang dijual di pasar dan faktor risiko yang berkaitan dan untuk menentukan profil rintangan antibiotik bakteria yang diasingkan daripada ayam dan daging ayam. Dua ratus sepuluh (210) ayam daging disampel di lima ladang dan 70 daging ayam di gerai jualan ayam di tujuh pasar. Lima (5) ladang tersebut telah dilawati sebanyak tiga kali dalam tempoh kajian. Lawatan pertama adalah untuk mengambil sampel daripada ayam pada umur pasaran (35-42 hari) sebelum dijual (populasi pertama) dan persekitaran ladang, lawatan kedua adalah untuk mengambil sampel persekitaran ladang dua (2) minggu selepas ladang ayam dikosongkan (semua ayam telah dijual), dibersihkan dan dibasmi kuman dan lawatan ketiga telah dijalankan dengan mengambil sampel ke atas kumpulan baru ayam pada umur pasaran juga sebelum dijual (populasi kedua) dan kawasan sekitarnya. Dalam setiap ladang, tiga puluh (30) sampel swab kloaka ayam dan dua belas (12) sampel kawasan sekitar, terdiri daripada tiga (3) sampel setiap jenis sampel iaitu air dari bekas minuman (3), sampel makanan daripada bekas makanan (3) sampel lantai rumah ayam (3) dan sampel lalat (disatukan 5-7 lalat dalam satu botol sebagai satu sampel) (3) dikumpulkan. Daging ayam telah dipilih secara rawak yang mengandungi bahagian dada, paha dan sayap, yang disaluti kulit. Bilangan sampel daripada setiap pasar adalah berbeza iaitu daripada 4 hingga 10 gerai jualan ayam yang ada di setiap pasar bergantung kepada jumlah gerai di pasar tersebut. Papan pemotong dan alat timbang juga disampel. Lima puluh lima pepuluhan tujuh (60.0% and 51.3%) ayam pada populasi satu dan dua dijangkiti *Campylobacter*. *Campylobacter* yang diasingkan daripada persekitaran ladang adalah 7.5% (1.7% dan 13.3%). Hanya satu sampel air yang positif *Campylobacter* (6.7%) pada lawatan pertama dan dua

(13.3%) positif pada lawatan ketiga. Lalat yang positif *Campylobacter* hanya pada lawatan ketiga (33.3%) dan hanya satu sampel lantai (6.7%) yang positif. Semua sampel makanan adalah negatif *Campylobacter*. Spesies yang paling kerap dipencilkan pada populasi pertama dan kedua adalah *C.jejuni* (74.9%), diikuti oleh *C.coli* (25.2%). Di pasar, kehadiran *Campylobacter* adalah 20.0% pada daging ayam, dan 5.7% daripada alat timbang dan papan pemotong dan spesies *Campylobacter* yang dikenalpasti adalah *C. jejuni*, *C.coli* dan *C.upsaliensis* masing-masing 78.6%, 17.9% dan 3.6%. Kehadiran *E. coli* pada ayam adalah 53.0%. Begitu juga, kehadiran *E.coli* pada sampel persekitaran pada lawatan pertama dan ketiga masing-masing 26.7% dan 40.0%, dan semua sampel adalah negatif pada lawatan kedua. Di antara sampel persekitaran, kadar pencilan pada makanan 13.3%, lantai 60.0%, air 13.3% dan lalat 73.3%. Kehadiran *E.coli* pada daging ayam di pasar adalah 45.0%. Di ladang, kehadiran *Campylobacter* dan *E. coli* pada lawatan populasi pertama dan kedua adalah hampir sama; ini mungkin kerana langkah-langkah amalan ladang adalah sama, biosekuriti yang diamalkan di ladang mungkin tidak mencukupi. Kehadiran lalat dan burung di sekitar ladang atau penggunaan air yang tercemar boleh memindahkan bakteria ini kepada ayam atau pekerja membawa bakteria tersebut ke dalam rumah ayam daripada persekitaran ladang. Di gerai jualan ayam di pasar, kontaminasi silang mungkin menjadi faktor semasa pengendalian dan memotong daging dan permukaan daging ayam boleh tercemar dengan *Campylobacter* dan *E.coli* daripada kandungan usus kerana usus terpecah atau daripada peralatan yang tercemar atau daripada air. Ketiadaan *Campylobacter* dalam sampel makanan mungkin kerana ketahanan *Campylobacter* yang lemah kepada keadaan dan tekanan persekitaran yang lain semasa penyimpanan sehingga mereka bertukar kepada bentuk *viable-but-not culturable*. Kebanyakan kelompok ayam adalah negatif sehingga berumur dua (2) minggu, dan apabila *Campylobacter* menjangkiti kelompok ayam daging, penyebaran bakteria adalah sangat cepat dan sehingga 100% daripada ayam dalam kelompok boleh dijangkiti dalam tempoh tiga (3) hari . Ujian kerintangan antibiotik telah dijalankan untuk menentukan corak rintangan antibiotik ke atas pencilan *Campylobacter* (n = 208) dan *E.coli* (n = 269) dengan menggunakan kaedah "disc diffusion" dan diuji terhadap 12 antibiotik yang berbeza. Pencilan *Campylobacter* menunjukkan rintangan yang tinggi terhadap *penicillin* adalah 75.5% (74.4% dan 76.6%) dan rintangan yang paling rendah adalah terhadap *amoxycillin_clavulanic acid* 33.7% (32.2% dan 35.1%). Pada daging ayam, *Campylobacter* menunjukkan rintangan yang tinggi terihadap *ampicillin* (62.5%) dan yang paling rendah adalah kepada *streptomycin* 3.1%. Didapati *E.coli* yang dipencil deripada ayam pada populasi pertama rintang terhadap *penicillin*, *erythromycin, ampicillin* dan *tetracycline* yang masing-masing pada 98.8%, 96.4%, 94.0% dan 92.8% dan rintangan yang paling rendah adalah terhadap *amoxicillin_cluvanic acid* 22.9% pada lawatan pertama. Manakala pada populasi kedua, rintangan yang paling tinggi adalah terhadap *erythromycin* dan *tetracycline* iaitu 100%, dan rintangan yang paling rendah adalah terhadap *cefotaxime* 20.0%. Bagi pencilan daripada daging ayam, rintangan yang paling tinggi adalah kepada *erythromycin* dan *penicillin* iaitu masing-masing 100% dan rintangan yang paling rendah adalah kepada *gentamicin* pada 40%. Rintangan yang tinggi mungkin terjadi hasil daripada penggunaan antibiotik yang kurang bijak untuk menggalakkan pertumbuhan dan untuk tujuan profilaksis di ladang dan dengan itu bakteria akan terbawa daripada ayam kepada daging ayam. Rintangan pelbagai drug (MDR) (rintangan kepada 3 atau lebih kelas antibiotik) adalah tinggi pada *Campylobacter* yang di pencilkan daripada ladang ayam iaitu 87.5% dan daging ayam 71.9%, manakala bagi *E.coli* adalah 99.5% dan untuk daging ayam 100%. Rintangan pelbagai drug paling kerap diperolehi untuk *Campylobacter* yang diasingkan dari ayam adalah kepada 7 (EPNaCtxSCipAmp) dan 10 (EPNaCipTeAmcSxtCnEnrAmp) kelas antibiotik, 13.2% setiap satu pada populasi pertama dan pada populasi kedua corak MDR yang kerap adalah kepada 9 (EPNaCtxSCipAmcEnrAmp) kelas iaitu 12.9%. Pada daging ayam, MDR ialah kepada 4 (TeAmcEnrAmp) dan 5 (EPTeCtxAmp) kelas iaitu pada 15.6% setiap satu. *E.coli* pada ayam, menunjukkan MDR paling kerap kepada 9 (EPNaSCipTeAmcSxtAmp) dan 10 (EPNaSCipTeAmcSxtEnrAmp) kelas antibiotik iaitu 16.0% setiap satu dalam populasi pertama dan populasi kedua. Corak yang paling kerap adalah kepada 8 (EPNaSCipTeAmcSxtEnrAmp) kelas antibiotik iaitu pada 19.2%. Pada daging ayam MDR adalah terhadap 8 (EPNaSCipTeEnrAmp) kelas antibiotik pada 21.4%. Hasil kajian memperlihatkan kehadiran organisma rintang antibiotik dalam persekitaran yang berpanjangan walaupun selepas pembersihan ladang. Lalat juga boleh memainkan peranan dalam membawa masuk bakteria rintang antibiotik ke ladang. Penggunaan antibiotik yang tidak bijak sebagai penggalak pertumbuhan atau untuk tujuan terapeutik yang digunakan secara tidak profesional boleh membawa kepada pembentukan rintangan oleh bakteria ini. Rekod kesihatan tidak terdapat di ladang-ladang dan maklumat tentang antibiotik yang digunakan tidak dapat diperolehi menyukarkan untuk membuat sesuatu kesimpulan dan hanya dapat membuat andaian sahaja. Faktor risiko ketara ($p < 0.05$) yang berkaitan dengan pencemaran *Campylobacter* pada ayam daging termasuklah: tiada pakaian kerja (OR 2.7, CI 1,144-6,374, p = 0,033), kekurangan penggunaan peralatan perlindungan yang baik (OR 12.6, CI 1,186-133,899, p = 0.036) dan (OR 38.50, CI 2,915-508,463, p = 0.006), kandang yang kurang basih (OR 44.00, CI 2,193-882,66, p = 0.013) dan penggunaan permukaan pemotong daripada kayu (OR 6.1, CI 1,198-31,164, p = 0.029). Faktor yang berkaitan tetapi tidak ketara adalah kerja yang kurang bersih (OR 5.250, CI 0,988-27,895, p = 0.05). Kajian ini mengenal pasti lima (5) faktor risiko pencemaran oleh *Campylobacter* dan jika diambil kira semuanya, mungkin boleh menerangkan sebab bagi kebanyakan kes-kes sporadik. Kehadiran MDR *Campylobacter* dan *E.coli* yang tinggi boleh menjejaskan rawatan pada manusia terutamanya jika antibiotik diperlukan dan jika organisma tersebut rintang kepada ubat dan ubat alternatif untuk rawatan dan dengan itu boleh menimbulkan risiko kesihatan yang ketara. Sudah tentu, di peringkat ladang, kehadiran *Campylobacter* dan *E.coli* pada ayam daging perlu dipantau untuk memastikan pelaksanaan langkah-langkah amalan penternakan, veterinar dan biosekuriti yang baik. Daripada kajian ini juga didapati bahawa terdapat keperluan untuk mewujudkan kesedaran dalam kalangan penternak untuk mematuhi amalan kebersihan yang baik dan penggunaan antibiotik secara berhemah untuk mengurangkan ancaman rintangan antibiotik.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory committee were as follows:

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

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ATCC	American Type Culture Collection
bp	Base pairs
CCUG	Culture Collection of the University of Goteborg
cueE	Siderophoreenterochelin
CLSI	Clinical Laboratory Standard Institute
^0C	Degree celcius
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic
flaA	Flagellin A gene
glyA	Serine hydroxyl methyl transferase gene
g	Gram(s)
h	Hour(s)
hip	Hippuricase gene
Kb	Kilobase
ml	Mililitre
mg	Milligram
mg	Milligram(s)
min	Minute(s)
MDR	Multidrug resistance
mPCR	Multiplex Polymerase Chain Reaction
NaCl	Sodium chloride
NCCLS	National Committee for Clinical Laboratory Standards
PFGE	Pulsed Field Gel Electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid

rRNA	Ribosomal RNA
s	Second(s)
Spp.	Species
TAE	Tris-acetate-EDTA
TBE	Tris-borate EDTA
UV	Ultraviolet
V	Volt
Taq	Thermophilus aquaticus
WHO	World Health Organization
µL	Micro liter
µg	Micro Gram
µM	Micro molar
EMB	Eosin Methylene Blue
SIM	Sulfide-Indole-Motility medium
MR-VP	Methyl Red- Voges-Proskaure
BPW	Buffered Peptone Water
NA	Nutrients Agar
$	Chi-square Tests
P	Probability

CHAPTER 1

INTRODUCTION

In livestock production, the use of antibiotics for disease treatment and prevention is important. It is recognised that the use of these agents in food animals creates the development of antibiotic resistant bacteria, which disseminate via the food chain. Globally, the increase and spread of antibiotic resistance especially to foodborne zoonotic bacteria with its reservoirs in healthy food animals such as poultry, pigs and cattle has become public health concern (EFSA, 2008a). In developed countries, there is increasing scientific reports regarding the widespread of antibiotic usage in food animal production that leads to the development of resistant pathogenic organisms in the food chain (Marshall & Levy, 2011; Philips et al., 2004).

The resistance 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 have gained much attention (Smith et al., 2007). In developed and developing countries, *Campylobacter* species are among the leading causes of zoonotic infections (EFSA, 2013; Anonymous, 2008) that the prevalence rate is increasing. In Europe, campylobacteriosis is the most reported zoonotic enteric disease surpassing salmonellosis (EFSA 2012; Fosse et al., 2007).

Poultry meat is regarded as the major source of campylobacteriosis in human, apart from chicken; other animals such as pigs and cattle also serve as reservoir hosts (Stanley & Jones, 2003). *Campylobacter* species is also found in various other domestic and wild animals, including goats, horses, cats, rodents and dogs (Man, 2011); they are also isolated from marine animals such as shellfish and dolphins. Poultry is considered as the main reservoir of *Campylobacter*. Among *Campylobacter*, *Campylobacter jejuni* and *Campylobacter coli* are the frequently cause of foodborne diseases in human (Uaboi-Egbenni et al., 2012). The risk to *Campylobacter* infection in human include 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 or travel are the highest risk associated with the infection (Everest and Ketley, 2002). In poultry, the occurrence of *Campylobacter* varies according to age and the type of farm; they are seldom detected in broiler chicken less than 2-3 weeks of age and in those managed under close 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 slaughter houses 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. 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 reported to develop resistance to a number of antibiotics including ciprofloxacin and other fluoroquinolones, macrolides and licosamides, chloramphenicols, aminoglycosides, tetracyclines, ampicillins and other β-lactams, cotrimoxazole, and tylosin (Moore et al., 2006; Padungton and Kaneene, 2003).

Escherichia coli is part of the normal enteric microbial flora in human, poultry and other animals and the pathogenic *E.coli* causes disease in both (Amin et al., 2012). Pathogenic *E. coli* cause a number of diseases in both poultry such as colibacilosis, aerosacculitis, polyserositis, septicemia and other mainly extraintestinal diseases. In humans diseases caused include hemorrhagic colitis, haemolytic uremic syndrome, acute and chronic endemic and epidemic diarrhoea. They are transmitted from person to person via direct contact with animal carriers, faeces, 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 faeces, it is 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). The chicken may also be infected with antibiotic resistance *E.coli* or may develop resistant to one or more antibiotics in the gut.

There is an alarming increase of antibiotic resistant bacteria in particular on MDR among human and animal population globally (Carlet et al., 2012; Marshal and Levy 2011).

However, limited data exists on the occurrence of multidrug resistant (MDR) *E.coli* and *Campylobacter* in poultry and poultry meat in Malaysia.

The hypothesis of this study was: There is a high occurrence of MDR *E.coli* and *Campylobacter* in broiler chicken (in farms) prior to harvesting and on chicken meat (retailed in markets).

Therefore, the objectives of this study were:

1. to determine the occurrence of *E.coli* and *Campylobacter* in chicken in the farm prior to harvesting and chicken meat retailed in the markets.
2. to determine the multidrug resistance (MDR) and antibiotic resistant profiles among the isolates.
3. to determine the associated risk factors in the occurrence of multidrug resistant (MDR) *Campylobacter* in chickens.

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