Agricultural Research Updates

Volume 32

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Editors

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PREFACE

This compilation opens with a review of the past and present literature related to conservation tillage practices in durum wheat production and analyses the problems that arise from conservation tillage practices.

Additionally, drought and salt tolerance are evaluated at an early stage of plant development in a collection of nine cultivated durum wheat cultivars (*Triticum durum* Desf.), using plants grown under hydroponic conditions.

The role of linear discriminant analysis and related chemometric methods in combination with liquid chromatography for the characterization, classification and authentication of foodstuffs and beverages is addressed.

The authors investigate the prevalence of *Campylobacter jejuni* and its toxin genes, namely the cytolethal distending toxin gene, in selected vegetables that are commonly consumed raw in Kuala Terengganu, Malaysia.

The *Campylobacter jejuni* isolated from selected vegetables is characterized against 10 types of antibiotics from six classes commonly used in clinical and agricultural settings, namely aminoglycosides, fluoroquinolones, glycopeptides, macrolides, beta-lactams, and tetracyclines.

The closing chapter discusses the case of the kwebo, a low-cost, multi-purpose farm structure designed to be typhoon-resistant yet easy to construct, making use of prefabricated structural elements and construction aids.

Chapter 1 - Conservation tillage practices, basically defined as tillage method that at least 30% of the crop residue should remain on the soil surface at the time of planting, are an important part of a sustainable agricultural system because they have many potential economic and environmental advantages. There are several types of conservation tillage practices, including mulch tillage, ridge tillage, strip tillage, and no-tillage. Also, reduced tillage and minimum tillage methods are considered within the scope of conservation tillage practices. The performance of conservation tillage practices is affected by numerous natural and cultural factors including management practices such as residue, fertilization, seeding methods, pest, diseases, weeds, weather and soil conditions, types of machine and tools, and the interaction of these and many other factors. Many studies have been conducted to investigate the effects of conservation tillage practices on yield and quality of durum wheat (*Triticum durum Desf.*). Some researchers reported that conservation tillage practices resulted in sustainable soil properties and increased crop productivity, but usually many problems in crop production due to weed, disease, pest was observed depending on crop rotation, soil and climate conditions, residue management and tillage methods, and many other factors. This chapter reviews past and present literature related to conservation tillage practices in durum wheat production and analysis the problems that arise from conservation tillage practices. Also, it discusses how conservation tillage practices influence the yield and quality of durum wheat in different climate and management conditions.

Chapter 2 - Salinity and drought are two important abiotic stresses that limit the production of food crops worldwide. Physiological responses of plants to drought and salinity stress can have common physiological and morphological characteristics. Many different traits contribute to salinity and drought tolerance, which are species-specific and developmental stage-dependent. In the present study, drought and salt tolerance were evaluated

at an early stage of plant development in a core collection composed of nine ancient and currently cultivated durum wheat cultivars (*Triticum durum* Desf.), using plants grown under hydroponic conditions. These wheat cultivars were subjected to a water stress using a treatment with polyethylene glycol (PEG) and to a salinity stress in the presence of NaCl during twenty-one days. A number of morphological and physiological traits representative of plant development and primary carbon and nitrogen metabolism were analyzed. Principal component analysis allowed the identification of genotypes exhibiting various level of tolerance to PEG and NaCl. Modern cultivars INRAT 69 and Om Rabiaa were the most tolerant to the two stresses respectively, whereas landraces Ben Bechir and Mahmoudi were the most sensitive. Among the nineteen morphological and physiological measured traits, shoot nitrate and shoot and root starch contents were found to be good markers that can be used to select plant resistant to water stress. Root ammonium, proline and soluble sugars contents of both shoot and root were those that can be used to select plants resistant to salinity.

Chapter 3 - Nowadays, society's interest in the quality of food products, especially those with beneficial health properties, is growing. Furthermore, consumers are increasingly interested in products with additional quality attributes such as their origin, cultivation techniques, varieties, etc. However, fraudulent practices such as adulteration of food products are booming, mainly due to the complexity of the food chain and the handling of foodstuffs from its production to its consumption. For this reason, the development of analytical methodologies for the characterization, classification and authentication of foods and beverages is necessary. Liquid chromatography with ultraviolet or fluorescence detection as well as coupled with low- and high-resolution mass spectrometry is one of the most widely used techniques for this purpose. Strategies to address food authentication rely on: (i) Targeted methods, in which a group of known compounds is quantified, and (ii) non-targeted methods, where the instrumental response is registered without assuming any previous knowledge of relevant or irrelevant food components. In both cases, due to the complexity of food sample matrices and the variability of

chemical components that can be present, the amount of chemical data that can be extracted is huge. Consequently, the use of chemometric data treatment methodologies is mandatory to extract relevant chemical information from the sample data sets to characterize, classify and authenticate foodstuffs and beverages. Among them, linear discriminant analysis (LDA) and other related chemometric approaches such as partial least squares regression-discriminant analysis (PLS-DA), orthogonal projections to latent structures-DA (OPLS-DA), canonical discriminant analysis (CDA), and soft independent modeling of class analogies (SIMCA) are employed to extract chemical descriptors to classify and authenticate foodstuffs and beverages. In this chapter, the role of LDA and related chemometric methods in combination with liquid chromatography for the characterization, classification and authentication of foodstuffs and beverages is addressed. An exhaustive coverage of all kind of applications in such an important field is beyond the scope of the present contribution, so the chapter will focus on some relevant applications published in the last years.

Chapter 4 - *Campylobacter jejuni* is being recognized as the leading pathogen that cause gastroenteritis in human. However, its' fastidious nature and difficulty to grow on conventional agar plates has led to low detection in food samples. The present study was conducted to investigate the prevalence *Campylobacter jejuni* and its toxin genes, namely cytolethal distending toxin (*cdt*) gene, in selected vegetables that are commonly consumed as raw in Kuala Terengganu, Malaysia. A total of 128 samples consisting of six types of vegetables were being investigated for the presence of *C. jejuni* and the cdt genes (*cdt*A, *cdt*B, and *cdt*C) by using a multiplex PCR method and conventional plating unto modified Charcoal-Cefoperazone Deoxycholate Agar (mCCDA). Multiplex PCR assay detected 63 samples (49.2%) were positive with *C. jejuni* and conventional plating on mCCDA agar detected 13 samples (10.2%) were positive with *C. jejuni*. Wild cosmos has the highest contamination and water spinach has the lowest contamination with 85.0% and 5.0%, respectively. *cdt*C gene presence at highest percentage with 22.2%, followed by *cdt*B gene with 12.7% and none *cdt*A detected. It can be concluded that the

vegetables popularly consumed in raw form pose risk of acquiring toxigenic *C. jejuni* which might lead to campylobacteriosis.

Chapter 5 - Multiple antibiotic resistance in *Campylobacter jejuni* has been increasing significantly due to the excessive use of antibiotics in poultry farming. The usage of manure as fertilizer for growing vegetables has posed a risk of contamination with antibiotics resistance *C. jejuni* strain. Several types of vegetables that are commonly consumed as raw will pose significant risk of contracting campylobacteriosis which is difficult to treat with conventional antibiotics. The goal of this study was to characterize the *C. jejuni* isolated from selected vegetables against 10 types of antibiotics from six classes of antibiotics commonly used in clinical and agricultural setting, namely aminoglycosides, fluoroquinolones, glycopeptides, macrolides, beta-lactams, and tetracyclines. Thirteen *C. jejuni* isolates were subjected to antibiotic resistance test using disk diffusion method on Muller Hinton agar. The antibiotic resistance profile was analysed and multiple antibiotic resistance (MAR) index was determined. *C. jejuni* isolates were found to be highly resistant towards beta-lactam class at 80.8% and glycopeptides at 76.9%. However, they were sensitive towards fluoroquinolones and aminoglycosides at 5.1 and 7.7%, respectively. Eleven *C. jejuni* isolates had MAR index more than 0.20 which indicated the contamination was from high risk sources. Thus, more biosafety measures are required to prevent *C. jejuni* cross contamination on farms and at retail outlets.

Chapter 6 - The crop damage and loss of farm investments in the Philippines are often due to typhoons. Thus, for farming operations that are shelter-dependent, it is necessary to make the accompanying structure typhoon-resistant. This paper discusses about the case of *Kwebo*, a low cost multi-purpose farm structure designed to be typhoon-resistant yet easy to construct, making use of prefabricated structural elements and construction aids. It documents the process of technology development up to the time it was deployed to a farming community wherein the farmers themselves were able to build the structure by themselves.

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In: Agricultural Research Updates. Volume 32 ISBN: 978-1-53618-974-2
Editors: Prathamesh Gorawala et al.

Chapter 5

ANTIBIOGRAM AND MULTIPLE ANTIBIOTIC RESISTANCE (MAR) INDEX OF *CAMPYLOBACTER JEJUNI* ISOLATED FROM FRESH PRODUCE

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ABSTRACT

Multiple antibiotic resistance in *Campylobacter jejuni* has been increasing significantly due to the excessive use of antibiotics in poultry farming. The usage of manure as fertilizer for growing vegetables has posed a risk of contamination with antibiotics resistance *C. jejuni* strain. Several types of vegetables that are commonly consumed as raw will pose significant risk of contracting campylobacteriosis which is difficult to treat with conventional antibiotics. The goal of this study was to characterize the *C. jejuni* isolated from selected vegetables against 10 types of antibiotics from six classes of antibiotics commonly used in clinical and agricultural setting, namely aminoglycosides, fluoroquinolones, glycopeptides, macrolides, beta-lactams, and tetracyclines. Thirteen *C. jejuni* isolates were subjected to antibiotic resistance test using disk diffusion method on Muller Hinton agar. The antibiotic resistance profile was analysed and multiple antibiotic resistance (MAR) index was determined. *C. jejuni* isolates were found to be highly resistant towards beta-lactam class at 80.8% and glycopeptides at 76.9%. However, they were sensitive towards fluoroquinolones and aminoglycosides at 5.1 and 7.7%, respectively. Eleven *C. jejuni* isolates had MAR index more than 0.20 which indicated the contamination was from high risk sources. Thus, more biosafety measures are required to prevent *C. jejuni* cross contamination on farms and at retail outlets.

Keywords: antibiotic resistance, MAR index, *Campylobacter jejuni*, vegetables

INTRODUCTION

Campylobacter cells are Gram negative mainly slender, spiral curved rods shape, 0.2 to 0.8 μm wide and 0.5 to 5 μm long. There are some species that are mostly curved or straight rods shape. Most cells of the species are motile with cork- screw motion performed by a single polar unsheathed flagellum at one or both ends of the cell. On the other hand, there are cells of some species that are non-motile or have multiple flagella. For biochemical characteristics, several species of *Campylobacter* grow in anaerobic condition with fumarate or nitrate as electron acceptor and hydrogen, formate or succinate are present to supplement electron to

the microorganisms. *Campylobacter* only grow under microaerobic condition (Nachamkin et al., 2007). *Campylobacter* spp. is microaerophilic and has to cope with oxidative stress and the toxic products produced from oxygen metabolism. However, these organisms do survive in food in sufficient numbers to cause infection in human, despite these limitations (Humphrey, 1995).

Campylobacter spp. continue to be the one of the pathogen frequently implicated with bacterial gastrointestinal infections in humans. They are fastidious microorganism but are capable of overcoming many stresses in the foods, in the environment and in the host defense mechanism to cause diseases (Murphy et al., 2006). There are 11 species within the genus *Campylobacter* but *C. jejuni* and *C. coli* are recognized to be pathogenic and responsible for campylobacteriosis in human. Between the two pathogens, cases of *C. jejuni* infections are more frequently reported. *C. jejuni* is known to be fragile microorganism that do not readily cultured in laboratory agar media. It is sensitive to environmental stresses such as atmospheric oxygen (Hoffman et al., 1979), high temperature (Blaser et al., 1980), and drying (Doyle and Roman, 1982).

Disease caused by *Campylobacter* spp. infection are generally self-limiting without the need of antibiotics prescription (Belanger and Shryock, 2007). Antibiotics treatment may be required to those who are immunocompromised, elderly, young children or pregnant women especially in cases of prolonged enteritis and septicemia (Allos, 2001). Among the available antibiotics, fluoroquinolones and macrolides are commonly administered in cases of prolonged campylobacteriosis (de Saussure, 2009; Hill et al., 2006). Besides, erythromycin and tetracycline is also popular and drug of choice for treating severe campylobacteriosis because it is highly effective with minimal toxicity (Trachoo, 2003; Adedayo and Kirkpatrick, 2008).

Antibiotic-resistance among foodborne pathogen is becoming a worldwide problem which pose significant threat to public health and it is also an important food safety issue. Different types of bacteria were becoming more resistant towards antibiotics with certain strains had developed resistance to different antibiotics (Mansouri-najand, 2012).

Introduction of flouroquinolones in the 1980s had offered a new means to treat acute gastroenteritis (Winstrom and Norrby, 1995; Dryden et al., 1996) but Chai et al. (2008) had found out *C. jejuni* isolates were resistance toward fluoroquinolone group of antibiotics and the resistance was related to commercial farm practices. After the reports of *Campylobacter* resistance to fluoroquinolones being reported since 1990's (Aarestrup and Engberg, 2001), macrolides are being considered as a favourable drug for treating campylobacteriosis even though there are also cases of *Campylobacter* resistance to this agent (Vlieghe et al., 2008).

The mechanisms of bacterial resistance against antibiotic include antibiotics degradation by bacterial enzyme, bacterial proteins alteration to avoid being targeted by antibiotics, and reduce membrane permeability to antibiotics (Dever and Demody, 1991). Over the years, increase number of antibiotics resistant cases has emerge in many bacteria strains. Antibiotics resistance has become an emerging problem as the number of resistance bacteria strain that isolated from patients has increase enormously. According to WHO (2011), bacterial antibiotic resistance data are important for microbial risk assessment study for a particular foodborne pathogen. The objectives of this study are to determine the antibiotic resistant profiles and the MAR index of *C. jejuni* isolates from salad vegetables.

METHODS

Bacterial Strains

This study included 13 isolates of *C. jejuni* isolated from various types of vegetable, from retail outlets around Terengganu, Malaysia. All isolates were revived with Bolton Selective Enrichment Broth (BB, CM0983B; Oxoid, Hampshire, England) without supplement and incubated at 42°C for 48 h in an anaerobic jar (AnaeroPack Rectangular Jar 0.4 L, FisherScientific) under microaerophilic condition using Anaerocult C (Merck, Germany).

Antimicrobial Susceptibility Testing

Antibiotics resistance pattern were performed using disk diffusion method (CLSI, 2006). *C. jejuni* isolates in glycerol stock were revived using Bolton Broth without lysed horse blood (Oxoid, Hampshire, England) for 48 h at 42ºC. The cultures were adjusted to 0.5 McFarland before being spread uniformly onto Mueller Hinton (MH) agar plates (Merck, Germany) using sterile cotton swab.

The selected antibiotics were amikacin (Ak) (30ug), gentamycin (Cn) (10ug), ciprofloxacin (Cip) (5ug), norfloxacin (Nor) (10ug), enrofloxacin (Enr) (5ug), vancomycin (Va) (5ug), erythromycin (E) (15ug), penicillin G (P) (10ug), ampicillin (Amp) (10ug), and tetracycline (Te) (30ug). Swab plates were incubated at 42ºC for 48 h under microaerophilic condition produced by Anaerocult C system (Merck, Germany). Antibiotic disc dispenser were used to place the antibiotics discs on the agar surface.

Data Interpretation

After 24 h incubation, the diameter of inhibition zones was measured and the levels of susceptible/resistance were determined based on Clinical and Laboratory Standard Institute guidelines (CLSI, 2006). Determination of strains as resistant, intermediate and susceptible was based on the size of the inhibition zones around every antimicrobial disc. The zone diameters were measured to the nearest millimetre. Intermediate-resistant isolates were classified together with resistant isolates for further interpretation of data (CLSI, 2006).

RESULTS AND DISCUSSION

From the Table 1, all 13 isolates of *C. jejuni* were tested against 10 types of antibiotics that are frequently used in clinical and agricultural practices. It was found that *C. jejuni* isolates were 100% susceptible to

Gentamicin 120 µg, Ciprofloxacin 5 µg, and Enrofloxacin 5 µg. Highest resistance of the isolates were found towards Penicillin G 10 µg (92.3%), followed by Vancomycin 5 µg (76.9%) and Erythromycin 15 µg (53.8%). Low antibiotic resistance was found against Amikacin 30 µg (13.4%), Norfloxacin 10 µg (13.4%), and Tetracycline 30 µg (23.1%). *C. jejuni* isolates showed highest resistance towards beta-lactams (80.8%), glycopeptides (76.0%), and macrolides (53.8%). The isolates were least resistant towards fluoroquinolones (5.1%), followed by aminoglycosides (7.7%) and tetracyclines (23.1%). Two of the isolates showed MAR index of 0.20 and the remaining 11 isolates were above 0.20 MAR index (Table 2) which indicated the isolates were possible from high risk source (Krumperman, 1983).

Table 1. Antibiotic resistance test of *Campylobacter jejuni*

Class of antibiotic	**Antibiotic**	**Disk content (µg)**	**n**	**No. (%) of samples**	
				Resistant	Sensitive
Aminoglycosides	Amikacin (Ak)	30 µg	13	2 (13.4)	11 (86.6)
	Gentamicin (Cn)	120 µg	13	0 (0.0)	13 (100.0)
TOTAL			*26*	*2 (7.7)*	*24 (92.3)*
Fluoroquinolones	Ciprofloxacin (Cip)	5 µg	13	0 (0.0)	13 (100.0)
	Norfloxacin (Nor)	10 µg	13	2 (13.4)	11 (86.6)
	Enrofloxacin (Enr)	5 µg	13	0 (0.0)	13 (100.0)
		TOTAL	*39*	*2 (5.1)*	*37 (94.9)*
Glycopeptides	Vancomycin (Va)	5 µg	13	10 (76.9)	3 (23.1)
		TOTAL	*13*	*10 (76.9)*	*3 (23.1)*
Macrolides	Erythromycin (E)	15 µg	13	7 (53.8)	6 (46.2)
		TOTAL	*13*	*7 (53.8)*	*6 (46.2)*
Beta-lactams	Penicillin (P)	10 µg	13	12 (92.3)	1 (7.7)
	Ampicillin (Amp)	10 µg	13	9 (69.2)	4 (30.8)
		TOTAL	*26*	*21 (80.8)*	*5 (19.2)*
Tetracyclines	Tetracycline (Te)	30 µg	13	3 (23.1)	10 (76.9)
		TOTAL	*13*	*3 (23.1)*	*10 (76.9)*

Table 2. Antibiogram of *Campylobacter jejuni* isolates

No.	Isolates	Source	Antibiotics resistance	MAR index
1	LB6	WM1	PVaAmpEAk	0.50
2	LB1	WM1	PAmpTeAk	0.40
3	LB2	WM2	PVaAmpNor	0.40
4	LB3	WM1	PVaAmpE	0.40
5	LB4	SP1	PVaAmpE	0.40
6	LB5	SP1	PVaENor	0.40
7	WB2	WM1	PVaAmpE	0.40
8	LB7	SP2	PVaAmp	0.30
9	WB1	WM1	PVaE	0.30
10	WD1	WM1	PVaTe	0.30
11	WB3	WM2	PVaAmp	0.30
12	WS1	WM1	PAmp	0.20
13	WD2	WM1	ETe	0.20

LN, Long Bean; WB, Winged Bean; WS, Water Spinach; WD, Water Dropwort WM, Wet Market; SP, Supermarket.

The exposure of bacteria to antimicrobial agents found in the environment become the important selective factor for bacterial resistance towards particular antibiotics. Thus, the antibiotic resistance profile differs geographically in which different practices in antibiotics administration. Antibiotic resistance in bacteria is resulted from complex reaction of environmental and genes mutations, bacterial genome transposition and genetic material exchange between bacteria (O'Brien, 2002).

Foodborne illness or infection caused by antibiotic resistant *C. jejuni* strains cause difficulty in clinical management of serious campylobacteriosis (Iovine, 2013). Antimicrobial resistant strain will prolong illness and render irresponsive treatment in patients with bacteremia. Developing countries recorded highest percentage of antibiotic resistant enteric infections. Such scenario was thought as a result from unrestricted usage of antimicrobial drugs in human and animal.

Patient suffering from campylobacteriosis may develop symptoms which include fever, diarrhoea (blood or without blood), vomiting, stomachache, muscle and joint pain. Infection in human from *Campylobacter* had been previously thought to be around 10,000 cells.

However, several studies have found smaller number of *Campylobacter* cells at around 500 cells are capable of causing disease in human. The difference in the infectious dose can be caused by several reasons such as the type of food consumed (e.g., meat or vegetables) and the overall wellbeing of the volunteer (FDA, 2012). The finding was supported by other researchers that suggest 500 to 800 of highly virulent *Campylobacter* strain were capable of infecting human (Black et al., 1988; Robinson, 1981). Once *Campylobacter* has been ingested, it will take 2 to 5 days before clinical symptoms appear. The disease will resolved by itself and may last around 2 to 10 days. Patient infected with *Campylobacter* may show distinct food poisoning symptoms of continuous abdominal pain to such intensity resembling acute appendicitis (Young and Mansfield, 2005; FDA, 2012).

The sequel for those who has contracted and recovered from camplybacteriosis may include developing a secondary autoimmune disease such as reactive arthritis or Guillain-Barre syndrome. These disease occurs were thought as result of antigen of *Campylobacter* similar the antigen on human nervous system which resulted immune cells attack host cells and cause weakness in muscle, arthritis or paralysis (Havelaar et al., 2009). *Campylobacter* characteristics such as motility, adherence, invasion and toxin production will determine its' virulence. The mechanism of pathogenicity on how *Campylobacter* adhere and invade the intestinal epithelial cells has not been fully elucidated (Levin, 2007). However, it was suggested that *Campylobacter* spiral shape and flagella which enable rapid motility help it to penetrate through the intestinal lining (Levin, 2007; Bhavasar and Kapadnis, 2007).

Thermotolerant *Campylobacter*, such as *Campylobacter jejuni* and *Campylobacter coli* are most frequently isolated from humans. Human illness caused by *C. jejuni* and *C. coli* estimated at 80% and 18.6%, respectively and small amount foodborne illness caused by *C. fetus* (Gurtler et al. 2005; FDA, 2012). Back in 1972, *C. jejuni* was mainly associated with animal disease that cause abortion and enteritis in cattle and sheeps.

Campylobacter may cause infection in individuals consuming contaminated food. However, high risk group such as toddler, children younger than 5 years old, immunocompromised patient (e.g., HIV/AIDS, cancer or under immunosuppressive medications) and elderly. It has been suggested that AIDS patients are 40 times more vulnerable from *Campylobacter* infection compared to those within the same age group with normal and healthy immune systems. Vertical transmission from pregnant mother to unborn child which cause miscarriages or stillbirths is extremely rare. It was estimated 0.1% of mortality rate from campylobacteriosis and infection in healthy person very unlikely to result in death (FDA 2012).

Bacterial susceptibility against antibiotic would determine the likelihood of effective treatment at the clinical dosage for severe cases of infection. Whereas, the resistant strains are more likely irresponsive towards antibiotics which result in therapeutic failure. Intermediate strains will pose unpredictable therapeutic success as certain strains are resistance to antibiotics which render ineffective treatment.

First isolation of aminoglycosides are streptomycin from *Streptomyces griseus* and it was effective antibiotic against Gram negative bacteria (Schatz et al., 1944). Additional aminoglycosides were discovered such as gentamicin from *Micromonospora purpurea* (Weinstein et al., 1963) and amikacin, a type of synthetic aminoglycosides which was synthesed in vitro (Kawaguchi, 1976). For the mechanism of action, it binds to the 30S ribosomal subunit and interfering translocation of tRNA from the A-site to the P-site. Therefore, results in bacteria cannot synthesize proteins that important for its growth.

The quinolones are synthetic antibiotics with a basic structure of dual ring. Nalidixic acid was first quinolones discovered in year 1960 as the starting of quinolones antibiotics (Lehtopolku, 2011). Quinolones consists of four groups which differentiated according to their mechanism of action (Andriole, 2003; Van Bambeke et al., 2005). Newer compound added into this family contain fluorine (fluoroquinolones) as well as other functional substitutions to enhance their efficacy against Gram-negative (second group), Gram-positive (third group) bacteria and also anaerobic bacteria

(fourth group). Quinolones antibacterial action targeted inhibition of bacterial DNA synthesis to prevent replication (Hooper, 2001; Yao and Moellering, 2003). It targeted DNA gyrase and topoisomerase IV found in most bacteria that are important in bacterial DNA repair, recombination, replication, and transcription (Jacoby, 2005). Fluoroquinolones will bind to these enzymes which render them useless and cause cell death (Hooper, 2001). Since the 1990's, bacterial resistance to fluoroquinolones has been reported to increase significantly (Aarestrup and Engberg, 2001).

Macrolides can be extracted from microorganisms such as *Streptomyces* and related bacteria or may be semisynthetic. Back in year 1952, the first macrolide isolated is erythromycin. It was found in *Streptomyces erythreus* (currently known as *Saccharopolyspora erythraea*) (Lehtopolku, 2010). All antibiotics within macrolides share a macrolactone ring and it is based on this ring structure 14-, 15-, or 16-membered compounds are being grouped (Bryskier and Butzler, 2005). Macrolides is considered safe and effective antibiotic against most of the Gram positive, Gram negative as well as anaerobes microorganism. Macrolides mechanism of action lies on interrupting proteins synthesis in bacterial ribosome which consist of large and small ribosomal subunits. The large 50S ribosomal subunit consists of the ribosomal proteins, 23S and 5S rRNA. The small 30S ribosomal subunits consists of ribosomal proteins and 16S rRNA. Macrolides inhibition of protein synthesis generally targeted the large 50S ribosomal subunit which causes ribosome three dimensional conformation changes and subsequent termination of the peptide chain elongation (Pfister et al., 2004; Poehlsgaard and Douthwaite, 2005; Yao and Moellering, 2003).

Campylobacters are known to be resistant to β-lactam group of antibiotics especially penicillins and cephaloporins (Van der Auwera and Scorneaux, 1985). They are seldom being used in clinical treatment for campylobacteriosis (Leibovitz et al., 2000). β-lactam targeted peptidoglycan of bacteria cell wall by disrupting the formation of peptidoglycan which eventually lead to cellular rupture and death (Martin and Kaye, 2004).

Streptomyces spp. also produce important antimicrobial agent such as tetracyclines. Tetracyclines is popular bacteriostatic antibiotic due to its broad-spectrum action (Chopra, 2003). Even though tetracyclines is popular antibiotic for campylobacteriosis treatment, it should not be given to children below nine years old (Moore et al., 2005). Opposite to macrolides, tetracyclines targeted the small 30S ribosomal subunit and inhibits peptide elongation (Connell et al., 2003). Tetracyclines resistance among *Campylobacter* isolates has been reported in which between year 1999 and 2002, approximately 50% of 203 clinical *C. jejuni* strains were resistant to tetracyclines (Gibreel et al., 2005).

C. jejuni resistance towards quinolones and erythromycin had caught attention of clinician and researcher around the world. Study by Chai et al., (2008) reported *C. jejuni* in '*ulam*' showed the same pattern as found in this study that resistancy of *Campylobacter* isolates towards erythromycin was high (>50%). Our study found fluoroquinolone, aminoglycosides and tetracycline were effective against *C. jejuni* isolates from retail outlets. These findings contradicted with the finding by Chai et al., (2008) and other reports that showed *C. jejuni* high resistance towards fluoroquinolone. This might be due to the use of quinolone as a prophylaxis in animal farming which also had affected in vegetable (Han et al., 2007; Kassa et al., 2007; Rodrigo et al., 2007).

The antibiotic resistance found in *C. jejuni* was suggested as a result from the abuse of antibiotic usage in agricultural and clinical setting (Chai et al., 2008). Wilson (2003) showed resistance to floroquinolones in *Campylobacters* can be due to these antibiotics being widely used in poultry and partly due to fluoroquinolones-resistant *Campylobacter* strains being biologically stronger in the chickens and outcompete majority of fluoroquinolone susceptible strains (Snelling et al., 2005). Chai et al. (2008) suggested the resistance trend of fluoroquinolone group was due to the widespread use of these antimicrobial drugs in agriculture industry. Such practice was thought to be one of the important factor that led to fluoroquinolone resistant strain in human isolates (Sanchez et al., 1994; Iovine et al., 2004).

However, low level of resistance observed in all *C. jejuni* isolates towards fluoroquinolone and aminoglycosides group suggested these antibiotics were not commonly used in agricultural farming. According to Reina et al., (1992), fluoroquinolones resistant strains has been increasingly rapidly since early 1990s. The study suggested this trend was partly contributed by indiscriminate use of these agents and cross-resistance from enrofloxacin.

Penicillin G had showed high level of antibiotics resistance which is nearly 92% in all *C. jejuni* isolates. It is due to the ability of *C. jejuni* to produce *b*-lactamase making the use of drugs from *b*-lactamase group suboptimal, especially in serious infections. Our study also found *C. jejuni* isolates resistance towards erythromycin is about 53.8% was significantly higher than other study which recorded 26.8% resistance (Rodrigo et al., 2007). National Antimicrobial Resistance Monitoring System reported slow changes in erythromycin resistance with only 2% (4 of 217) in 1997 and 2% (8 of 384) in 2001 (Gupta et al., 2004).

High resistance to erythromycin and tetracycline was reported in Thailand at 38.3 and 66.2%, respectively (Padungtod et al., 2006). The study isolated *Campylobacter* spp. from chicken, pig, dairy and human. Our finding is in agreement with the study and this suggest the pathogen contamination might be from high risk source (e.g., animal farming). Over the years, studies have found the resistance rate of *C. jejuni* toward erythromycin isolated from human has changed very little (Navarro et al., 1993; Sjogren, 1997) even though there is a report of increased percentage of erythromycin-resistance *C. jejuni* isolates (Gibreel et al., 2006).

CONCLUSION

Antibiotic resistance profile in *C. jejuni* isolated from vegetables differs geographically and greatly affected by the agricultural activities around the area. Since antibiotics are not commonly used in vegetable farm, the antibiotic resistance in *C. jejuni* was thought to be originated from animal as indicated by MAR index.

ACKNOWLEDGMENTS

This research was supported by a research grant from the International Foundation of Sciences (IFS), Sweden (E/5237-2F).

REFERENCES

Adedayo, O., and Kirkpatrick, B. D. 2008. *Campylobacter jejuni* infections: update on presentation, diagnosis, and management. *Hospital Physician* 44:9-15.

Allos, B. M. 2001. *Campylobacter jejuni* Infections: update on emerging issues and trends. *Clinical Infectious Disease* 32(8):1201-1206.

Andriole V. T. 2003. Quinolones. In: Finch R., Greenwood D., Norrby S., Whitley R., editors. *Antibiotic and Chemotherapy*. 8th edition. UK: Churchill Livingstone, pp. 348-352.

Arrestrup, F. M., and Engberg, J. E. 2001. Antimicrobial resistance of thermophilic *Campylobacter. Veterinary Research* 32:311–321.

Belanger, A. E. and Shryock, T. R. 2007. Macrolide-resistant *Campylobacter*: the meat of the matter. *Journal of Antimicrobial Chemotherapy* 60(4):715-723.

Bhavasar, S. P., and Kapadnis, B.P. 2007. Virulence factors of *Campylobacter*. *The Internet Journal of Microbiology* 3:2

Black, R. E., Levine, M. M., Clements, M. L., Hughes, T. P., and Blaser, M.J. 1988. Experimental *Campylobacter jejuni* infection in humans. *The Journal of Infectious Diseases* 157:472-479.

Blaser, M. J., Hardesty, H. L., Powers, B. and Wang, W. L. 1980. Survival of *Campylobacter fetus* subsp. *jejuni* in biological milieus. *Journal of Clinical Microbiology* 11:309–313.

Bryskier A. and Butzler J. P. 2005. Macrolides. In: Finch R., Greenwood D., Norrby S., Whitley R., editors. *Antibiotic and Chemotherapy*. 8th edition. Philadelphia: Churchill Livingstone. pp. 310-327.

Chai, L. C., Fatimah, A. B., Laila, R. A. S., Ghazali, F. M., Thahirahtul, A.Z., and Lee, H. Y., Malakar, P. M., Son, R., Tunung, R., Nakaguchi, Y., Shamsinar, A., and Nishibuchi, M. 2008. Biosafety of *Campylobacter jejuni* from raw vegetables consumed as *Ulam* with reference to their resistance to antibiotics. *International Food Research Journal* 15:125-134.

CLSI, 2006. *Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline M45-A.* Clinical and Laboratory Standards Institute, Wayne, PA.

Chopra I. 2003. Tetracyclines. In: Finch R, Greenwood D, Norrby S, Whitley R, editors. *Antibiotic and Chemotherapy*. 8th edition. UK: Churchill Livingstone, pp. 393-394.

Connell S. R., Trieber C. A., Dinos G. P., Einfeld E., Taylor D. E., and Nierhaus K. H. 2003. Mechanism of Tet(O)- mediated tetracycline resistance. *EMBO Journal* 22(4):945- 953.

de Saussure, P. P. 2009. Management of the returning traveller with diarrhea. *Therapy Advance Gastroenterology* 2:367-375.

Dever, L. A. and Dermody, T. S. 1991. Mechanisms of bacterial resistance to antibiotics. *Archive of Internal Medicine* 151:886-895.

Doyle, M. P. and Roman, D. J. 1982. Growth and survival of *Campylobacter fetus* subsp. *jejuni* as a function of temperature and pH. *Journal of Food Protection* 44:596–601.

Dryden, M. S., Gabb, R. J., Wright, S. K. 1996. Empirical treatment of severe acute community-acquired gastroenteritis with ciprofloxacin. *Clinical Infectious Disease* 22:1019-1025.

FDA, 2012. *Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook*, 2nd ed. US Food and Drug Administration, Silver Spring, p.17–20. http://www.fda.gov/Food/FoodborneIllness Contaminants/CausesOfIllnessBadBugBook/ucm2006773.htm. Accessed 27 June 2019.

Gibreel A., Kos V. N., Keelan M., Trieber C. A., Levesque S., Michaud S., and Taylor D. E. 2006. Macrolide Resistance in *Campylobacter jejuni* and *Campylobacter coli*: Molecular Mechanism and Stability of the

Resistance Phenotype. *Antimicrobial Agents and Chemotherapy* 49(7):2753-2759.

Gupta, A., Nelson, J. M., Barrett, T. J., Tauxe, R. V. Rossiter, S. P., Friedman, C. R., Joyce, K. W., Smith, K. E. Jones, T. F., Hawkins, M.A., Shiferaw, B., Beebe, J. L., Vugia, D. J., Rabatsky-HER, T., Benson, J. A., Root, T. P., Angulo, F. J., and NARMS Working Group. 2004. Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. *Emerging Infectious Diseases* 10(6):1102-1109.

Gurtler, M., Alter, T., Kasimir, S., and Fehlhaber, K. 2005. The importance of *Campylobacter coli* in human campylobacteriosis: Prevalence and genetic characterization. *Epidemiology and Infection* 133:1081–1087.

Han, K., Jang, S. S., Choo, E., Heu, S. and Ryu, S. 2007. Prevalence, genetic diversity and antibiotic resistance patterns of *Campylobacter jejuni* from retail raw chicken in Korea. *International Journal of Food Microbiology* 114:50-59.

Havelaar, A. H, Van Pelt, W., and Ang, C. W. 2009. Immunity to *Campylobacter*: Its role in risk assessment and epidemiology. *Critical Reviews in Microbiology* 35:1–22.

Hill, D. R., Ericsson, C. D., Pearsons, R. D., Keystone, J. S., Freedman, D. O., Kozarsky, P. E., Dupont, H. L., Bia, F. J., Fischer, P. R., and Ryan, E. T. 2006. The practice of travel medicine: guidelines by the Infectious Disease Society of America. *Clinical Infectious Disease* 43(12):1499-1539.

Hoffman, P. S., George, H. A., Krieg, N. R. and Smibert, R. M. 1979. Studies of the microaerophilic nature of *Campylobacter fetus* susp. *jejuni*. II. role of exogenous superoxide anions and hydrogen peroxide. *Canadian Journal of Microbiology* 25:8-16.

Hooper D. C. 2001. Mechanisms of action of antimicrobials: focus on fluoroquinolones. *Clinical Infectious Diseases* 32(S1):S9-S15.

Humphrey, T., Mason, M., and Martin, K. 1995. The isolation of *Campylobacter jejuni* from contaminated surfaces and its survival in diluents. *International Journal of Food Microbiology* 26:295-303.

Iovine, N. M. 2013. Resistance mechanism in *Campylobacter jejuni*. *Virulence* 4(3):230-240.

Iovine, N. M., and Blaser, M. J. 2004. Antibiotics in animal feed and spread of resistant Campylobacter from poultry to humans. *Emerging Infectious Disease*. Available at http://dx.doi.org/10.3201/eid1006.040403 Accessed 24 July 2019.

Jacoby G. A. 2005. Mechanisms of resistance to quinolones. *Clinical Infectious Diseases* 41(S2):S120-126.

Kassa, T., Gebre-Selassie, S. and Asrat, D. 2007. Antimicrobial susceptibility patterns of thermotolerant *Campylobacter* strains isolated from food animals in Ethiopia. *Veterinary Microbiology* 119:82-87.

Kawaguchi H. 1976. Discovery chemistry and activity of amikacin. *Journal of Infectious Diseases* 134:S242-S248.

Krumperman, P. H. 1983. Multiple antibiotic resistance indexing of *Escherischia coli* to identify high-risk sources of fecal contamination of food. *Applied and Environmental Microbiology* 46:165-170.

Lehtopolku, M., Nakari, U.-M., Kotilainen, P., Huovinen, P., Siitonen, A., and Hakanen, A. J. 2010. Antimicrobial susceptibilities of multidrug-resistant *Campylobacter jejuni* and *C. coli* strains: in vitro activities of 20 antimicrobial agents. *Antimicrobial Agents Chemotheraphy* 54(3):1232-1236.

Leibovitz, E., Janco, J., Piglansky, L., Press, J., Yagupsky, P., Reinhart, H., Yaniv, I., and Dagab, R. 2000. Oral ciprofloxacin vs. intramuscular ceftriaxone as empiric treatment of acute invasive diarrhea in children. *Pediatric Infectious Disease Journal* 19(11):1060-1067.

Levin, R. E., 2007. *Campylobacter jejuni*: A review of its characteristics, pathogenicity, ecology, distribution, subspecies characterization and molecular methods of detection. *Food Biotechnology* 21:271–347.

Mansouri-najand, L., Saleha, A. A, and Wai, S. S. 2012. Prevalence of multidrug resistance *Campylobacter jejuni* and *Campylobacter coli* in chickens slaughtered in selected markets, Malaysia. *Tropical Biomedicine* 29:231–238.

Martin S. I. and Kaye K. M. 2004. Beta-lactam antibiotics: Newer formulations and newer agents. *Infectious Disease Clinics of North America* 18(3):603-619.

Moore J. E., Corcoran D., Doolay J. S., Fanning S., Lucey B., Matsuda M., McDowell D. A., Mégraud F., Millar B. C., O'Mahony R., O'Riordan L. O'Rourke M., Rao J. R., Rooney P. J., Sails A., and Whyte P. 2005. *Campylobacter*. *Veterinary Research* 36(3):351-382.

Murphy, C., Carroll, C., and Jordan, K. N. 2006. Environmental survival mechanisms of the foodborne pathogen *Campylobacter jejuni*. *Journal of Applied Microbiology* 100(4): 623–632.

Nachamkin, I. 2007. *Campylobacter jejuni*. Ch 11 In: Doyle MP, Beuchat LR (eds) *Food microbiology: Fundamentals and frontiers*. 3rd ed, ASM Press, Washington D.C., p. 237–248.

Navarro, F., Miro, E., Mirelis, B., and Prats, G. 1993. *Campylobacter* spp. antibiotic susceptibility. *Journal of Antimicrobial Chemotherapy* 32:906-907.

O'Brien, T. F. 2002. Emergence, spread and environmental effect of antimicrobial resistance, how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clinical Infectious Disease* 34:78–84.

Pfister P., Jenni S., Poehlsgaard J., Thomas A., Douthwaite S., Ban N., and Böttger E. C. 2004. The structural basis of macrolide-ribosome binding assessed using mutagenesis of 23S rRNA positions 2058 and 2059. *Journal of Molecular Biology* 342(5): 1569-1581.

Poehlsgaard J. and Douthwaite S. 2005. The bacterial ribosome as a target for antibiotics. *Nature Reviews Microbiology* 3(11):870-881.

Padungtod, P., Kaneene, J. B., Hanson, R., Morita, Y. and Boonmar, S. 2006. Antimicrobial resistance in *Campylobacter* isolated from food animals and humans in northern Thailand. *FEMS Immunology and Medical Microbiology* 47:217-225.

Reina, J., Borrell, N., and Serra, A. 1992. Emergence of resistance to erythromycin and fluoroquinolones in thermotolerant *Campylobacter* strains isolated from feces 1987-1991. *European Journal of Clinical Microbiology Infection Disease* 11:1163.

Robinson, D. A. 1981. Infective dose of *Campylobacter jejuni* in milk. *British Medical Journal* 282:1584.

Rodrigo, S., Adesiyun, A., Asgarali, Z. and Swanston, W. 2007. Antimicrobial resistance of *Campylobacter* spp. isolated from broilers in small poultry processing operations in Trinidad. *Food Control* 18:321-325.

Sanchez, R., Fernandez-Baca, V., Diaz, M. D., Munoz, P., Rodriguez-Crexems, M. and Bouza, E. 1994. Evolution of susceptibilities of *Campylobacter* spp. to quinolones and macrolides. *Antimicrobial Agents and Chemotherapy* 38:1879-1882.

Schatz A., Bugie E. and Waksmann A. 1944. Streptomycin a substance exhibiting antibiotic activity against Gram positive and Gram negative. *Proceedings of the Society for Experimental Biology and Medicine* 55:66-69.

Sjogren, E., Lindblom, G. B., and Kaijser, B. 1997. Norfloxacin resistance in *Campylobacter jejuni* and *Campylobacter coli* isolates from Swedish patients. *The Journal of Antimicrobial Chemotherapy* 40:257-261.

Snelling, W. J., Matsuda, M., Moore, J. E. and Dooley, J. S. G. 2005. Under the microscope: *Campylobacter jejuni*. *Letters in Applied Microbiology* 41:297-302.

Trachoo, N. 2003. *Campylobacter jejuni*: An emerging pathogen. *Songklanakarin Journal of Science Technology* 25(1):141-157.

Van der Auwera P. and Scorneaux B. 1985. In vitro susceptibility of *Campylobacter jejuni* to 27 antimicrobial agents and various combinations of beta-lactams with clavulanic acid or sulbactam. *Antimicrobial Agents and Chemotheraphy* 28(1):37-40.

Van Bambeke F., Michot J. M., Van Eldere J., and Tulkens P. M. 2005. Quinolones in 2005: an update. *Clinical Microbiology Infection* 11(4): 256-280.

Vlieghe, E. R., Jacobs, J. A., Van Ebsroeck, M., Koole, O., and Van Gompel, A. 2008. Trends of norfloxacin and erythromycin resistance of *Campylobacter jejuni/Campylobacter coli* isolates recovered from international travelers, 1994 to 2006. *Journal of Traveler Medicine* 15(6):419-425.

Weinstein M. J., Luedemann G. M., and Oden E. M. 1963. Gentamicin a new antibiotic complex from Micromonospora. *Journal of Medicinal Chemistry* 6(4):463-464.

WHO, 2011. *Fact sheet No. 125-Enterohaemorrhagic Escherichia coli (EHEC).* World Health Organisation, Geneva. Available at http://www.who.int/mediacentre/factsheets/fs125/en/. Accessed 30 June 2019.

Wilson, I. G. 2003. Antibiotic resistance of *Campylobacter* in raw retail chickens and imported chicken portions. *Epidemiology and Infection* 131:1181-1186.

Wistrom, J. and Norrby, S. R. 1995. Fluoroquinolones and enteritis, when and for whom? *Journal of Antimicrobial Chemotherapy* 36:23-39.

Yao J. D. and Moellering R. C. Jr. 2003. Antimicrobial Agents. In: Murray P., Baron E., Jorgensen J., Pfaller M., Yolken R., editors. *Manual of Clinical Microbiology*. 7th edition. Washington D.C. ASM Press, pp. 1047- 1050.

Young, V. B., and Mansfield, L. S. 2005. *Campylobacter* infection - Clinical context. Ch 1 In: Ketley J. M, and Konkel M. E. (eds) *Campylobacter*: Molecular and cellular biology. *Horizon Bioscience.* Wymondham, p. 1–12.