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Quantification and antibiotic susceptibility of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in raw vegetables *(ulam)*

[1*]Najwa, M.S., [1]Rukayadi, Y., [1]Ubong, A., [1]Loo, Y.Y., [1]Chang, W.S., [1]Lye, Y.L., [1]Thung, T.Y, [1]Aimi, S.A., [1]Malcolm, T.T.H, [1]Goh, S.G., [1]Kuan, C.H., [2]Yoshitsugu, N., [2]Nishibuchi, M. and [1]Son, R.

[1]*Center of Excellence for Food Safety Research, Faculty of Food Science and Technology, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor Darul Ehsan, Malaysia*
[2]*Center for Southeast Asian Studies, Kyoto University, Kyoto 606-8501, Japan*

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Abstract

Salmonella has been reported to be presence both in raw and processed foods worldwide. In this study, the prevalence, quantification and antibiotic susceptibility of *Salmonella* isolated from raw vegetables or locally known as *ulam* such as asiatic pennywort (*Centella asiatica* (L) Urb), water dropwort (*Oenanthe javanica* (Blume) DC), long bean (*Vigna sinensis* EndL), and winged bean (*Psophocarpus tetragonolobus* (L) DC) obtained from retail markets in Selangor, Malaysia were carried out. From 96 samples tested, the overall prevalence of *Salmonella* spp. was 97.9%, *Salmonella* Enteritidis was 54.2% and *Salmonella* Typhimurium was 82.3% respectively. Samples were contaminated with *Salmonella* ranging from <3 to 2400 MPN/g. *Salmonella* Enteritidis and *Salmonella* Typhimurium isolates obtained from the raw vegetables (*ulam*) were found to exhibit high resistance against ampicillin (100%), erythromycin (100%), amoxicillin/clavunic acid (81.3%), cephalothin (75%), streptomycin (50%) and ciprofloxacin (50%). All *Salmonella* isolates showed multi drug resistant (MDR) profile with each isolate being resistant to 3 or more antibiotics. The multiple antibiotic resistance (MAR) index of *Salmonella* isolates ranged from 0.27 to 0.55 for *Salmonella* Enteritidis and 0.27 to 0.82 for *Salmonella* Typhimurium. The presence of *Salmonella* on raw vegetables (*ulam*) and high antibiotic resistance isolates indicated that raw vegetables could be contaminated and thus imposes possible health risk to local consumers.

Introduction

Salmonella is a bacterium that causes infection known as salmonellosis. There are 2,463 serovars of *Salmonella* in which *Salmonella* Enteritidis and *Salmonella* Typhimurium are two most important serovars of salmonellosis. They are transmitted from animals to humans in most parts of the world (Montville and Matthews, 2008; Pui *et al.*, 2011; WHO, 2013). Poultry, egg, meat and dairy products continue to be the most common food vehicles for the infection. However, human salmonellosis has also been associated with fresh produce such as bean sprouts, mangoes, cantaloupe, papaya, alfafa sprouts, and tomatoes (CDC, 2014). In Malaysia, some of fresh vegetables are consumed raw including asiatic pennywort, water dropwort, long bean and winged bean. These fresh vegetables are known as *ulam* or in other countries they are equivalent to salad. *Ulam*s are consumed to add variety and flavour to the diet as well as for their health benefits. Usually these vegetables are mixed with other ingredients such as chillies, grated coconut and rice. *Ulam* is rich in carbohydrate, protein, mineral, vitamin, carotenoid and well known as anti-aging agent (Salleh *et al.*, 2003; Fatimah *et al*., 2012). There is an increase of fruit and vegetable consumption worldwide including Malaysia due to rising health consciousness of people (Olaimat and Holley, 2012; DAN, 2015). However, numbers of research detecting prevalence of *Salmonella* in fresh produce including vegetables and fruits have been published (Arumugaswamy *et al.*, 1995; Salleh *et al*., 2003; Pui *et al.*, 2011; Nillian *et al*., 2011; Diana *et al*., 2012). Since most fresh produce receives minimal processing and is often eaten raw, pathogen contamination could pose possible risks. Even though, salmonellosis is common worldwide, its association with vegetables sources is rarely reported in Malaysia (Yoke-Kqueen *et al*., 2008).

Nontyphoid *Salmonella* is the most common bacterial pathogen causing gastrointestinal infection worldwide. Every year, *Salmonella* spp. contributes 1 million illnesses, 19,000 hospitalizations and 380 deaths in United States (CDC, 2014). Although

*Corresponding author.
Email: *shahrilnajwa@yahoo.com*

most *Salmonella* infections cause mild-to-moderate gastroenteritis that usually resolves with or without treatment, some lead to severe invasive infections (e.g., bacteremia meningitis, and osteomyelitis) which need antibiotic treatments (Vugia *et al.*, 2004; Chen *et al*., 2013). Invasive *Salmonella* infections can be life threatening, leading to death and are more common in young children, the elderly and the immunocompromised.

Controlling *Salmonella* infection could be challenging due to its high tolerance to environmental stresses, widespread distribution, multiple drug resistance, and adaptability (Chen *et al*., 2013). Excessive and improper uses of antibiotics are the main factor attributed to increasing of antibiotic resistant bacteria. The antibiotic resistant bacteria will survive and continue to multiply through several mechanisms which allow them to survive antibiotic treatments.

Periodic surveillance to determine the prevalence and quantity of *Salmonella* spp. in food is important to control human salmonellosis. Hence, the objective of this study was to quantify *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in *ulam* from wet markets and hypermarkets in a limited geographical location, mainly Selangor, Malaysia. Another objective was to determine and evaluate the antibiotic resistance profiles of *Salmonella* isolated from these samples since most of the antimicrobial resistance studies of *Salmonella* focused mainly on isolates from clinical and animal sources (Miko *et al*., 2005; Adzitey *et al.*, 2012; Ngoi and Thong, 2013; Thong and Modarressi 2011; Budiati *et al*., 2013).

Material and Methods

Sample collection

A total of 96 samples of *ulam* were purchased randomly from wet markets and hypermarkets at Seri Kembangan and Bangi, Selangor, Malaysia. In this study, four types of *ulam* were analysed (Table 1). All the samples were transported to the laboratory in an ice box and examined immediately after purchased.

Most probable number (MPN) method

A 10 g of *ulam* was aseptically weighed and transferred into a sterile stomacher bag. A 90 mL of sterile buffered peptone water (BPW; Merck, Darmstadt, Germany) was added into stomacher bag and pummeled for 60 s. A three-tube most probable number (MPN) method was employed where 100 fold and 1000 fold dilutions of the stomacher fluids were prepared by using RVS (Rappaport-Vassiliadis *Salmonella)* broth as diluent. All the tubes were incubated at 37ºC for 24 h in a shaker incubator with 100 rpm rotation. After incubation, MPN tubes were checked for turbidity where turbid tubes were subjected for streaking on Xylose Lysine Deoxycholate Agar (XLD; Eiken Chemical Co., Tochigi, Japan), and DNA extraction followed by multiplex PCR.

Table 1. Types of *ulam*

English name	Local name	Botanical name	Number of samples (n)
Asiatic pennywort	Pegaga nyonya	*Centella asiatica* (L) Urb	24
Water dropwort	Selom	*Oenanthe javanica* (Blume) DC	24
Long bean	Kacang panjang	*Vigna sinensis* EndL	24
Winged bean	Kacang botol	*Psophocarpus tetragonolobus* (L) DC	24
		TOTAL	**96**

Plating method

A loopful of turbid MPN tubes (1 mL) was streaked on Xylose Lysine Deoxycholate Agar (XLD; Eiken Chemical Co., Tochigi, Japan) and incubated at 37ºC for 24 h. Presumptive colonies were confirmed by multiplex PCR assay.

DNA extraction

A boil cell method with some modifications as described by Pui *et al*., (2011) was used to extract DNA for all turbid MPN tubes and presumptive colonies on agar plates.

Multiplex PCR

Amplification of DNA was performed on a thermocycler (Applied Biosystem 2720 Thermal Cycler, USA) by using three sets of primers; *Salmonella* spp. (Styinva-JHO-2-F, 5'- AAA CGT TGA AAA ACT GAG GA-3') and (Styinva-JHO-2-R, 5'- TCG TCA TTC CAT TAC CTA CC-3'), *Salmonella* Enteritidis (ENT-F, 5'-AAA TGT GTT TTA TCT GAT GCA AGA GG-3') and (ENT-R, 5'-GTT CGT TCT TCT GGT ACT TAC GAT GAC-3'), *Salmonella* Typhimurium (STM4492-F, 5'-ACA GCT TGG CCT ACG CGA G-3') and (STM4492-R, 5'-AGC AAC CGT TCG GCC TGA C-3') which produced amplicons of 119 bp, 299 bp and 759 bp respectively (Saeki *et al.*, 2013). Total PCR mixtures was 20 µL containing 5 µL of 5x PCR buffer, 4 mM of $MgCl_2$, 0.6 mM of deoxynucleoside triphosphate mix, 0.4 mM of each primer used, 1 U of Taq DNA polymerase and 4 µL DNA template. Amplification

Table 2. Incidence of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in *ulam* from wet markets and hypermarkets

Ulam	Wet markets			Hypermarkets			TOTAL		
	[a]Spp/n, (%)[g]	[b]SE/n, (%)[g]	[c]ST/n, (%)[g]	[a]Spp/n, (%)[g]	[b]SE/n, (%)[g]	[c]ST/n, (%)[g]	[d]Spp/n, (%)[g]	[e]SE/n, (%)[g]	[f]ST/n, (%)[g]
Asiatic pennywort	10/12, (83.3)	1/12, (8.3)	8/12, (66.7)	12(12), (100)	7/12, (58.3)	11/12, (91.7)	22/24, (91.7)	8/24, (33.3)	20/24, (83.3)
Water dropwort	12/12, (100)	6/12, (50.0)	10/12, (83.3)	12(12), (100)	8/12, (66.7)	11/12, (91.7)	24/24, (100)	14/24, (58.3)	21/24, (87.5)
Long bean	12/12, (100)	8/12, (66.7)	10/12, (83.3)	12(12), (100)	8/12, (66.7)	10/12, (83.3)	24/24, (100)	16/24, (66.7)	20/24, (83.3)
Winged bean	12/12, (100)	6/12, (50.0)	8/12, (66.7)	12(12), (100)	8/12, (66.7)	10/12, (83.3)	24/24, (100)	14/24, (58.3)	18/24, (75.0)
TOTAL (%)	46/48, (95.8)	21/48, (43.8)	36/48, (75.0)	48(48), (100)	31/48, (64.6)	42/48, (87.5)	94/96, (97.9)	52/96, (54.2)	79/96, (82.3)

Note: [a]Number of positive samples for *Salmonella* spp./number of *ulam* samples; [b]Number of positive samples for *S*.Enteritidis/number of *ulam* samples; [c]Number of positive samples for *S*.Typhimurium/number of *ulam* samples; [d]Number of positive samples for *Salmonella* spp./number of total *ulam* samples; [e]Number of positive samples for *S*.Enteritidis/number of total *ulam* samples; [f]Number of positive samples for *S*.Typhimurium/number of total *ulam* samples; [g]Percentage (%)

were carried out with initial denaturation at 95°C for 10 min; 35 cycles of denaturation at 94°C for 60 s, primer annealing at 60°C for 90 s, and extension at 72°C for 90 s; final extension at 72°C for 10 min. PCR products were subjected to electrophoresis on 1.5% agarose gel with 0.5x TBE buffer (pH 8.0) at 100 V for 30 min.

Standard disk diffusion method

The antibiotic susceptibility test was conducted based on standard disk diffusion method of Kirby-Bauer (Bauer *et al*., 1966) and Clinical Laboratory Standards Institute (CLSI) guidelines. Eleven common antibiotics were used including chloramphenicol (30 µg), ciprofloxacin (5 µg), ampicillin (10 µg), erythromycin (15 µg), cephalothin (30 µg), streptomycin (10 µg), amoxicillin/clavunic acid (30 µg), nalidixic acid (30 µg), gentamicin (10 µg), trimethoprim-sulphamethoxazole (25 µg), and tetracycline (30 µg). All the antimicrobial discs were purchased from Oxoid (England). *Escherichia coli* ATCC 25922 was used as a control based on CLSI guidelines (Clinical and Laboratory Standards Institute, 2013).

Data interpretation

Diameter of inhibition zones were measured including the diameter of disk in milimeter (mm). Data were interpreted as susceptible, intermediate and resistant based on CLSI guidelines (Clinical and Laboratory Standards Institute, 2013).

Multiple antibiotic resistance (MAR) indexing

Result obtained for each isolates was analysed for MAR indexing. MAR indexing defined as a/b where 'a' indicates number of resistant antibiotics while 'b' indicates total number of tested antibiotics (Krumperman, 1983).

Statistical analysis

SPSS software (Version 22.0) was applied to determine statistic significant difference between the prevalence of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in *ulam* for wet markets and hypermarkets, and between leafy vegetables (asiatic pennywort, water dropwort) and non-leafy vegetables (long bean, winged bean).

Result and Discussion

The prevalence of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium was higher in hypermarkets compared with wet markets where 100% of *Salmonella* spp., 64.6% of *Salmonella* Enteritidis and 87.5% of *Salmonella* Typhimurium were detected while for wet markets, 95.8%, 43.8% and 75% for *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium were detected respectively (Table 2). These findings was confirmed when statistical analysis was carried out where $p<0.05$. There was no obvious pattern in density of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium. The density ranged from <3MPN/g to >2400 MPN/g for wet markets and hypermarkets (Table 3). According to previous researchers (Kuan *et al*., 2013; Puspanadan *et al*., 2012; Ponniah *et al*., 2010 and Tunung *et al*., 2010), improper handling and poor hygienic practices play a major role as source of contamination of bacteria on food at retail level such as using contaminated equipment and containers during transportation, cross contamination during processing, and places

Table 3. Density of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in *ulam* samples

		Salmonella spp.			*Salmonella* Enteritidis			*Salmonella* Typhimurium		
Location	Ulam	[a]Min	[b]Med	[c]Max	[a]Min	[b]Med	[c]Max	[a]Min	[b]Med	[c]Max
Wet markets	Asiatic pennywort	<3	68.5	>2400	<3	<3	6	<3	6.4	23
	Water dropwort	9.3	47.5	1100	<3	1.5	6.1	<3	10.6	43
	Long bean	3	571.5	>2400	<3	3.6	35	<3	8.2	93
	Winged bean	3.6	252	>2400	<3	1.5	29	3	3.6	43
Hypermarkets	Asiatic pennywort	9.1	112.5	>2400	<3	3	6.1	<3	9.1	43
	Water dropwort	6	39	1100	<3	3	23	<3	11	93
	Long bean	16	85	>2400	<3	3.6	93	<3	16.1	93
	Winged bean	23	180	>2400	<3	3.3	23	<3	23	43

[a]Min=Minimum MPN/g value; [b]Med=Median MPN/g value; [c]Max=Maximum MPN/g value

where vegetables were displayed which are not properly cleaned and sanitized. Moreover, longer holding time at hypermarkets than wet markets which are fast selling increase opportunity for the pathogenic bacteria to multiply and accumulate in food (Puspanadan *et al*., 2012; Goh *et al*., 2012; Kuan *et al*., 2012).

Longer holding time will also increase cross contamination between food and food contact surfaces since *Salmonella* spp. has ability to form biofilm at various types of surfaces such as stainless steel, glass, high-density polyethylene, polystyrene, PVC, Teflon, Buna-N rubber, acrylic, polyurethane and cement (Joseph *et al*., 2001; Solomon *et al*., 2005; Manijeh *et al*., 2008; Chia *et al*., 2009; Pui *et al*., 2011; Valeriano *et al*., 2012; Singla *et al*., 2014; Nguyen *et al*., 2014). Moreover, *Salmonella* spp. also can form biofilm on fresh produce (Lapidot *et al*., 2006; Patel and Sharma, 2010) and long storage time (one week at 4°C and 25°C) contribute to the formation of biofilm on parsley and cause persistence of *Salmonella* due to the resistency against disinfectants and water rinsing (Lapidot *et al*., 2006). Pui *et al*., 2011, stated that the ability of *Salmonella* Typhimurium to form biofilm on plastic cutting board can transfer the bacteria to dragon fruit with transfer rate range from 0.68 to 0.77. It is possible that the cross contamination may occur from contaminated display site. According to Puspanadan *et al*., (2012), vegetables in hypermarket are being display for one week and wash using normal water without further treatment to reduce pathogenic bacteria. Cleaning the site with just running of water and soap is not efficient enough to remove bacteria. Soares *et al*., (2012), suggested that cleaning with dish soap and mechanical scrubbing, and followed by disinfection with hypochlorite is able to reduce bacterial pathogen on difference surfaces.

Other than that, natural habitats where the plants are growing also contribute high prevalence of *Salmonella*. Asiatic pennywort is a top soil creeper while water dropwort grows at banks of ponds, irrigation ditches and swamps and usually these places are for liquid waste disposal from slaughtering house and processing plants (Salleh *et al*., 2003). Long bean and winged bean also show high prevalence of *Salmonella* even though these plants are not in direct contact with soil. There is no statistical difference in prevalence of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium between leafy vegetables (asiatic pennywort and water dropwort) and non-leafy vegetables (long bean and winged bean) in this study ($p>0.05$). This result is similar to Quiroz-Santiago *et al*., (2009), in which there is no difference in prevalence of *Salmonella* spp. between leafy and non-leafy vegetables. It shows that *Salmonella* spp. might has the ability to attach and grow on both type of produce. Other than *Salmonella* spp., *Vibrio parahaemolyticus, Listeria monocytogenes* and *Campylobacter* spp., were detected on long bean and winged bean at retail level (Chai *et al*., 2007; Tunung *et al*., 2010; Ponniah *et al*., 2010). High prevalence of *Salmonella* spp. in long bean and winged bean indicate poor sanitation occurred at post-harvest stage. Contamination can still occur through internalization of *Salmonella* into fresh produce. Internalization of human pathogens such as *Salmonella* spp. and *E.coli* O157:H7 in fresh produce is reported (Bordini *et al*., 2007; Deering *et al*., 2012; Ge *et al*., 2012; Nicholson *et al*., 2015). These pathogens can internalize into plant tissue through contaminated soil, irrigation water, wash water, water used for hydroponic plants, seed, seedling and manure applied to soil.

Table 4. Percentage of *Salmonella* isolates resistant to antimicrobial agents

Antibiotics used	*Salmonella* Enteritidis (%)[a]	*Salmonella* Typhimurium (%)[b]	Total resistant (%)[c]
Chloramphenicol	0	10	6.3
Ciprofloxacin	50	50	50
Ampicillin	100	100	100
Erythromycin	100	100	100
Cephalothin	66.7	80	75
Streptomycin	50	50	50
Amoxycillin/clavunic acid	83.3	80	81.3
Nalidixic acid	0	20	12.5
Gentamicin	0	0	0
Trimethoprim-sulphamethoxazole	0	10	6.3
Tetracycline	0	20	12.5

[a] Total isolates of *Salmonella* Enteritidis (6); [b] Total isolates of *Salmonella* Typhimurium (10); [c] Total isolates of *Salmonella* (16)

All *Salmonella* isolates (100%) were resistant against erythromycin (Table 4). This finding was similar to other researchers done by Yoke-Kqueen *et al*., (2008); Learn-Han *et al*., (2009); Thong *et al*., (2002); Adzitey *et al*., (2012); and Nipa *et al*., (2011). The high resistance of *Salmonella* isolates against erythromycin was contributed by its molecules size which is too large to pass through the bacterial cell wall outer membrane (Learn-Han *et al*., 2009). The present study showed low resistance against nalidixic acid, tetracycline, chloramphenicol and trimethoprim-sulphamethoxazole. Low resistance against nalidixic acid, chloramphenicol, and trimethoprim-sulphamethoxazole were also reported by Yoke-Kqueen *et al*., (2008); Learn-Han *et al*., (2009); and Lertworapreecha *et al*., (2013). Low resistance against chloramphenicol (6.3%) in this study was supported by Learn-Han *et al*., (2009), which suggested the increase efficiency of this antibiotic was due to the restricted use in certain countries because of the fear of its serious side effects. In this study high resistance percentage was observed against ampicillin (100%), amoxicillin/clavunic acid (81.3%), cephalothin (75%), streptomycin (50%), and ciprofloxacin (50%) for both *Salmonella* serovars isolates.

Higher resistance percentage for streptomycin and ampicillin were in concordance with studies done by Learn-Han *et al*., (2009); Yoke-Kqueen *et al*., (2008); and Lertworapreecha *et al*., (2013). Higher resistance against ampicillin is alarming since this drug can be used in the treatment of human salmonellosis (de Oliveira *et al*., 2006). Moreover, all these studies also found higher resistance percentage of *Salmonella* isolates against tetracycline (27%, 33%, and 42%) which was inconsistent with the present study. In this study only 12.5% *Salmonella* isolates were resistant against tetracycline. This is similar with findings reported by de Oliveira *et al*., (2006) and higher resistance was expected as this drug is one of the widely used for livestock production. Abakpa *et al*., (2015) reported on higher resistance of *Salmonella* isolates against cephalothin and lower resistance of amoxicillin/ clavunic acid and ciprofloxacin compared to the present study. The emergence of resistance to fluoroquinolones among nontyphoid *Salmonella* is of particular concern, since this class of antimicrobial agents constitutes the drug of choice for treating severe *Salmonella* infections caused by multiple-antibiotic resistant strains in adults (Parry and Threlfall, 2008). All *Salmonella* isolates in this study were susceptible against gentamicin. This result is in concordance with Lertworapreecha *et al*., (2013) which found all *Salmonella* isolates from fresh vegetables exhibited no resistance against gentamicin.

Several studies have shown that *Salmonella* can exhibit multidrug resistant patterns (Abakpa *et al*., 2015; Yoke-Kqueen *et al*., 2008; Miko *et al*., 2005; Lertworapreecha *et al*., 2013; Thong *et al*., 2002; Learn-Han *et al*., 2009). All *Salmonella* isolates from the present study showed multidrug resistance profiles (MDR) (Table 5). Multidrug resistance profiles (MDR) used in this study is defined as resistant to more than two drugs tested (Yang *et al*., 2002). The common antibiotic multi resistant pattern displayed by *Salmonella* Enteritidis and *Salmonella* Typhimurium isolates was a combination of

Table 5. Antibiograms of *Salmonella* Enteritidis and *Salmonella* Typhimurium isolated from *ulam*

Salmonella serovars	Antibiograms	Sources and isolate's code	MAR index
S. Enteritidis	AMCAmpCipEKfS	P5	0.55
	AMCAmpEKfS	P6	0.46
	AMCAmpCipEKf	S6	0.46
	AmpCipEKfS	P7	0.46
	AMCAmpE	W1, W2	0.27
S. Typhimurium	AMCAmpCCipEKfNaSTe	S3	0.82
	AMCAmpEKfNaSxtTe	S5	0.64
	AMCAmpCipEKfS	P2, S4	0.55
	AMCAmpCipEKf	P4	0.46
	AMCAmpCipE	P1	0.36
	AMCAmpEKf	P3	0.36
	AmpEKfS	S2, L1	0.36
	AMCAmpE	S1	0.27

P- Asian pennywort; S- Water dropwort; L- Long bean; W- Winged bean *AMC- Amoxycillin/clavunic acid; Amp- Ampicillin; C- Cholaramphenicol; Cip- Ciprofloxacin; Cn- Gentamicin; E- Erythromycin; Kf- Cephalothin; Na- Nalidixic acid; S- Streptomycin; Sxt- Trimethoprim-Sulphamethoxazole; Te- Tetracycline

amoxicillin/clavunic acid, ampicillin, erythromycin and cephalothin due to its high resistant percentage. According to Cruchaga *et al*., (2001), the spread of multi-resistant clones will have a greater potential for infections and for the development of additional resistance to new antibiotics. This will eventually leads to the development of multi-resistant serotypes of *Salmonella* in different parts of the world. Results from current study could suggest that *Salmonella* Enteritidis and *Salmonella* Typhimurium isolates that are resistant against amoxicillin/clavunic acid, ampicillin, erythromycin and cephalothin are also at increasing risk for becoming resistant to additional antibiotics agents.

Overall, *Salmonella* isolates from the present study exhibited MAR index ranging from 0.27 until 0.8. This MAR index for *Salmonella* isolated from vegetables was similar to findings described by Yoke-Kqueen *et al*., (2008) which ranged from 0.1 until 0.8. Surprisingly, these two MAR index were higher than MAR index of *Salmonella* isolated from poultry which ranged from 0.1 to 0.7. Moreover, Thong *et al.*, (2002) also reported that all the vegetables isolates were resistant to at least two antibiotics and 11% were resistant against multiple antibiotics while most of human and well water isolates which were 85% and 87% respectively were remained sensitive to all antimicrobial tested. The emergence of high antimicrobial resistance among all *Salmonella* isolates is alarming since these vegetables are commonly eaten raw and has obvious implications for public health since multidrug resistance limits the possible effectiveness of therapeutic treatments (Thong *et al*., 2002; NIH, 2015).

A study conducted in Malawi, Africa showed that increased incidence of bacteremia among adults and children was associated with the acquisition of multidrug resistance to ampicillin, cotrimoxazole and chloramphenicol by each *Salmonella* serovar. Moreover, this study showed that *Salmonella* Typhimurium and *Salmonella* Enteritidis were among the most common NTS (nontyphoidal salmonellae) isolated in which 76% and 75% of *Salmonella* Typhimurium were isolated from children and adults while 21% of *Salmonella* Enteritidis were accounted for both (Gordon *et al*., 2008).

Salmonella Enteritidis exhibited five multidrug resistant profiles with multiple antibiotic resistant (MAR) index ranged from 0.27 until 0.55 while *Salmonella* Typhimurium exhibited eight multidrug resistant profiles with larger multiple antibiotic resistant (MAR) index ranged from 0.27 until 0.82. These indicated that *Salmonella* Typhimurium isolates were more resistant than *Salmonella* Enteritidis isolates. The resistance rate, however, varies with different serotypes and different antibiotics (Parry and Threlfall, 2008; Lee *et al*., 2009). *Salmonella* Enteritidis, one of the most prevalent *Salmonella* serotypes, is relatively more susceptible to antimicrobial agents than other serotypes including *Salmonella* Typhimurium (Lee *et al*., 2009; Su *et al*., 2004). This is in agreement with the present study which all *Salmonella* Enteritidis isolates were susceptible against chloramphenicol, nalidixic acid, trimethoprim-sulphamethoxazole and tetracycline.

It is not surprising that *Salmonella* Typhimurium exhibited higher resistance against antimicrobial tested as this serovar considered as the highest occurrence of multiple resistances among the human strains and has been found in animal and food products (de Oliveira *et al*., 2006). Cruchaga *et al*., (2001) reported that the chromosomal genes coding for resistance to ACSSuT of *Salmonella* Typhimurium DT104 could have been transferred horizontally to other *Salmonella* Typhimurium strains, contributing to the increasing frequency of resistance in this serotype.

Two main mechanisms involved in antibiotic resistant bacteria which are gene mutation and acquiring antibiotic resistance genes. Antibiotic resistance genes exist naturally in the chromosomes of environmental microorganisms and several antibiotics are produced by them. Antibiotic resistance can be caused by the extensive antibiotic usage in animal farming, agriculture and human therapy. These entire antibiotic residues will be release to natural environment and cause the selection of resistant bacteria under selective pressure (Martinez, 2009).

Many resistance genes are located on mobile genetic elements such as plasmids, integrons and transposons. The resistance traits located on mobile genetic elements can be transferred to other bacteria. For instance, the location of the non-SGI1 class 1 integrons on large transferable plasmids can makes their intra- and interspecies horizontal transfer very efficient, thus disseminate the antibiotic resistance traits. Moreover, the detection of mutations in the quinolone resistance-determining region (QRDR) of the subunit A of DNA gyrase of nalidixic acid contributed to resistance against nalidixic acid of *Salmonella* isolates from food (Miko *et al*., 2005).

Conclusion

The high presence of *Salmonella* in *ulam* indicates that these vegetables could be contaminated during harvesting, processing and/or distribution under

inadequate hygiene conditions. Therefore, thorough surveillance is needed in order to ensure the safety and quality of these vegetables. The emergence of antibiotic resistance suggesting excessive use of antibiotic in human and agriculture purposes and resulting in increasing risk to human health. Controlling the use of antibiotic and wisely usage of it is needed to reduce spread of antibiotic resistance among *Salmonella* serovars.

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