

## Prevalence and antimicrobial susceptibility of non-typhoidal *Salmonella* (NTS) from salad vegetables at farms and retail markets in Terengganu, Malaysia

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### Abstract

In recent years, the consumption of vegetables has increased due to their health benefits. There are several foodborne outbreaks related to raw vegetable consumption and non-typhoidal *Salmonella* (NTS) is one of them. In connection with the worrying issue, this study was conducted to determine the occurrence of NTS from salad vegetables from farms, wet markets and supermarkets, and antimicrobial susceptibility profiling of NTS isolates. Samples were collected from farms (n = 270), wet markets (n = 216) and supermarkets (n = 180) in Kuala Terengganu and Kuala Nerus, Terengganu. The samples were analysed according to International Standard protocol ISO 6579:2002(E) and serotyping was performed by Ipoh Public Health Laboratory, Perak. The isolated NTS from the samples were tested resistant against 20 antibiotics of clinical and agricultural importance. Out of 666 samples analyzed, 58 (8.71%) samples were NTS positive. The prevalence of NTS in samples from farms, wet markets and supermarkets were found to be 2.96% (n = 8), 20.37% (n = 44) and 3.33% (n = 6), respectively. The serotyping analysis had identified 23 NTS serovars, the most predominant was *Salmonella enterica* serovar Weltevreden (20.69%), followed by *Salmonella enterica* serovar Albany (10.34%), *Salmonella enterica* serovar Hvittingfoss (8.62%) and *Salmonella enterica* serovar Aberdeen (6.90%). Among the isolates, 34.48% (20/58) showed resistance toward at least one antibiotic tested with 95% of them were the isolates from wet markets. Resistances among isolates were observed in the highest rate for ampicillin (20.69%), followed by tetracycline (18.97%), doxycycline (17.24%), trimethoprim-sulfamethoxazole (17.24%) and chloramphenicol (15.52%). Multidrug-resistant (MDR) characteristic was found in six serovars and *S. enterica* serovar Albany represents the highest prevalence of 83.33%. One and two isolates were also found resistant toward ertapenem and colistin (last resort antibiotics), respectively. Based on the findings, salad vegetables can be considered as one of the important vehicles of MDR NTS to cause salmonellosis in humans. The wet market shows a significant risk of transmitting MDR NTS through vegetables. Thus, it is important to have proper handling to prevent cross-contamination of *Salmonella* in vegetables.

## 1. Introduction

The government of Malaysia through the Ministry of Agriculture and Food Industry has highlighted the importance of salad vegetables (locally known as *ulam*) consumption among Malaysian due to their nutritional value (Roshila-Murni, 2020) such as vitamins, minerals, dietary fibre and polyphenolic compounds (Sebastian *et*

*al.*, 2019). Salad vegetables such as *ulam raja* (cosmos), *selom* (water parsley), *pegaga* (pennywort), *timun* (cucumber), *kacang botor* (winged bean) and *bayam brazil* (sissoo spinach) are commonly available at retail markets all over the country. The recommended daily intake of vegetables for Malaysians is at least three servings which are equal to 240 g (Malaysian Dietary

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Guidelines, 2010).

Despite the health benefits, salad vegetables have also been associated with contamination of enteric pathogens such as *Salmonella* spp., *Listeria monocytogenes*, thermophilic *Campylobacter* spp. and enterohaemorrhagic *Escherichia coli* (Khalid et al., 2015; Tang et al., 2016; Koukkidis and Freestone, 2018; Mohd Noor et al., 2020). Pathogens contaminate vegetables via direct or indirect contact with humans, livestock, wild animals, manure and irrigation water (Mir et al., 2018). Thus, the microbial safety of salad vegetables is of concern as these foods are consumed raw. Salad vegetables have been recognized as one of the most common sources of foodborne illness (Crowe et al., 2015). Several reports indicated that foodborne outbreaks associated with fresh vegetables were mostly caused by non-typhoidal *Salmonella* (NTS) (Yang et al., 2020). According to the European Food Safety Authority (EFSA), ready-to-eat leafy vegetables were ranked in the first place in terms of outbreak occurrence risk due to the presence of *Salmonella* (McLauchlin, 2014).

NTS is a major foodborne pathogen causing diarrhoeal illness (Chang et al., 2020). Each year, NTS causes approximately 93.8 million cases of acute gastroenteritis and 155,000 human deaths worldwide (Chang et al., 2020). Gastroenteritis caused by NTS is commonly mild, self-limiting and does not require treatment, however, antibiotic therapy is necessary for severe and invasive infections such as bacteremia, septic arthritis, and meningitis mostly in children and the immunocompromised (Chang et al., 2020; Yang et al., 2020).

During the last few years, the prevalence of antibiotic-resistant and multidrug-resistant (MDR) *Salmonella* isolated from fresh vegetables was increased in Malaysia (Abatcha et al., 2018; Thung et al., 2020) as well as in other countries worldwide (Abatcha et al., 2020; Yang et al., 2020). A review by Abatcha et al. (2020) involving reports from several countries found relatively high resistances among *Salmonella* from raw vegetables towards erythromycin (82.3%), furazolidone (62.9%), streptomycin (49.6%), cefoperazone (48.6%), kanamycin (48.5%), cephalothin (44.7%) and amoxicillin-clavulanic acid (37.8%). Of particular concern is the probability of *Salmonella* strains to show resistance toward medically important classes of antibiotics such as extended-spectrum cephalosporins, fluoroquinolones, macrolides, aminoglycosides and penicillins which might compromise the treatment of complicated infections (Scott et al., 2019).

Therefore, this study was conducted to determine the prevalence of *Salmonella* in salad vegetables from farms

and retail outlets in Terengganu, Malaysia and their antibiotic susceptibility patterns. The findings in this study were expected to describe the role of salad vegetables as a vehicle of *Salmonella* transmission with their MDR characteristic in the food chain.

## 2. Materials and methods

### 2.1 Sample collection

A total of 666 samples were collected from farms (270), wet markets (216) and supermarkets (180) of Kuala Terengganu and Kuala Nerus city in Terengganu, Malaysia during the period of January to December 2020. Five commercial farms located at Gong Badak (5.36853,103.08281; 5.36788,103.08352; 5.36785,103.08279; 5.36697,103.08273) and Manir (5.30918,103.08382), four wet markets at Bukit Tunggal (5.33705,103.10704), Batu Enam (5.34656,103.09182), Manir (5.30945,103.08198), Chabang Tiga (5.31743,103.12511) and four supermarkets in Kuala Terengganu (5.31115,103.13057; 5.31057,103.12814) and Kuala Nerus (5.35539,103.09601; 5.35923,103.09881) were selected as sampling locations. Descriptions of the samples were summarized in Table 1. Sample of approximately 250 g was aseptically transferred into sterile bags (3M™ plain sample bag) and transported to the laboratory in an icebox to maintain a temperature of 1-4°C.

### 2.2 *Salmonella* isolation and identification

Isolation of *Salmonella* from samples was carried out according to the International Standard protocol ISO 6579:2002(E) (ISO, 2002). For the first enrichment, 25 g of sample was weighed in a sterile stomacher bag and mixed thoroughly with 225 g of buffered peptone water (BPW) (Merck, 107228, Germany) prior to incubation at 37±1°C for 18±2 hr. Then, 0.1 mL and 1 mL of the pre-enriched cultures were incubated in 10 mL of Rappaport-Vassiliadis medium with soy (RVS) (Oxoid, CM0669, UK) at 41.5±1°C for 24±3 hrs and 10 mL of Muller-Kauffmann Tetrathionate novobiocin (MKTTn) broth (Merck, 105878, Germany) at 37±1°C for 24±3 hrs, respectively. Cultures obtained were then inoculated on the surface of Xylose Lysine Deoxycholate (XLD) agar (Merck, 105287, Germany) and Bismuth Sulphite (BS) agar (Oxoid, CM0201, UK) prior to incubation at 37±1°C for 24±2 hrs and 48±2 hrs, respectively to obtain well-isolated colonies. Suspected colonies of *Salmonella* were subjected to biochemical (TSI, urea hydrolysis, lysine decarboxylation, β-galactosidase and indole reactions) and serological (O, H, and Vi antigens) tests for confirmation. *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 and *Citrobacter freundii* ATCC 43864 were used as positive and negative

Table 1. Descriptions of samples taken at farms, wet markets and supermarkets

Type of sample			No. of sample		
Local name	Common name	Scientific name	Farms	Wet markets	Supermarkets
<i>Ulam raja</i>	Cosmos	<i>Cosmos caudatus</i>	27	36	36
<i>Selom</i>	Water parsley	<i>Oenanthe javanica</i>	36	36	27
<i>Pegaga</i>	Pennywort	<i>Centella asiatica</i>	18	36	36
<i>Timun</i>	Cucumber	<i>Cucumis sativus</i>	9	36	36
<i>Kacang botor</i>	Winged bean	<i>Psophocarpus tetragonolobus</i>	9	36	36
<i>Bayam brazil</i>	Sissoo spinach	<i>Alternanthera sissoo</i>	36	36	9
	Irrigation water		45	– <sup>a</sup>	–
	Soil		45	–	–
	Manure		45	–	–
	Total		270	216	180

<sup>a</sup> –, not applicable.

control, respectively. All the isolates were sent to the reference laboratory (Ipoh Public Health Laboratory) in Perak, Malaysia for serotyping.

### 2.3 Antimicrobial susceptibility testing

All *Salmonella* isolates were tested for their susceptibility against 20 antimicrobial agents (Oxoid, UK) viz. ampicillin (AMP, 10 µg), Amoxicillin-clavulanic acid (AMC, 20 µg/10 µg), ampicillin-sulbactam (SAM, 10µg/10 µg), piperacillin-tazobactam (TZP, 100 µg/10 µg), aztreonam (ATM, 30 µg), ertapenem (ETP, 10 µg), imipenem (IPM, 10 µg), meropenem (MEM, 10 µg), cefotaxime (CTX, 30µg), ceftazidime (CAZ, 30 µg), cefoperazone (CFP, 75 µg), cefepime (FEP, 30 µg), ceftriaxone (CRO, 30µg), tetracycline (TE, 30 µg), doxycycline (DO, 30 µg), minocycline (MH, 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25 µg/23.75 µg), azithromycin (AZM, 15 µg), chloramphenicol (C, 30 µg) and colistin (COL). The standard Kirby-Bauer disc diffusion method (Bauer et al., 1966) was used except for the colistin test. Standards and interpretive criteria described by the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018) was referred for the interpretation of the disc diffusion method of testing with *Escherichia coli* ATCC 25922 as the reference strain. Susceptibility toward colistin was tested by the minimum inhibitory concentration (MIC) method using the Sensititre® FRCOL plates (TREK Diagnostic Systems, UK). The interpretation was done according to the breakpoint tables for interpretation of MICs and zone diameters version 9.0, recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2019). The MICs of colistin against *E. coli* ATCC25922

and *Pseudomonas aeruginosa* ATCC27853 were determined as quality control. The multiple antibiotic resistance (MAR) index was determined according to the method stated by Krumperman (1983). MAR index = a/b (a = number of antibiotics to which the particular isolate was resistant, b = total number of antibiotics tested).

### 3. Results

All farms involved in this study were operated traditionally in a controlled environment with proper management. Their farming areas were well separated from other nearby activities with appropriate fencing. The farmers used treated animal waste manure for fertilization and untreated water for irrigation. Their preference for untreated water is due to cost-saving and non-chlorinated properties as the chlorine toxicity to plants is a concern for them. The overall characteristics of each farm were summarized in Table 2.

The isolation of *Salmonella* in this study was presented in Table 3. Overall, *Salmonella* was found in 8 (2.96%), 44 (20.37%) and 6 (3.33%) samples collected from farms, wet markets and supermarkets, respectively. The wet market showed the highest incidence of *Salmonella* compared to the supermarket and farm, all varieties of salad vegetables from wet markets were contaminated at different percentages with the most contaminated were *selom* and *pegaga* (36.11%). Among samples taken at the farm, *Salmonella* was mostly found in irrigation water (11.11% of water samples tested). In soil samples, the percentage of *Salmonella* detection was 2.22%. Interestingly, *Salmonella* was not found in all manure samples tested.

Table 2. Characteristics of each farm involved as sampling point

Characteristics	Farm I	Farm II	Farm III	Farm IV	Farm V
Location	Flat land, village				
Management	Traditional	Traditional	Traditional	Traditional	Traditional
Fertilizer	Treated manure				
Irrigation source	Well water	Stream water	Well water	Well water	Well water
Fencing	Yes	Yes	Yes	Yes	Yes

Table 3. Contamination of *Salmonella* in samples from farms, wet markets and supermarkets

Location	Sample	Sample size	Positive rate of <i>Salmonella</i> (%)	<i>Salmonella</i> serovars (no. of strains)
Farms	<i>Ulam raja</i>	27	2 (7.41)	<i>S. enterica</i> ser. Augustenborg (2)
	<i>Selom</i>	36	0 (0)	
	<i>Pegaga</i>	18	0 (0)	
	<i>Timun</i>	9	0 (0)	
	<i>Kacang botor</i>	9	0 (0)	
	<i>Bayam brazil</i>	36	0 (0)	
	Irrigation water	45	5 (11.11)	<i>S. enterica</i> ser. Weltevreden (3), Subsp. Iiib Ser 47; C; Z35 (1), Subsp Ii Ser47:enx,z15:1,6 (1)
	Soil	45	1 (2.22)	<i>S. enterica</i> ser. Weltevreden (1)
	Manure	45	0 (0)	
Total	270	8 (2.96)		
Wet markets	<i>Ulam raja</i>	36	7 (19.44)	<i>S. enterica</i> ser. Weltevreden (2), <i>S. enterica</i> ser. Albany (3), <i>S. enterica</i> ser. Newport (1), <i>S. enterica</i> ser. Hindmarsh (1)
	<i>Selom</i>	36	13 (36.11)	<i>S. enterica</i> ser. Weltevreden (4), <i>S. enterica</i> ser. Virginia (1), <i>S. enterica</i> ser. Aberdeen (2), <i>S. enterica</i> ser. Bareilly (1), <i>S. enterica</i> ser. Hvittingfoss (2), <i>S. enterica</i> ser. Virchow (1), <i>S. enterica</i> ser. Saintpaul (1), <i>S. enterica</i> ser. Kentucky (1)
	<i>Pegaga</i>	36	13 (36.11)	<i>S. enterica</i> ser. Rubislaw (1), <i>S. enterica</i> ser. Rissen (1), <i>S. enterica</i> ser. Newport (1), <i>S. enterica</i> ser. Saintpaul (1), <i>S. enterica</i> ser. Albany (2), <i>S. enterica</i> ser. Poona (3), <i>S. enterica</i> ser. Hvittingfoss (1), <i>S. enterica</i> ser. Aberdeen (1), <i>S. enterica</i> ser. Corvallis (2)
	<i>Timun</i>	36	2 (5.56)	<i>S. enterica</i> ser. Weltevreden (1), <i>S. enterica</i> ser. Stanley (1)
	<i>Kacang botor</i>	36	2 (5.56)	<i>S. enterica</i> ser. Stanley (1), <i>S. enterica</i> ser. Muenchen (1)
	<i>Bayam brazil</i>	36	7 (19.44)	<i>S. enterica</i> ser. Weltevreden (1), <i>S. enterica</i> ser. Hvittingfoss (1), <i>S. enterica</i> ser. Albany (1), <i>S. enterica</i> ser. Corvallis (1), <i>S. enterica</i> ser. Aberdeen (1), <i>S. enterica</i> ser. Agona (1),
Total	216	44 (20.37)		
Supermarkets	<i>Ulam raja</i>	36	3 (8.33)	<i>S. enterica</i> ser. Mountpleasant (1), <i>S. enterica</i> ser. Braenderup (1), <i>S. enterica</i> ser. Lexington (1)
	<i>Selom</i>	27	1 (3.70)	<i>S. enterica</i> ser. Lexington (1)
	<i>Pegaga</i>	36	2 (5.56)	<i>S. enterica</i> ser. Hvittingfoss (1), <i>S. enterica</i> ser. Rissen (1)
	<i>Timun</i>	36	0 (0)	
	<i>Kacang botor</i>	36	0 (0)	
	<i>Bayam brazil</i>	9	0 (0)	
Total	180	6 (3.33)		
Grand total	666	58 (8.71)		

The distribution of *Salmonella* serovars isolated in this study was shown in Table 4. Of 58 isolates, 23 nontyphoidal *Salmonella* (NTS) serovars were identified, the most predominant serovar was *S. enterica* ser. Weltevreden (20.69%), followed by *S. enterica* ser. Albany (10.34%), *S. enterica* ser. Hvittingfoss (8.62%) and *S. enterica* ser. Aberdeen (6.90%).

Tables 5 and 6 show the antimicrobial susceptibility profiles and MAR index of the NTS isolates against 20

antibiotics. Among all, 20 (34.48%) isolates showed resistance toward at least one antibiotic tested with 19 (95%) and one (5%) of them were the isolates from wet markets and supermarkets, respectively. All isolates from farms were susceptible to all antibiotics tested. The highest rate of resistance was observed for ampicillin (20.69%), followed by tetracycline (18.97%), doxycycline (17.24%), trimethoprim-sulfamethoxazole (17.24%) and chloramphenicol (15.52%). Intermediate resistance was also detected for the antibiotics including

Table 4. Serovars of *Salmonella* isolates from farms, wet markets and supermarkets

Serovars	No. of isolates (%)	Farms			Wet market					Supermarket			
		Ulam raja	Irrigation water	Soil	Ulam raja	Selom	Pegaga	Timun	Kacang botor	Bayam brazil	Ulam raja	Selom	Pegaga
<i>S. enterica</i> ser. Weltevreden	12 (20.69)	- <sup>a</sup>	3	1	2	4	-	1	-	1	-	-	-
<i>S. enterica</i> ser. Albany	6 (10.34)	-	-	-	3	-	2	-	-	1	-	-	-
<i>S. enterica</i> ser. Hvittingfoss	5 (8.62)	-	-	-	-	2	1	-	-	1	-	-	1
<i>S. enterica</i> ser. Aberdeen	4 (6.90)	-	-	-	-	2	1	-	-	1	-	-	-
<i>S. enterica</i> ser. Poona	3 (5.17)	-	-	-	-	-	3	-	-	-	-	-	-
<i>S. enterica</i> ser. Corvallis	3 (5.17)	-	-	-	-	-	2	-	-	1	-	-	-
<i>S. enterica</i> ser. Augustenborg	2 (3.45)	2	-	-	-	-	-	-	-	-	-	-	-
<i>S. enterica</i> ser. Newport	2 (3.45)	-	-	-	1	-	1	-	-	-	-	-	-
<i>S. enterica</i> ser. Saintpaul	2 (3.45)	-	-	-	-	1	1	-	-	-	-	-	-
<i>S. enterica</i> ser. Rissen	2 (3.45)	-	-	-	-	-	1	-	-	-	-	-	1
<i>S. enterica</i> ser. Stanley	2 (3.45)	-	-	-	-	-	-	1	1	-	-	-	-
<i>S. enterica</i> ser. Lexington	2 (3.45)	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. enterica</i> ser. Hindmarsh	1 (1.72)	-	-	-	1	-	-	-	-	-	-	-	-
<i>S. enterica</i> ser. Virginia	1 (1.72)	-	-	-	-	1	-	-	-	-	-	-	-
<i>S. enterica</i> ser. Bareilly	1 (1.72)	-	-	-	-	1	-	-	-	-	-	-	-
<i>S. enterica</i> ser. Virchow	1 (1.72)	-	-	-	-	1	-	-	-	-	-	-	-
<i>S. enterica</i> ser. Kentucky	1 (1.72)	-	-	-	-	1	-	-	-	-	-	-	-
<i>S. enterica</i> ser. Rubislaw	1 (1.72)	-	-	-	-	-	1	-	-	-	-	-	-
<i>S. enterica</i> ser. Muenchen	1 (1.72)	-	-	-	-	-	-	-	1	-	-	-	-
<i>S. enterica</i> ser. Agona	1 (1.72)	-	-	-	-	-	-	-	-	1	-	-	-
<i>S. enterica</i> ser. Heidelberg	1 (1.72)	-	-	-	-	-	-	-	-	1	-	-	-
<i>S. enterica</i> ser. Mountpleasant	1 (1.72)	-	-	-	-	-	-	-	-	-	1	-	-
<i>S. enterica</i> ser. Braenderup	1 (1.72)	-	-	-	-	-	-	-	-	-	1	-	-
Subsp. Iiib Ser 47; C; Z35	1 (1.72)	-	1	-	-	-	-	-	-	-	-	-	-
Subsp Ii Ser47:enx,z15:1,6 (1)	1 (1.72)	-	1	-	-	-	-	-	-	-	-	-	-
Total	58 (100)	2	5	1	7	13	13	2	2	7	3	1	2

<sup>a</sup> -, No *Salmonella* isolated.

Table 5. Antibiotic resistance profiles and MAR index of *Salmonella* serovars from different sources

Isolate code <sup>a</sup>	<i>Salmonella</i> serovars	Resistance profile <sup>b</sup>	MAR index
FUR1	<i>S. enterica</i> ser. Augustenborg	– <sup>c</sup>	NA <sup>d</sup>
FUR2	<i>S. enterica</i> ser. Augustenborg	–	NA
FIW1	<i>S. enterica</i> ser. Weltevreden	–	NA
FIW2	<i>S. enterica</i> ser. Weltevreden	–	NA
FIW3	<i>S. enterica</i> ser. Weltevreden	–	NA
FIW4	Subsp. Iiib Ser 47; C; Z35	–	NA
FIW5	Subsp. Ii Ser47:enx,z15:1,6	–	NA
FSO1	<i>S. enterica</i> ser. Weltevreden	–	NA
WUR1	<i>S. enterica</i> ser. Weltevreden	DO-AZM-COL	0.15
WUR2	<i>S. enterica</i> ser. Weltevreden	–	NA
WUR3	<i>S. enterica</i> ser. Albany	AMP-SAM-CFP-TE-SXT-C	0.30
WUR4	<i>S. enterica</i> ser. Albany	AMP-SXT-C	0.15
WUR5	<i>S. enterica</i> ser. Albany	AMP-SXT-C	0.15
WUR6	<i>S. enterica</i> ser. Newport	–	NA
WUR7	<i>S. enterica</i> ser. Hindmarsh	AMP-TE-DO-MH-C	0.25
WSE1	<i>S. enterica</i> ser. Weltevreden	ATM-AZM	NA
WSE2	<i>S. enterica</i> ser. Weltevreden	AMC-FEP	NA
WSE3	<i>S. enterica</i> ser. Weltevreden	AMP-AZM	NA
WSE4	<i>S. enterica</i> ser. Weltevreden	–	NA
WSE5	<i>S. enterica</i> ser. Virginia	TE-DO-MH	NA
WSE6	<i>S. enterica</i> ser. Aberdeen	–	NA
WSE7	<i>S. enterica</i> ser. Aberdeen	–	NA
WSE8	<i>S. enterica</i> ser. Bareilly	–	NA
WSE9	<i>S. enterica</i> ser. Hvittingfoss	–	NA
WSE10	<i>S. enterica</i> ser. Hvittingfoss	–	NA
WSE11	<i>S. enterica</i> ser. Virchow	–	NA
WSE12	<i>S. enterica</i> ser. Saintpaul	–	NA
WSE13	<i>S. enterica</i> ser. Kentucky	–	NA
WP1	<i>S. enterica</i> ser. Rubislaw	–	NA
WP2	<i>S. enterica</i> ser. Rissen	AMP-ATM-CTX-CAZ-CFP-FEP-CRO-TE-DO-SXT-C	0.55
WP3	<i>S. enterica</i> ser. Newport	–	NA
WP4	<i>S. enterica</i> ser. Saintpaul	–	NA
WP5	<i>S. enterica</i> ser. Albany	AMP-SXT-C	0.15
WP6	<i>S. enterica</i> ser. Albany	AMP-AMC-SAM- ATM-ETP-CFP- CRO-TE-SXT-C	0.50
WP7	<i>S. enterica</i> ser. Poona	–	NA
WP8	<i>S. enterica</i> ser. Poona	–	NA
WP9	<i>S. enterica</i> ser. Poona	–	NA
WP10	<i>S. enterica</i> ser. Hvittingfoss	AMP-CTX-TE-DO-MH-SXT-AZM-C-COL	0.45
WP11	<i>S. enterica</i> ser. Aberdeen	–	NA
WP12	<i>S. enterica</i> ser. Corvallis	TE-DO-MH	NA
WP13	<i>S. enterica</i> ser. Corvallis	–	NA
WT1	<i>S. enterica</i> ser. Weltevreden	AZM	NA
WT2	<i>S. enterica</i> ser. Stanley	–	NA
WKB1	<i>S. enterica</i> ser. Stanley	–	NA
WKB2	<i>S. enterica</i> ser. Muenchen	–	NA
WBB1	<i>S. enterica</i> ser. Weltevreden	–	NA
WBB2	<i>S. enterica</i> ser. Hvittingfoss	–	NA
WBB3	<i>S. enterica</i> ser. Albany	TE-DO-MH-SXT	NA
WBB4	<i>S. enterica</i> ser. Corvallis	AMP-SAM-TE-DO-SXT	0.25
WBB5	<i>S. enterica</i> ser. Aberdeen	–	NA
WBB6	<i>S. enterica</i> ser. Agona	TE-DO	NA
WBB7	<i>S. enterica</i> ser. Heidelberg	AMP-AMC	NA
SUR1	<i>S. enterica</i> ser. Mountpleasant	–	NA
SUR2	<i>S. enterica</i> ser. Braenderup	–	NA
SUR3	<i>S. enterica</i> ser. Lexington	–	NA
SSE1	<i>S. enterica</i> ser. Lexington	–	NA
SP1	<i>S. enterica</i> ser. Hvittingfoss	–	NA
SP2	<i>S. enterica</i> ser. Rissen	AMP-AMC-TE-DO-MH-SXT-C	0.35

<sup>a</sup> Isolate code: F (farm), W (wet market), S (supermarket), UR (*ulam raja*), IW (irrigation water), SO (soil), SE (*selom*), P (*pegaga*), T (*timun*), KB (*kacang botor*), BB (*bayam brazil*).

<sup>b</sup> Antimicrobial abbreviations: ampicillin (AMP), amoxicillin-clavulanic acid (AMC), ampicillin-sulbactam (SAM), piperacillin-tazobactam (TZP), aztreonam (ATM), ertapenem (ETP), imipenem (IPM), meropenem (MEM), cefotaxime (CTX), ceftazidime (CAZ), cefoperazone (CFP), cefepime (FEP), ceftriaxone (CRO), tetracycline (TE), doxycycline (DO), minocycline (MH), trimethoprim-sulfamethoxazole (SXT), azithromycin (AZM), chloramphenicol (C) and colistin (COL).

<sup>c</sup> –, not resistant to any antimicrobial agents tested.

<sup>d</sup> NA: not available

Table 6. Antimicrobial profiles of *Salmonella* toward different classes of antibiotic

No	Antibiotic class	Antibiotics	Antimicrobial profiles		
			Susceptible (%)	Intermediate (%)	Resistant (%)
1	Penicillins	Ampicillin	46 (79.31)	- <sup>a</sup>	12 (20.69)
2	β-lactam/ β-lactamase combination	Amoxicillin-Clavulanic acid	51 (87.53)	3 (5.17)	4 (6.90)
		Ampicillin-sulbactam	50 (86.21)	5 (8.62)	3 (5.17)
		Piperacillin-tazobactam	58 (100)	-	-
3	Monobactams	Aztreonam	54 (93.10)	1 (1.72)	3 (5.17)
4	Carbapenems	Ertapenem	57 (98.28)	-	1 (1.72)
		Imipenem	58 (100)	-	-
		Meropenem	56 (96.55)	2 (3.45)	-
		Cefalosporins (3 <sup>rd</sup> and 4 <sup>th</sup> generation)	Cefotaxime	56 (96.55)	-
5		Ceftazidime	57 (98.28)	-	1 (1.72)
		Cefoperazone	49 (84.48)	6 (10.34)	3 (5.17)
		Cefepime	55 (94.83)	3 (5.17)	3 (5.17)
		Ceftriaxone	56 (96.55)	-	2 (3.45)
		6	Tetracyclines	<u>Tetracycline</u>	44 (75.86)
Doxycycline	47 (81.03)			1 (1.72)	10 (17.24)
Minocycline	50 (86.21)			3 (5.17)	5 (8.62)
7	Folate pathway antagonists	Trimethoprim-sulfamethoxazole	48 (82.76)	-	10 (17.24)
8	Macrolides	Azithromycin	53 (91.38)	-	5 (8.62)
9	Phenicols	Chloramphenicol	49 (84.48)	-	9 (15.52)
10	Colistin	Colistin	56 (96.55)	-	2 (3.45)

<sup>a</sup> -, None of the isolates showed related profile.

cefoperazone (10.34%), ampicillin-sulbactam (8.62%), amoxicillin-clavulanic acid, cefepime, tetracycline and minocycline (5.17% each). Piperacillin-tazobactam and imipenem were effective in preventing the growth of NTS in this study with 100% susceptibility. An isolate of *S. enterica* ser. Albany from *pegaga* in the wet market was found resistant toward ertapenem. Colistin resistance was also demonstrated by one *S. enterica* ser. Weltevreden and *S. enterica* ser. Hvittingfoss from wet markets, isolated from *ulam raja* and *pegaga*, respectively. As shown in Table 5, 11 isolates (18.97%) were MDR and *S. enterica* ser. Rissen was detected with the highest MAR index value of 0.55. Both *S. enterica* ser. Rissen isolated in this study were MDR which exhibit resistance to at least five different classes of antibiotic. Among all different NTS serovars, six serovars showed MDR characteristics and *S. enterica* ser. Albany represent the highest prevalence with 5 (83.33%) isolates exhibit MDR (Table 5).

#### 4. Discussion

Finding on the prevalence of *Salmonella* in this study was in line with the previous study which reported a relatively high incidence of *Salmonella* in salad vegetables from the wet market including 21.5% in the northern and middle region of Malaysia (Abatcha et al., 2018), 12.9% in Mekong Delta, Vietnam (Nguyen et al., 2021) and 17.59% in Hue City, Vietnam (Chau et al., 2014). Cross-contamination through improper handling and poor hygienic practices might play a significant role as a source of *Salmonella* contamination to salad

vegetables in the wet market, in addition, with the open display under ambient temperature, growth and multiplication of pathogenic bacteria including *Salmonella* will be promoted. In Malaysia, Nidaullah et al. (2017) reported consistent contamination of *Salmonella* (86.18% detection) in the environmental samples collected along the wet market chicken processing line, suggesting that poultry from the wet market can be an important vehicle for the transmission of *Salmonella*. Since selling locations for poultry and vegetables are adjacent in the wet market, *Salmonella* might be transferred to the salad vegetables through unhygienic and poor sanitation practices among retailers and consumers. Rodents that inhabit the wet markets could also be considered as the reservoirs and transmitters of *Salmonella* to salad vegetables. A study by Ribas et al. (2016) reported 49.10% prevalence of *Salmonella* in rats from wet markets in Thailand, they have belonged to three serovars: *S. enterica* ser. Typhimurium (30%), *S. enterica* ser. Weltevreden (12.7%) and *S. enterica* ser. 4,[5],12:i:- (6.4%). This finding indicates that rodents could act as a potential transmitter of *Salmonella* due to the contact they have with food products stored and sold in wet markets.

Overall occurrence of *Salmonella* in salad vegetables from a supermarket in this study was low, comparable to other studies in Malaysia (3.0%) and Turkey (3.38%) (Buyukunal et al., 2015; Saw et al., 2020). A supermarket is an organized grocery store selling a variety of goods. The goods were systematically arranged in different zones under specific conditions

depending on the type of goods to avoid cross-contamination. The environment in a supermarket is commonly clean, tidy and cool with proper maintenance, these conditions are required to maintain the quality of goods, especially food items (Saw et al., 2020). All these circumstances might contribute to the low incidence of *Salmonella* in salad vegetables sold in a supermarket.

In this study, only two (1.48%) *Salmonella* were detected from vegetable samples collected from the farm, this finding could be explained by the probability of low survivability of *Salmonella* on the vegetables during cultivation due to direct exposure to sunlight. According to Strawn, Gröhn, Warchocki et al. (2013), pathogens that present in soil are likely to contaminate vegetables, however, the presence of pathogens will decrease over time after cultivation, due to environmental conditions exposure such as ultraviolet light that reduces pathogen loads. Furthermore, the use of pesticides might be one of the causes of the low detection of *Salmonella* in farm vegetables, the pesticides have been shown to inhibit growth and kill soil microorganisms (Filimon et al., 2015). This finding was further evidenced by a study by Ottesen et al. (2015) which observed lower counts of *Salmonella* on tomato fruits and leaves that were regularly sprayed with pesticides as compared to controls that were not exposed to pesticides.

The prevalence of *Salmonella* from irrigation water in vegetable farms was also demonstrated by several other studies such as 12.5% in New York, USA, (Strawn, Gröhn, Warchocki et al., 2013), 7.7% in California, USA (Benjamin et al., 2013) and 3.4% in Kano, Nigeria (Abakpa et al., 2015). Through observation during the sampling process, all vegetable farmers involved in this study were found using irrigation pumps to water their crops from wells and streams (Table 3), sprinkler irrigation is the most common form of irrigation practised in the farms. Due to the usage of untreated water which is found to have *Salmonella*, vegetables might be contaminated through irrigation. Adetunde et al. (2015) revealed a strong positive correlation between the microbial counts on vegetables and in irrigation water, which makes it one of the main sources of vegetable contamination. Furthermore, the sprinkler method of irrigation used also contributed to the higher transfer and retention of bacteria on the edible portion of vegetables (Gupta and Madramootoo, 2017). Contamination of pathogens into streams and wells is commonly attributable to household or small industries wastewater (Keraita et al., 2014) and wild or domestic animals (Adetunde et al., 2015).

The detection of *Salmonella* in soil samples collected from farms was in agreement with other studies in Kano,

Nigeria (2.5%) and New York, USA (2.0%) (Strawn, Fortes, Bihn et al., 2013; Abakpa et al., 2015). *Salmonella* may enter the soil environment from various sources such as through contaminated water, manure, livestock and wildlife (Alegbeleye et al., 2018), their survivability in the soil is influenced by various factors such as temperature, moisture, soil type, presence of plants, pH, nutrient availability, exposure to sun (ultraviolet) light and protozoan predation (Jacobsen and Bech, 2012). With regard to this study, the most probable source of *Salmonella* contamination into the soil is from untreated water used for irrigation, based on the prevalence of *Salmonella* in water samples analyzed. This finding could be related to a study by Leifert et al. (2008) which demonstrated the exposure of water through precipitation and irrigation as one of the most critical factors influencing microbial transport and survival in soil.

Our results suggest that the application of treated manure to fields can significantly decrease the risk of *Salmonella* contamination as none of the manure samples tested in this study were contaminated with *Salmonella*. All manure samples taken for this study were in the form of processed pellets of chicken faeces. The manure management practices, with regards to the collection, transport, handling, treatment, disposal and utilization might affect the survival of foodborne pathogens. Treatment methods for manure such as thermal, chemical, biological and physical are commonly used, all these types of treatment may reduce pathogen loads in manure before releasing into the environment (Alegbeleye and Sant'Ana, 2020). Different farms handle manure using various types of systems and their combinations, each treatment is capable of reducing pathogen loads in manure, even though the efficiency of different methods may vary (Millner, 2014).

*S. enterica* ser. Weltevreden was also reported as the most found serovar in salad vegetables from other studies in Vietnam (Nguyen et al., 2021), Malaysia (Abatcha et al., 2018) and Indonesia (Kusumaningrum et al., 2012). This serovar has been identified as the common pathogen associated with human salmonellosis in the South and South-East Asian regions (Gunasena and De Silva, 2021). *S. enterica* ser. Weltevreden was recognized for the first time to cause a salmonellosis outbreak related to plant products in Scandinavia (Norway, Denmark and Finland), this outbreak occurred in 2007 involving 45 cases resulting from consumption of contaminated alfalfa sprouts (Gunasena and De Silva, 2021). The capability of *S. Weltevreden* to grow and survive in a plant is possibly due to the presence of additional carbohydrate metabolism clusters in their genomes as compared to other serovars, which enable

this serovar to metabolize carbohydrates better in plant tissue (Brankatschk *et al.*, 2012). Brankatschk *et al.* (2012) also demonstrated the presence of genes for myo-inositol utilization in *S. enterica* ser. Weltevreden isolates tested, the capability of this serovar to grow on stereoisomers of myo-inositol might also contribute to their survival on plants as this carbohydrate is commonly present in soil and the phosphorylated form of myo-inositol is stored in plant tissue. The presence of these types of genes in *S. enterica* ser. Weltevreden might be considered as the reason for their capability to become prevalent in salad vegetable samples involved in this study.

A study on the prevalence of *Salmonella* in salad vegetables from Peninsular Malaysia by Abatcha *et al.* (2018) found a relatively comparable percentage of *S. enterica* ser. Albany (4.60%), *S. enterica* ser. Hvittingfoss (5.75%) and *S. enterica* ser. Aberdeen (4.60%) with this present study. These serovars are rarely associated with salmonellosis outbreaks, however, in 2016, *S. enterica* ser. Hvittingfoss has been reported to cause outbreaks associated with fresh produce (cantaloupes) in Australia (Flynn, 2016).

On the other hand, *S. enterica* ser. Newport is one of the most important NTS serovars that has been associated with several major outbreaks due to consumption of raw vegetables such as alfalfa sprouts, tomatoes, lettuce and cucumber (El-DougDoug *et al.*, 2019; Yang *et al.*, 2020). Latest in 2020, a multistate outbreak of *S. enterica* ser. Newport infections linked to onions affecting 1127 people from 48 states in the USA has been reported (CDC, 2020). This serovar has previously been listed among the three highest ranks of *Salmonella* serovars associated with foodborne outbreaks in the USA since 1970 (CDC, 2013). Furthermore, the Public Health Agency of Canada has listed *S. enterica* ser. Newport in the ten most commonly reported *Salmonella* serotypes in Canada accounting for human illness in 2018 (Government of Canada, 2020). In Malaysia, there is no published report of a foodborne outbreak associated with *S. enterica* ser. Newport so far. However, *S. enterica* ser. Newport was present in salad vegetables tested in this study and also in a study by Abatcha *et al.* (2018) with 3.45% and 2.30% detection, respectively. This indicates the probability for the occurrence of salmonellosis associated with salad vegetables contaminated with *S. Newport* in Malaysia.

Multiple types of other serovars were also found among samples tested (Table 4), the diversity of these serovars might have been influenced by the different sampling locations that were exposed to different sources of contamination (different farms, transports, retail

outlets or retailers).

A few reports were published concerning the antibiotic-resistant *Salmonella* associated with vegetables in Malaysia and other Southeast Asian countries (Abatcha *et al.*, 2018; Nguyen *et al.*, 2021). Among the isolates recovered in this study, resistance to ampicillin, tetracycline and trimethoprim-sulfamethoxazole occurred most frequently, these results are consistent with the report from a study of resistance among *Salmonella* isolates from raw vegetables in China (Yang *et al.*, 2020). It is noteworthy that the highest resistance is toward ampicillin as this antibiotic is categorized as the critically important antibiotic with high priority in treating serious bacterial infections in people (Scott *et al.*, 2019; WHO, 2019). Resistance toward tetracycline, doxycycline, trimethoprim-sulfamethoxazole and chloramphenicol could also be of concern since these antibiotics are categorized as highly important for human medicine by the WHO (2019). All the resistant NTS found in this study were isolated from wet markets and supermarkets, thus, the most probable source of antibiotic resistance is from direct contamination of resistant isolates from humans during harvesting and handling of salad vegetables due to insufficient hygiene measures. Similarly, Mesbah Zekar *et al.* (2017) demonstrated that raw eaten vegetables at the market constitute a reservoir of resistant and MDR bacteria that might be reflected from improper hygiene practices during handling.

Attention should be given to the presence of ertapenem resistant *S. enterica* ser. Albany since carbapenems are the last line of antibiotics used to treat human infections caused by MDR bacteria (Monte *et al.*, 2019). However, this finding is relatively low as compared to the finding by Igbinsosa *et al.* (2017), who reported the presence of 16 (22.22%) imipenem (carbapenem class of antibiotic) resistant *Salmonella* isolated from fresh vegetables. According to WHO (2019), there is still limited transmission of carbapenem-resistant *Salmonella* from non-human sources, however, based on reports from several countries, the spread in the food chain is increasing. Carbapenem-resistant bacteria have caused severe outbreaks, the infections are difficult to treat and associated with high costs and mortality (Malchione *et al.*, 2019). The increasing trend of carbapenem-resistance in bacteria necessitates a move to the usage of colistin as the last-resort antibiotic where this antibiotic is active against carbapenem-resistant bacteria (Malchione *et al.*, 2019; Wakabayashi *et al.*, 2020). The finding of colistin-resistant NTS in this study is comparable to a report by Verma *et al.* (2018) which found two (7.7%) isolates of colistin-resistant *Salmonella* from vegetable samples examined. The

emergence of colistin-resistant bacteria is a worldwide concern as this antibiotic is recognized as the last choice of antibiotic for the treatment of infections in hospitalized patients caused by carbapenem-resistant Gram-negative bacteria (Elbediwi *et al.*, 2019). The presence of carbapenem and colistin-resistant NTS isolated from salad vegetables in this study, even in low percentage, indicate the emerging spread of resistance toward these last-resort antibiotics in the food chain.

The level of MDR NTS (18.97%) in this study was lower than that previously reported by Abatcha *et al.* (2018) in Malaysia (55.1%). Abatcha *et al.* (2018) also reported the presence of two (50%) MDR isolates of *S. Albany* from vegetables with high MAR index of 0.66. Several other studies also reported the high prevalence of MDR among *S. enterica* ser. Albany such as in the chicken food chain in Cambodia (96.1%), poultry processing plant and retail markets in South Korea (93.2%) and chicken meat in Korea (80%) (Shang *et al.*, 2019; Vuthy *et al.*, 2017; Sin *et al.*, 2020). The MDR characteristics among most of *S. enterica* ser. Albany isolated in this study and also from others might indicate the specific *S. enterica* ser. Albany in the food chains have acquired resistance to various types of antimicrobial agents including the medically important antibiotics.

## 5. Conclusion

Findings in this study reveal the relatively high occurrence of NTS contamination of salad vegetables from wet markets in Terengganu, Malaysia, suggesting that salad vegetables could represent a potential source of salmonellosis. Furthermore, the resistance toward medically important antibiotics among NTS in this study highlight the potential risk to public health. More attention should be given to the presence of carbapenem and colistin-resistant isolates as these antibiotics are categorized as last-resort antibiotics in the treatment of severe foodborne illness cases. These data may contribute to providing useful information for the development of effective strategies to ensure the safety of salad vegetables sold at retail outlets in Malaysia.

## Conflict of interest

The authors declare no conflict of interest.

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